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Pharmacological approaches for a novel GABA_A receptor agonist

Valk, Beatrijs Irene

DOI:
[10.33612/diss.182479404](https://doi.org/10.33612/diss.182479404)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2021

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Valk, B. I. (2021). *Pharmacological approaches for a novel GABA_A receptor agonist: the long and winding road of drug development in anesthesia*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen. <https://doi.org/10.33612/diss.182479404>

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Pharmacological approaches for a novel GABA_A receptor agonist

*The long and winding road of drug
development in anesthesia*

Beatrijs Irene Valk

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Cover & lay-out: Karin van der Vegt, Revista ontwerp

Printed by: Ridderprint BV, Ridderkerk

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Pharmacological approaches for a novel GABA_A receptor agonist

The long and winding road of drug development in anesthesia

Proefschrift

ter verkrijging van de graad van doctor aan de
 Rijksuniversiteit Groningen
 op gezag van de
 rector magnificus prof. dr. C. Wijmenga
 en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

maandag 25 oktober 2021 om 16.15 uur

door

Beatrijs Irene Valk

geboren op 24 september 1994
 te Amsterdam

Promotores

Prof. dr. M.M.R.F. Struys

Prof. dr. A.R. Absalom

Copromotor

Dr. D.J. Eleveld-Ufkes

Beoordelingscommissie

Prof. dr. R.H. Henning

Prof. dr. A. Dahan

Prof. dr. K. Meissner

Voor papa

Besondere Vorkommnisse: keine.

Reinhard Mey

Paranimfen

Lisa Wagner

Minke M. de Wolf

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CHAPTER 1

Introduction and aims of this thesis

Partially modified from *Etomidate and its analogues: A Review of Pharmacokinetics and Pharmacodynamics*, *Clinical Pharmacokinetics*, 2021, online ahead of print

Beatrijs I. Valk, Michel M.R.F. Struys

General introduction

Although anesthesiology is a relatively young medical specialty, the concept of anesthesia dates back to pre-historic times. The oldest known sedative is one that is still notorious today for its mind-numbing effect: alcohol, which was first distilled at least 10,000 years ago.¹ Other well-known sedatives and hypnotics described in ancient records include opium (derived from the juice of the *Papaver somniferum* plant), cocaine (from the leaves of the plant *erythroxylon coca*), cannabinoids (from the plant *cannabis sativa*) and leaves of the aconite plants, which were used in regions ranging from ancient China, to Mesopotamia, Ancient Greece and the Roman Empire, to deep within the jungles of South America. Modern history of Anesthesiology is generally considered to start in the 19th century, culminating in the first successful public demonstration of anesthesia on the 16th of October 1846 in the Massachusetts General Hospital, Boston, when dentist William Thomas Green Morton administered diethyl ether to a man undergoing a procedure to remove a tumor from his neck.² Since then, many agents have been developed for both inhalational and intravenous anesthesia. However, the ideal anesthetic agent has not yet been discovered, although we are getting closer.

The ideal intravenous anesthetic agent

The ideal intravenous anesthetic agent has several characteristics. Most importantly, the ideal i.v. anesthetic agent shows a rapid onset of action and an equally (organ independent) rapid clearance, so that its effect can be tightly controlled. Furthermore, the ideal i.v. anesthetic agent does not affect circulatory and respiratory systems in a negative way and has a minimum amount of side effects. Ideally, it also has additional desirable pharmacologic effects, for example as an anti-emetic.³

In the next paragraphs, the aims of this thesis are presented and detailed background information on the mechanism behind anesthesia, and the current state of intravenous anesthesia is provided.

Aims of this thesis

This thesis presents various pharmacological approaches used in the drug development process of GABA_A receptor agonist ABP-700. It uses them to assess the safety and efficacy of this novel anesthetic agent, but also to show the use of both pharmacology at a receptor level and advanced population pharmacokinetic-pharmacodynamic modelling in assessing and interpreting side effects.

- The first dose regimen of a novel intravenous drug is a single bolus dose. A phase I study of ascending single bolus doses of ABP-700 was executed and is presented in chapter 2.
- In daily anesthetic practices, anesthesia or sedation is maintained by a continuous infusion of the anesthetic. Safety and efficacy of ascending continuous infusion doses of ABP-700 were assessed in a phase I study and are presented in chapter 3.
- During both clinical studies, involuntary muscle movements were a regularly observed phenomenon. To assess the cause of these movements, pre-clinical studies in beagle dogs and additional receptor studies of the GABA_A receptor were performed. The results of these studies are presented in chapter 4.
- A recirculatory pharmacokinetic/pharmacodynamic model was developed to further characterize the pharmacokinetic and pharmacodynamic properties of ABP-700, but also to assess whether a relationship exists between clinical measures of depth of anesthesia and the occurrence of involuntary muscle movements. This model is presented in chapter 5.

The GABA_A-receptor

For a long time, the molecular mechanisms behind the clinical drug effect of anesthetic agents were an enigma for researches, not in the least because there is a great chemical diversity among anesthetics. Indeed, only during the last three decades⁴ it was discovered that the γ -aminobutyric acid type A (GABA_A) receptor is the likeliest molecular target for most intravenous general anesthetic agents.⁵⁻⁷

The GABA_A receptor is a transmitter-gated ion channel. It is composed of five transmembrane-crossing subunits, which are surrounding a central ion channel pore, and is located on synapses throughout the central nervous system (CNS).

Figure 1-1. A schematic figure of GABA_A receptor, containing five subunits, indicating the most important anesthetic binding sites. Propofol binds at both the etomidate binding sites and the barbiturate binding sites.

The GABA_A receptor produces its effect when it is activated by the matching neurotransmitter, GABA. The receptor undergoes a conformational change, allowing the center ion channel pore to open. This permits chloride ions to pass from the extracellular to the intracellular space, resulting in hyperpolarization of the neuron, inhibiting the activity of that particular cell (**Figure 1-2**).⁸ When anesthetic agents bind to a certain site somewhere on the receptor, it enhances the response of the receptor to GABA, thus enhancing the inhibiting effect on the CNS. These are the so-called positive allosteric modulators of the GABA_A receptor. The mechanism behind and the extent of this enhancement depends on the anesthetic and its binding site on the GABA_A receptor.

Figure 1-2. The influx of chloride ions through the GABA_A receptor. After binding of GABA, a moderate influx of chloride ions takes place. After binding of both GABA and an anesthetic, in this case etomidate, the influx of chloride ions is increased.

In order to identify these binding sites, it is important to understand the configuration of the GABA_A receptor. About 19 different types of GABA_A subunits exist (α_{1-6} , β_{1-3} , γ_{1-3} , δ , ϵ , θ , π and ρ_{1-3}).⁹ Usually, the receptor is composed of two copies of an α - and two copies of a β subunit, supplemented by one copy of another subunit type.⁹ In total, 20 to 30 isoforms exist throughout the mammalian CNS, each with its own distinctive distribution throughout the CNS and matching behavioral profile. The most abundant GABA_A receptor in the mammal CNS are the $\alpha_1\beta_2\gamma_2$ and the $\alpha_1\beta_3\gamma_2$ isoforms.⁸

Important binding sites in anesthesia (Figure 1-1)

The neurotransmitter, GABA, binds to the β +/ α - interface in the extracellular domain of the GABA_A receptor.^{10,11} Due to the configuration of the receptor, the presence of two α - and two β -subunits, there are generally two binding sites for GABA. For general anesthetic agents, multiple binding sites have been identified. These binding sites are located in the trans-membrane domain of the receptor. Propofol, one of the most widely used i.v. anesthetics, binds to the β +/ α - interface, the α +/ β - interface and the γ +/ β - interface.¹² Barbiturates have been shown to bind to the α +/ β - and the γ +/ β - interfaces¹³, whereas etomidate solely binds to the β +/ α - interface.^{14,15} Furthermore, whilst most general anesthetics show little selectivity for the different GABA_A receptor subtypes, etomidate selectively enhances

the GABA response of receptors containing the β_2 or β_3 subunits.¹⁶ For benzodiazepines, the binding site on the receptor is located at the $\alpha+\gamma-$ interface, also in the extracellular domain.^{17,18} Note that in order for benzodiazepines to bind to the receptor, a γ -subunit has to be present.

An overview of intravenous anesthetic agents

In order to appreciate the reason why it might be desirable to develop a novel anesthetic agent, it is important to know which intravenous anesthetic agents are currently available, along with their advantages and disadvantages.

Barbiturates

Barbiturates were discovered in the early twentieth century and were the first intravenous anesthetic agents to be used clinically. The most well-known barbiturates are thiopental, which was introduced into clinical practice in 1934,¹⁹ and methohexital. This class of drugs binds to two sites on the GABA_A receptor (**Figure 1-1**) where it acts as an agonist. Barbiturates are in general metabolized in the liver. Barbiturates produce dose-dependent sedation and anesthesia. Distribution to the brain is quick, resulting in a fast onset of action, namely less than 30 seconds. Offset of anesthesia takes usually five to ten minutes after administration of a single dose. Clinical effects of barbiturates are sensitive to alterations in the pharmacokinetic parameters, regularly leading to a delayed recovery in patients, especially the elderly.²⁰

Barbiturates produce a dose-related central respiratory depression, as well as cardiovascular depression due to peripheral vasodilatation. Baroreceptor reflexes are still intact, leading to an increase in heart rate as a response to the decrease in blood pressure.²¹ A major disadvantage of barbiturates is that they cannot be mixed with other acidic solution because it can result in precipitation as free acids in the infusion line. This means that many other drugs used in clinical anesthesia, such as many muscle relaxant agents, S-ketamine and midazolam, cannot be co-administered with barbiturates.²² Furthermore, barbiturates produce an increase in circulating histamine with the potential of causing an anaphylactoid reaction.²³

Propofol

Propofol is currently the best-known general anesthetic agent. As previously mentioned, it is a GABA_A receptor agonist and binds to several binding sites on the receptor. It is insoluble in water and is currently mostly formulated as an Intralipid-based emulsion.²⁴ Propofol was

developed in the 1970's and has been available for commercial use in Europe since 1986, with the USA following suit in 1989.²⁵ It is used for induction and maintenance of general anesthesia or sedation. Its most prominent side effects are pain on injection, cardiovascular depression and metabolic side effects, such as hyperlipidemia, attributable to the lipid formulation of propofol, 6-diisopropylphenol.²⁶ The most notorious side effect of propofol is the so-called "Propofol-Infusion-Syndrome". This is a rare but often fatal phenomenon that characterized by severe metabolic acidosis, rhabdomyolysis, hyperkalemia and cardiovascular depression poorly responsive to catecholamine administration.

The pharmacokinetic properties of propofol are relatively fast. Due to the intravenous administration route, there is no first-pass effect, as is the case in all intravenous drugs. Furthermore, it swiftly crosses the blood-brain barrier hereby causing rapid loss of consciousness. It is also characterized by a fast initial distribution which causes a short time to offset of clinical effect after a single bolus dose. Additionally, propofol has a relative short context-sensitive half-life after prolonged infusion compared to the other clinically-available intravenous hypnotic agents. Elimination of propofol is mainly via hepatic metabolism, with renal metabolism playing a lesser but still significant role.

Propofol has numerous effects on the central nervous system (CNS). Of course, the most obvious effect is its hypnotic effect, which occurs within 30 seconds after a bolus dose, through its function as a GABA_A positive allosteric modulator. Additionally, propofol has amnestic²⁷ and anxiolytic²⁸ effects as well as a remarkable antiemetic action.²⁹ It decreases cerebral blood flow, intracranial pressure (ICP) and cerebral metabolic rate, making it a popular hypnotic agent for anesthesia during neurosurgery.³⁰

The cardiovascular system is significantly affected by propofol. The most prominent effect is a drop in systemic blood pressure due to vasodilation, together with a decrease in cardiac output and an inhibition of physiological baroreceptor response, which enhances the cardiovascular depression. This property of propofol is even more pronounced in elderly patients and in patients who suffer from pre-existing cardiovascular instability.³¹ Also, a concentration-dependent ventilatory depression is observed with propofol, with apnea occurring in higher doses.³² In contrast to the barbiturates, no effects on histamine are observed upon administration of propofol.³³

Etomidate

Etomidate, like the barbiturates and propofol, is an agonist of the GABA_A receptor. It is based on an imidazole ring. It was initially developed as an antifungal agent in 1964, but during animal studies, its hypnotic effect was observed.³⁴ Originally, it was synthesized as a racemic mixture, but it was found that the R(+)-enantiomer had higher hypnotic potency. Etomidate was introduced into clinical practice in 1972.³⁵

The pharmacokinetic properties of etomidate are fast, and distribution to the brain occurs rapidly. Metabolism of etomidate occurs mostly through hydrolysis by esterases, resulting in an inactive carboxylic acid metabolite. These esterases are present throughout the body, however, the metabolism mostly takes place in the liver.³⁶

Etomidate produces a swift onset of hypnotic effect, similarly to barbiturates and propofol, through its action on the GABA_A receptor.³⁷ It has no analgesic effect. A major advantage of etomidate is that it barely impacts the cardiovascular system. It produces minimal changes in systemic blood pressure and heart rate, which makes it an excellent drug to use in patients who are hemodynamically unstable, who suffer from cardiac disease, or even those in hemorrhagic shock.^{38,39} Effects on the respiratory system are smaller than in propofol and barbiturates, although a depression of response to CO₂ still exists.⁴⁰ No histamine release occurs upon administration of etomidate.⁴¹

Based on the properties described, etomidate seems to be a suitable drug for continuous infusion. However, in 1983, a decade after the clinical introduction of etomidate into clinical practice, it was first reported that mortality among patients in the ICU who received prolonged infusions of etomidate increased.⁴² Subsequently, it was discovered that etomidate caused suppression of the adrenal axis, by inhibiting the cytochrome P450 enzyme 11 β -hydroxylase. Etomidate's potency for inhibiting this enzyme is 100-fold greater than its hypnotic potency. This inhibition results in a prolonged adrenal suppression, which lasts 6-8 hours after administration of a single bolus etomidate, and more than 24 hours after an infusion.⁴³⁻⁴⁷ Especially critically ill patients are prone to this effect after prolonged infusion of etomidate.⁴⁸⁻⁵⁰ Other significant side effects include postoperative nausea and vomiting, pain on injection and myoclonic movements^{51,52} or involuntary muscle movements during induction and emergence. These movements can be (partly) attenuated by split-dose induction⁵³ or premedication with benzodiazepines and/or opioids.⁵⁴

Benzodiazepines

Benzodiazepines are widely used in anesthesia and in the medical field in general. They work as anxiolytics, sedatives and hypnotics. They have a specific binding site on the GABA_A receptor, as described previously, where they act as positive allosteric modulators. The most frequently used benzodiazepine in daily anesthesia practice is midazolam. The other benzodiazepines, diazepam, lorazepam and temazepam, are more used outside of anesthesia practice.

The first benzodiazepine, diazepam, was discovered in 1954, and first used as an IV anesthetic in 1965. Midazolam was the first benzodiazepine primarily produced for use in anesthesia in 1976.⁵⁵ It is the most short-acting benzodiazepine clinically available, with

lorazepam and temazepam being intermediate-acting agents and diazepam a long-acting agent. After absorption, benzodiazepines are rapidly distributed. Metabolism takes place in the liver.⁵⁶⁻⁵⁸

Because they enhance GABA_A activity, benzodiazepines produce hypnosis/sedation, anxiolysis and amnesia. Furthermore, they have anticonvulsant and centrally produced muscle-relaxing properties. Interestingly, it depends on the composition of the GABA_A receptor whether benzodiazepines have sedative, amnestic and anticonvulsant, or anxiolytic and muscle-relaxant effect, with the responsible α -receptor being the α_1 and the α_2 subunits.⁵⁹ Time to recovery is usually slower with benzodiazepines compared to the other hypnotics, especially when co-administered with other anesthetic drugs, hereby often producing a synergistic effect. Like other anesthetic agents, benzodiazepines have a suppressive effect on the respiratory system, enhanced when co-administration with opioids occurs.^{60,61} When administered alone, benzodiazepines have little effect on the cardiovascular system producing only a mild decrease in systemic blood pressure. Benzodiazepines have no analgesic properties.

Ketamine

Ketamine is the odd one out under the general anesthetic agents. As opposed to most general anesthetic agents, it has no affinity for the GABA_A receptor. Instead, ketamine acts like a noncompetitive antagonist of the *N*-methyl-D-aspartate (NMDA) receptor, which has a stimulating effect on the CNS.⁶² In short, ketamine blocks the stimulation of the CNS, whereas most other general anesthetic agents enhance the inhibition of the CNS.

Ketamine was introduced into clinical use in 1970, and initially widely used as a battlefield anesthetic agent.⁶³ Ketamine is a racemic mixture of an R(-)- and an S(+)-isomer. It was discovered, that the S(+)-isomer has more favorable pharmacokinetic and pharmacodynamic properties and the S(+)-ketamine is increasingly used in anesthesia.⁶⁴

Ketamine is usually administered through the intravenous route, however, other formulations, for example sublingual or nasal, are being developed.^{65,66} Ketamine is rapidly distributed into the brain, after systemic absorption. Metabolism mainly takes place through oxidative metabolism by hepatic microsomal enzymes.⁶⁷

Ketamine causes so-called “dissociative anesthesia”, where patients appear to be in a cataleptic state. Upon emergence, it produces psychedelic effects.⁶⁸ Therefore, psychiatric disease is a contraindication for ketamine. Furthermore, ketamine can increase ICP, and use in patients who already have an increased ICP should be evaluated cautiously. It has a stimulating effect on the cardiovascular system, increasing arterial blood pressure and heart rate through central sympathetic stimulation and inhibition of neuronal catechol-

amine uptake.^{63,69} Ketamine has little effect on the respiratory system, preserving central respiratory drive.⁷⁰ Interestingly, ketamine also has an effect on opioid-receptors, making it the only general anesthetic agent that also has a potent analgesic effect.^{71,72} This property makes it a very suitable drug for use for emergency medicine.

Dexmedetomidine⁷³

Dexmedetomidine is also not a GABA_A receptor agonist. It acts on the α_2 receptor and has sedative, anxiolytic, sympatholytic and analgesic effects.⁷⁴ It is a relatively new drug and has been approved recently for use in ICU sedation in the European Union in 2011, and for procedural sedation in 2017.

Dexmedetomidine is only registered for us as an IV agent, however, alternative routes of administration such as intranasal and buccal are being investigated.⁷⁵ After absorption, dexmedetomidine has a fast distribution to the brain. Metabolism mainly takes place in the liver.⁷⁶

Dexmedetomidine produces a sleep-like sedation. A unique effect of dexmedetomidine is that patients are easily arousable during sedation.⁷⁷ This makes dexmedetomidine an attractive agent to use in conscious sedation and awake craniotomies,⁷⁸ but also for sedation in the ICU. Dexmedetomidine has minimal effect on the respiratory drive and the ventilatory response to CO₂ is preserved. It produces a biphasic hemodynamic response: at low plasma concentrations hypotension is observed and at high plasma concentrations hypertension is observed.⁷⁹

Recent developments in anesthetic drug discovery

During the last two decades, several attempts were made to develop a novel anesthetic agent that would be able to outperform propofol in daily anesthesia practice, especially regarding the hemodynamic depression that propofol produces upon induction. Several strategies have been tried to develop a drug that answers to the criteria of an ideal anesthetic agent as described previously.

Softdrugs

One strategy used by various researchers is that of the “soft drug”. A soft drug is a molecule which is derived of a parent compound and is specifically designed to undergo predictable and rapid metabolism into inactive metabolites.⁸⁰ The pharmacology of such drugs is, in theory, optimal for use in daily anesthesia practice as the drug can be titrated to swiftly

reach the desired effect. Hereby, anesthesiologists can quickly respond to the needs of patients under anesthesia and adapt rapidly fluctuating depths of anesthesia.⁸¹ Also, a growing need of efficiency in modern medical practice demands more on demand on- and offset of anesthesia. Currently approved and commonly used soft drugs in anesthesia include remifentanyl,⁸² a short-acting opioid, and esmolol, a short-acting β -adrenergic blocker.⁸³

Presently, several soft drugs are under pre-clinical and clinical development for use in anesthesia. The most notable of these are remimazolam, a short-acting benzodiazepine⁸⁴ and AZD3043, a propranolol-related compound.⁸⁵ Both are rapidly metabolized by esterases into an inactive metabolite. Both present a rapid on- and offset of sedation and relatively stable hemodynamics and ventilation. During infusions of AZD3043, involuntary muscle movements were observed.^{85,86} Additionally, another soft analogue of etomidate, ET-26, is currently in pre-clinical development stages.⁸⁷

Other strategies

Other compounds currently in development as a potential anesthetic agent are fospropofol, a prodrug of propofol,⁸⁸ and phaxan, a new formulation of the pre-existing steroidal anesthetic agent alphaxalone.⁸⁹

The search for a new analogue of etomidate

MOC-Etomidate

As described previously, etomidate is an imidazole-based anesthetic agent with rapid on- and offset of anesthetic drug effect and a remarkable preservation of hemodynamic stability. Unfortunately, the occurrence of suppression of the adrenal axis upon prolonged administration makes it unsuitable for continuous infusion.

Cotton et al. hypothesized that it would be possible to design a soft analogue of etomidate that would retain etomidate's favorable pharmacological properties whilst diminishing its adrenal suppressive effects. The first of these soft analogues of etomidate developed by Cotton et al. was Methoxycarbonyl-etomidate (MOC-etomidate).⁹⁰ The objective of this molecule was to mostly alter the design of etomidate to enable ultra-rapid metabolism by non-specific esterases to a carboxylic acid. This would prevent it from binding to the hydrophobic catalytic site of the 11β -hydroxylase enzyme⁹¹ hereby reducing the possibility of adrenocortical suppression. This was achieved by the addition of a new ester moiety that would be prone to fast hydrolysis.

In vitro and in vivo studies of MOC-etomidate show that it functions as a potent positive allosteric modulator of the GABA_A receptor in rats and tadpoles resulting in rapid onset of hypnosis and retaining etomidate's rapid onset of action and hemodynamic stability. Furthermore, its metabolism is ultra-rapid, which causes an equally ultra-rapid hypnotic recovery. Also, 30 minutes after a single bolus administration, MOC-etomidate did not cause significant reduction in the ACTH-stimulated serum corticosterone concentration in rats, whilst an equipotent dose of etomidate did so significantly.⁹⁰ Upon administration of a continuous infusion, it was found that because of the ultra-rapid metabolism of MOC-etomidate, large quantities of drug were necessary in order to maintain an acceptable depth of anesthesia in rats. As a result, MOC-etomidate did cause adrenocortical suppression, however, recovery from it was more than twice as fast compared to equipotent doses of etomidate.⁹² Furthermore, because large quantities of drug have to be administered and metabolism is ultra-rapid, equally large quantities of metabolite are being produced. Although the potency of the metabolite of MOC-etomidate, MOC-CA, is approximately 300-fold lower than that of MOC-etomidate,⁹³ this amount of metabolite was sufficient to cause burst suppression and delayed recovery of the rats after prolonged infusions.⁹⁴ Hence, because of its ultra-rapid pharmacokinetic properties, MOC-etomidate was not deemed suitable for further clinical development.

Carboetomidate

Where MOC-etomidate presented a potential pharmacokinetic strategy, i.e. ultra-rapid metabolism to circumvent the adrenocortical suppression caused by etomidate, the same team developed a potential pharmacodynamic strategy. The imidazole ring in the molecular structure of etomidate is thought to be responsible for this suppression. A high affinity interaction occurs between the basic nitrogen in this imidazole ring and the heme group which the cytochrome P450 enzyme 11 β -hydroxylase contains.⁹¹ This interaction is thought to lead to suppression of 11 β -hydroxylase, thus inhibiting the adrenal axis. Cotten et al. hypothesized that this high affinity binding could be “designed out” of etomidate, whilst keeping intact the positive allosteric modulation of the GABA_A receptor. In order to do so, the basic nitrogen in the imidazole ring would have to be replaced with another atom or group that would not interact with the heme iron present in cytochrome P450 enzymes.⁹⁵ This hypothesis of Cotten et al. led to the synthesis of carboetomidate, a pyrrole-based sedative hypnotic analog of etomidate.⁹⁶ In vitro studies demonstrated that the potency with which carboetomidate inhibits cortisol is three orders of magnitude weaker than that of etomidate. In tadpoles and rats however, it has one-third and one-seventh the hypnotic potency of etomidate respectively. Also, onset of hypnosis in rats was slower with carboetomidate than with etomidate. Hemodynamic stability was maintained during dosing. Unlike etomidate, carboetomidate inhibits the nicotinic acetylcholine receptor.⁹⁷ A molecule that attempted to combine the properties of MOC-etomidate and carboetomidate, MOC-carboetomidate, was subsequently investigated. However, this drug was found to be very

hydrophobic, leading to problems with the formulation for administration.⁹⁸ As a result, this development route was aborted.

In the end, it was decided to move forward on the development of a soft analogue of etomidate, i.e. they took the pharmacokinetic route. Because MOC-etomidate's pharmacokinetic properties were too fast, MOC-etomidate was adapted into 13 new analogs which attempted to slow down its fast pharmacokinetics. This was done by adding various aliphatic substituents onto the two-carbon spacer in MOC-etomidate, that would sterically protect the 'bare' ester-moiety so that hydrolysis could slow down.⁹⁹ Of these 13 new analogues, two molecules, dimethyl-methoxycarbonyl metomidate (DMMM) and cyclopropyl-methoxycarbonyl metomidate (CPMM) showed high hypnotic potencies and a duration of action between that of MOC-etomidate and etomidate upon single bolus administration in rats. Upon prolonged infusions of both compounds, it was found that especially CPMM demonstrated a context insensitive and swift recovery profile.¹⁰⁰ Upon assessment of adrenocortical suppression during and after a continuous infusion of hypnotic doses CPMM, it was found that during infusion, it did suppress adrenocortical function, however, this effect ceased within 30 minutes after terminating the infusion. Conversely, adrenocortical suppression lasted more than three hours after termination of an infusion of hypnotic doses of etomidate.¹⁰¹

Subsequent studies on the metabolite of CPMM, CPMM-CA, showed that it does produce a dose-dependent hypnotic effect, however, its potency compared to its parent compound was more than 1:1500; whereas that of MOC-etomidate's principle metabolite, MOC-CA, was approximately 1:400 compared to its parent compound. Furthermore, after prolonged infusions of CPMM in rats, plasma concentrations of CPMM-CA did not reach hypnotic concentrations by 2-3 orders magnitude. Elimination of CPMM-CA was swift, especially compared to that of MOC-CA, where plasma concentrations of CPMM-CA decreased by 94% in 60 minutes, compared to 33% in MOC-CA.¹⁰²

Figure 1-3.
Structure of
cyclopropyl-
methoxy-
carbonyl
metomidate
(CPMM), or
ABP-700

As up to this point, only rats were used as an animal model, CPMM was also tested in a non-rodent species, as required by regulatory authorities. CPMM showed similar pharmacokinetic and pharmacodynamic properties in beagle dogs, to when it was administered to rats: rapid metabolism, ultra-rapid hypnotic action and a swift recovery profile, regardless of the duration of the infusion.¹⁰³ Furthermore, CPMM showed an adrenocortical recovery profile which was similar to that of propofol, the current standard of care, where 90 minutes after ending a continuous infusion of 2 hours, adrenocortical function was equivalent. One side effect observed during administration to beagle dogs, was the occurrence of involuntary muscle movements, similar to those observed with etomidate. The incidence of these movements was higher during CPMM infusion than it is during etomidate infusion. They were successfully attenuated by midazolam.¹⁰³

After these animal experiments, CPMM was considered to have the most promising pharmacology of the analogues of etomidate and was put forward as a lead candidate for clinical trials. In this thesis, the results of these clinical trials of CPMM will be described extensively.

Drug Development¹⁰⁴

The development of a new drug is a long, costly, and complicated process and can take up to more than 10 years. After a drug is discovered, either through new mechanistic insights, testing of several compounds, serendipity of existing drugs or new technologies, it enters the development stages. Before a new drug can be tested in humans, extensive pre-clinical testing through in vitro and in vivo experiments have to be conducted. During these experiments, compounds are tested on dosing and toxicity levels, in mediums from a small petri-dish to non-rodent animals. After review of the findings of pre-clinical testing, researchers evaluate whether the drug can be moved forward to clinical trials, which occur in four different phases.

- **Phase I:** the goals of phase 1 studies are mainly to evaluate the safety and dosage of a new drug. Typical study participants are healthy volunteers. The group size is about 20 to 100 participants. Average length of this phase is several months.
- **Phase II:** in this phase the goals are mainly to study the efficacy and potential side effects. Typical study participants are patients who have the disease or condition for which the drug is developed. Group size is up to several hundred participants. Average length of this phase is several months up to two years.
- **Phase III:** here, the goal is mainly to study the efficacy and monitors potential adverse reactions. Typical study participants are patients who have the disease or condition for which the drug is developed. Group size is up to several thousand participants. Average length of this phase is one to four years.
- **Phase IV:** these are studies about the side effects and efficacy of a drug after it has become available on the market.

After Phase III, the drug is evaluated by regulatory authorities, such as the Food and Drug Administration in the USA, or the European Medicines Agency in Europe, who decide whether a compound is approved for use in daily clinical practice or if more research is required.

An important fact to keep in mind, is that after each step and each phase of drug development, only a small number of novel compounds proceed to the next step or phase. In the end, for any new drug that actually makes it to market, researchers start with about 5,000 to 20,000 chemical compounds in the drug discovery phase.

■ **Figure 1-4.** The course of drug development

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CHAPTER 2

A Phase 1, Single-center, Double-blind, Placebo-controlled Study in Healthy Subjects to Assess the Safety, Tolerability, Clinical Effects, and Pharmacokinetics–Pharmacodynamics of Intravenous Cyclopropyl-methoxycarbonyl metomidate (ABP-700) after a Single Ascending Bolus Dose

Modified from *Anesthesiology*. 2017 Jul;127(1):20-35.

Michel M. R. F. Struys*, Beatrijs I. Valk*, Douglas J. Eleveld, Anthony R. Absalom, Peter Meyer, Sascha Meier, Izaak den Daas, Thomas Chou, Kai van Amsterdam, Jason A. Campagna, Steven P. Sweeney,

**both authors contributed equally to this article and should both be considered as first authors.*

Abstract

Background: Cyclopropyl-methoxycarbonyl metomidate (ABP-700) is a new “soft” etomidate analog. The primary objectives of this first-in-human study were to describe the safety and efficacy of ABP-700 and to determine its maximum tolerated dose. Secondary objectives were to characterize the pharmacokinetics of ABP-700 and its primary metabolite (cyclopropyl-methoxycarbonyl acid), to assess the clinical effects of ABP-700, and to investigate the dose–response and pharmacokinetic/pharmacodynamic relationships.

Methods: Sixty subjects were divided into ten cohorts and received an increasing, single bolus of either ABP-700 or placebo. Safety was assessed by clinical laboratory evaluations, infusion-site reactions, continuous monitoring of vital signs, physical examination, adverse event monitoring, and adrenocorticotrophic hormone stimulation testing. Clinical effects were assessed with modified observer’s assessment of alertness/sedation and Bispectral Index monitoring. Pharmacokinetic parameters were calculated.

Results: Stopping criteria were met at 1.00 mg/kg dose. No serious adverse events were reported. Adverse events were dose-dependent and comprised involuntary muscle movement, tachycardia, and ventilatory effects. Adrenocorticotrophic hormone stimulation evoked a physiologic cortisol response in all subjects, no different from placebo. Pharmacokinetics were dose-proportional. A three-compartment pharmacokinetic model described the data well. A rapid onset of anesthesia/sedation after bolus administration and also a rapid recovery were observed. A quantitative concentration–effect relationship was described for the modified observer’s assessment of alertness/sedation and Bispectral Index.

Conclusions: This first-in-human study of ABP-700 shows that ABP-700 was safe and well tolerated after single-bolus injections up to 1.00 mg/kg. Bolus doses of 0.25 and 0.35 mg/kg were found to provide the most beneficial clinical effect versus side-effect profile.

Cyclopropyl-methoxycarbonylmetomidate (CPMM, now ABP-700; The Medicines Company, USA) is a new intravenous anesthetic agent that currently is being developed for the induction and/or maintenance of general anesthesia and sedation in patients undergoing diagnostic or therapeutic procedures. ABP-700 is a second-generation analog of etomidate, a γ -aminobutyric acid (GABA) type A receptor agonist.¹ It contains an ester bond that is subject to rapid hydrolysis by nonspecific tissue esterases, a property that should result in a predictable dose–response relationship. The *in vivo* ester hydrolysis of the methoxycarbonyl moiety in ABP-700 generates a principle active metabolite, cyclopropyl-methoxycarbonyl acid (CPM-acid), which is a 1,000-fold less potent activator of the GABA type A receptor compared to ABP-700.² This chemical approach to modulation of pharmacology has been used previously in approved drugs such as remifentanyl, esmolol, and clevidipine to cause rapid inactivation of the compound. This approach also is being applied to sedative-hypnotics to create so-called “soft analogs” that show faster pharmacokinetics and a high therapeutic index.^{3,4}

Etomidate was introduced into clinical practice to induce and maintain the hypnotic component of anesthesia while preserving hemodynamic and respiratory stability.⁵ However, large population variability in recovery times and significant suppression of adrenocortical steroid synthesis has limited its clinical use.⁶ In an attempt to eliminate these side effects while retaining its beneficial cardiovascular and respiratory profile, various new analogs of etomidate have been synthesized and tested in preclinical and animal settings.² Of these, ABP-700 showed promising pharmacology in rats.⁷ When tested in beagle dogs, it was observed that recovery after ABP-700 was rapid both after single-bolus and continuous infusion.¹ Also, adrenocortical recovery occurred within approximately 90 min, which is not significantly different from propofol.¹

In this article, results from a first-in-human, Phase 1, single-center, double-blind, placebo-controlled study of ABP-700 after a single ascending bolus dose are reported. The primary objectives were to describe the safety and efficacy of ABP-700 and to determine its maximum tolerated dose (MTD). Secondary objectives were to characterize the pharmacokinetics of ABP-700 and its primary metabolite (CPM-acid), to assess the clinical effects of ABP-700, and to investigate the pharmacokinetic–pharmacodynamic relationships. We also tested the influence of a single-dose fentanyl pre-treatment on the clinical and side effect profile with two of the most promising ABP-700 dosages.

Materials and Methods

Study Management and Registration

This trial was conducted at the QPS early Phase 1 unit, Groningen, The Netherlands, in cooperation with the Department of Anesthesiology at the University Medical Center Groningen, University of Groningen, The Netherlands, in accordance with the Declaration of Helsinki, in compliance with good clinical practice and applicable regulatory requirements. Ethics committee approval was obtained (Medische Ethische Toetsings Commissie Stichting Bebo, Assen, The Netherlands, NL48312.056.14, being the responsible ethics committee for QPS), and the study was registered at a public registry (Dutch Trial Register, NTR4545) before the start of the study. All volunteers provided written informed consent before participation.

Subjects

Healthy nonsmoking men and women aged between 18 and 45 yr with a body mass index (BMI) between 17.5 and 30 kg/m², American Society of Anesthesiologists physical status of I, and without risk of a difficult airway (modified Mallampati score I or II) were eligible for this study. Women were included if they were of nonchildbearing potential, i.e., had to have undergone one of the following sterilization procedures at least 6 months before the first dose: hysteroscopic sterilization, bilateral tubal ligation or bilateral salpingectomy, hysterectomy, bilateral oophorectomy, or be postmenopausal with amenorrhea for at least 1 yr before the first dose and have follicle-stimulating hormone serum levels consistent with postmenopausal status (follicle-stimulating hormone levels of less than 30 IU/l). Subjects were excluded in case of a history or presence of significant disease or disease risk. In addition, volunteers must have refrained from, or not anticipate, the use of any medication, alcohol, or (illicit) drug abuse. Participants should not have had surgery within 90 days before drug dosing, a history of febrile illness within 5 days before dosing, not have participated in another clinical trial within 90 days before dosing, and not be pregnant or lactating. Subjects with a history or presence of adrenal insufficiency as defined by serum cortisol level less than 6 µg/dl at screening also were excluded from participation.

Study Execution

This study was set up as a Phase 1, single-center, double-blind, placebo-controlled, single ascending-dose study of ABP-700. In total, 60 volunteers were divided into 10 cohorts. In each cohort, six subjects received a single IV bolus dose of ABP-700 or placebo in a 5:1 ratio. The actual dosages of ABP-700 were 0.03, 0.10, 0.25, 0.35, 0.50, 0.75, and 1 mg/kg. The starting dose of 0.03 mg/kg was chosen as a conservative starting point based on preclinical efficacy and toxicology data and following guidelines from the European Medicines Agency (London, United Kingdom). Two cohorts of six volunteers received a single IV bolus dose

of 0.25 or 0.35 mg/kg ABP-700 or placebo preceded by 1 µg/kg fentanyl in a 5:1 ratio. An additional cohort (cohort 10) of six volunteers was added, receiving a single IV bolus dose of ABP-700, 0.175 mg/kg or placebo in a 5:1 ratio to finetune the dose–response relationship.

Subjects were admitted to the clinical pharmacology unit 1 day before study drug administration. Subjects were required to fast for a minimum of 8 h overnight before study drug administration and continued to fast for at least 4 h thereafter. Water was not permitted from 2 h before until 1 h after dosing. Consumption of foods and beverages containing caffeine, alcohol, or grapefruit was prohibited 24 h, 48 h, and 10 days before dosing, respectively, and throughout subjects' admission to the study facility. Before dosing, all subjects were transported to a dedicated treatment room equipped similarly to an operating room used in the University Medical Center Groningen that included monitoring equipment as well as respiratory support, including a tracheal intubation kit, anesthesia machine (Primus®; Dräger, Germany), and a fully equipped emergency “crash” cart immediately available. Subjects breathed room air before, during, and postdosing of ABP-700. In the event that an oxygen saturation (SpO₂) level less than 90% was not resolved by stimulation or jaw thrust, additional oxygen (5 l/min) was delivered via nasal prongs (Microstream®; Medtronic, Ireland). If required, brief manual ventilatory support was allowed with the use of a tight-fitting face mask. Before drug administration, two intravenous cannulas were inserted. The intravenous cannula for study drug administration was placed downstream of the arterial sampling and monitoring cannula, which was inserted into the radial artery after local anesthesia. The second intravenous cannula was placed in the opposite arm to draw the venous blood samples. All subjects received minimal crystalloid intravenous fluids during the drug administration period via the cannula for drug administration.

Because ABP-700 is derived from etomidate, a drug associated with involuntary muscle movements (IMMs), and because of the preclinical findings that ABP-700 also is associated with IMM, including myoclonic jerking,¹ midazolam was made available as safety medication at an initial 1-mg bolus dose for amelioration of severe myoclonus if observed. Additional midazolam doses of 1 mg could be administered at the discretion of the anesthesiologist-investigator. During the conduct of the study, a board-certified anesthesiologist was present throughout the dosing period until full recovery of the subject.

Subjects remained supine until recovery and were supine or semirecumbent in bed until the removal of the arterial line. When supine or semirecumbent, subjects were allowed to rise for brief periods under supervision. Subjects did not engage in strenuous activity at any time during the confinement period. Subjects were asked to avoid exercise 72 h before clinical laboratory tests at screening, check-in (1 day before dosing), and follow-up. Subjects remained in the clinic through completion of all scheduled postdose procedures on day 2 and returned for a follow-up visit 4 to 6 days after dosing.

After each cohort, the principal investigator (PI), sponsor, and ethics committee evaluated all available data relevant to the safety, tolerability, pharmacokinetics, and clinical effects of ABP-700 to proceed to the next dose level. Once an MTD was identified, no further dose escalation occurred. Stopping criteria for dose escalation were a grade 3 or higher dose-limiting toxicity event as defined in the September 2007 Food and Drug Administration Guidance; SpO₂ less than 90% not resolved by simple stimulation, jaw thrust, or supplemental oxygen administered via nasal prongs; any serious adverse events (AEs) that were considered by the PI to be related to study drug; and any clinically significant AEs that the sponsor and PI considered a safety concern. Because methanol is a byproduct of the ABP-700 metabolism and was detected during animal studies (data on file), it was indicated to search for significant changes in clinical or laboratory parameters indicative of methanol exposure with the potential for toxicity including, but not limited to, evidence of metabolic acidosis.

Primary Study Endpoints

Safety and tolerability of ABP-700 were assessed by evaluation of AEs, physical examination, safety laboratory tests (serum chemistry, hematology, arterial blood gas, urinalysis, and coagulation), serum methanol concentration, intermittent 12-lead electrocardiograms, temperature, and infusion-site reaction monitoring. Electrocardiograms were evaluated for PR interval, QRS interval, and QTcF interval. AEs were defined as the incidences of treatment-emergent adverse experiences per system organ class according to MedDRA (version 16.1; MedDRA MSSO, USA) from the period of arrival at the clinic up to the follow-up visit.

All volunteers were monitored continuously with a Philips MP50 monitor (Philips, The Netherlands) measuring continuous three-lead electrocardiogram and for heart rate, continuous pulse oximetry, noninvasive blood pressure every minute (at lower limb level), continuous invasive blood pressure via the radial artery cannula, respiration rate, respiration pattern, and end-tidal carbon dioxide (Microstream; Medtronic). High-frequency electronic data were captured from 1 min predose until 15 min after full recovery.

An adrenocorticotrophic hormone (ACTH) stimulation test was performed to evaluate the effect of ABP-700 on adrenal function. Screening and baseline cortisol levels were attained before 9:00 AM after 1-h rest in the supine position and after at least an 8-h fast. Adrenocortical stimulation commenced with an IV bolus administration of 250 µg synthetic ACTH 60 min after ABP-700 administration. Cortisol levels were measured at 1 and 2 h after ACTH administration. The MTD was reached when two or more volunteers met stopping criteria in any particular dosing cohort.

Secondary Study Endpoints

The clinical hypnotic–anesthetic drug effect of ABP-700 was evaluated by means of the Modified Observer’s Assessment of Alertness/Sedation (MOAA/S) score, as shown in [table 2-1](#). Clinical effect was defined as a MOAA/S less than 5. Onset of deep sedation/anesthesia was defined as the first postdose transition to a MOAA/S value less than 3 and offset of deep sedation/anesthesia as the transition to a MOAA/S value greater than 2. Duration of sedation/anesthesia was defined as the time between onset and offset. MOAA/S scoring was performed 1 min before dosing and at approximately 15 s, 30 s, 1 min postdose, and every minute thereafter for a minimum of 15 min after full recovery. Subjects were considered recovered when three consecutive MOAA/S scores of 5 were obtained. In addition, a processed electroencephalographic measure, the Bispectral Index (BIS), was applied as a continuous measure of cerebral drug effect. BIS (software revision 1.13; Medtronic) was derived from two-channel frontal electroencephalogram using an Fpz-F7 and Fpz-F8 referential montage and calculated with the BIS-VISTA monitor (Medtronic) using the bilateral electrodes BIS® Sensor electrodes (Medtronic). An average BIS value was calculated by use of data from the left and right BIS values. Electrode impedance was less than 5 kOhm. The smoothing interval of the BIS® monitor (Medtronic) was set at 15 s. BIS values range from 100 to 0, with lower values denoting more drug effect.

Responsiveness	Score
Responds readily to name spoken in normal tone	5 (alert)
Lethargic response to name spoken in normal tone	4
Responds only after name is called loudly and/or repeatedly	3
Responds only after mild prodding or shaking	2
Responds only after painful trapezius squeeze	1

Table 2-1. Modified Observer’s Assessment of Alertness/Sedation (MOAA/S) score

Pharmacokinetic–Pharmacodynamic and Clinical Effect Evaluation

The pharmacokinetics of ABP-700 and its metabolite (CPM-acid) were studied. Arterial and venous blood samples were collected. Arterial samples were drawn before ABP-700 injection and at 0.5, 1, 2, 3, 4, 8, 12, 20, 30, 45, 60, 90, 120, and 180 min after ABP-700 injection. Venous samples were drawn predose, 1.5, 3.5, 7, 13, 21, 35, 75, 125, 185, 240 min and 6, 8, and 12 h postdosing. Blood samples were collected in prechilled Vacutainers (Becton Dickinson,

USA) containing NaF/Na₂EDTA to inhibit nonspecific esterase activity (BD cat. No. 367587; Becton Dickinson). Samples were stored on wet ice for no longer than 30 min until centrifuged at 5°C for 7 min at 1,800g to separate the plasma. Plasma was transferred by the use of disposable pipettes into cryovials and placed immediately on dry ice until transferred to a -80°C freezer within a total allotted time of 60 min. Plasma concentrations of ABP-700 and metabolite CPM-acid were measured by high-performance liquid chromatography with tandem mass spectrometric detection using the SCIEX API 4000 LC/MS/MS System with a TurbolonSpray® interface (AB SCIEX, Canada) in positive mode at QPS.

Deuterium-labeled D5-ABP-700 and D5-CPM-acid were used as internal standards. To 0.05 ml plasma, the internal standard solution and acetonitrile were added. After mixing and centrifuging, part of the supernatant was transferred onto the Ostro Protein Precipitation and Phospho-lipid Removal Plate, 25 mg (Waters Chromatography B.V., The Netherlands). By the use of positive pressure, the super-natant was eluted and collected in a 96-well plate. Before analysis, a dilution step with Milli-Q ultrapure water (Merck Milliport, The Netherlands) and acetonitrile was applied. Liquid chromatography was performed on a C18 column (50 × 3.0 mm, 5 µm; Advanced Chromatography Technologies Ltd, UK) mounted in line with a 4 × 3.0-mm C18 guard column (Advanced Chromatography Technologies Ltd) on an Agilent 1100/1200 LC system (Agilent Technologies, USA). The column temperature was maintained at 50°C. The mobile phase A was 0.1% formic acid in water, and the mobile phase B was 0.1% formic acid in 50% aceto-nitrile. For sample elution, a gradient of 30 to 95% B was applied over a period of 1 min at a flow rate of 1 ml/min. The approximate elution times for ABP-700 and CPM-acid were 2.1 and 1.7 min, respectively. The nominal mass transitions monitored were 315.2 to 211.1 and 301.3 to 197.0 m/z for ABP-700 and CPM-acid, respectively. The method was validated over a concentration range of 5.00 to 1,250 ng/ml for ABP700 and 25.0 to 6,250 ng/ml for CPM-acid. Precision and accuracy were demonstrated for the validation samples within a single run of six aliquots (within-run for repeatability) and between different runs (between-run for reproducibility) distributed over at least 2 days. Validation samples were prepared in blank human NaF/Na₂EDTA plasma by spiking known concentrations of ABP-700 and CPM-acid. For precision, acceptance criteria of coefficient of variation (CV%) not to exceed 20.0% at lower limit of quantification (LLOQ) or 15% at all other levels was met for all samples. For accuracy, acceptance criteria of percentage of relative error not to exceed 20.0% at LLOQ or 15% at all other levels was met for all samples. LLOQ for ABP-700 and CPM-acid was determined at 5 and 25 ng/ml, respectively.

Noncompartmental pharmacokinetic analyses of concentration–time data of both arterial and venous plasma ABP-700 and its primary metabolite (CPM-acid) were conducted with Phoenix® WinNonlin®, version 6.3 (Pharsight Corporation, USA). Only plasma pharmacokinetic profiles that contained more than five consecutive data points with a quantifiable concentration value were considered evaluable. Actual elapsed times from dosing were

used to estimate all individual plasma pharmacokinetic parameters for evaluable subjects. Observed predose concentrations were set as missing to generate C0 values, which were calculated as the extrapolated concentration at time 0 (computed for parent only). Systematic exposure was calculated with the area under the drug concentration–time curve. More detailed information on the applied methods to analyze the noncompartmental pharmacokinetics of the concentration–time data of plasma ABP-700 (both arterial and venous) and its primary metabolite can be found in *supplemental material 2-1*.

In addition, compartmental pharmacokinetic and pharmacodynamic models were developed with NONMEM (Icon Development Solutions, USA). The time course of ABP-700 was modeled with a three-compartmental pharmacokinetic model with volumes V1, V2, V3; elimination clearance CL; and intercompartmental clearances Q2 and Q3. Arterial observations were related to the central compartment. Venous and metabolite concentrations were not used. All parameters were scaled linearly with total body weight. Mixing delay for the pharmacokinetic was set to 15 s. Residual error in pharmacokinetic observations was assumed to be proportional to the predicted concentration. Pharmacokinetic observations reported as lower than the LLOQ were ignored.

We modeled BIS using a sigmoidal E_{max} model driven by an effect compartment concentration (Ce) connected to the plasma compartment by a first-order rate constant ($k_{e0, BIS}$). The equation of the pharmacodynamic model was as follows:

$$\frac{dCe}{dt} = k_{e0, BIS} \cdot (C - Ce)$$

Where C and Ce are the concentrations in the central (V1) and effect compartments, E0 is the baseline pharmacodynamic measure when no drug is present, E_{max} is the maximum possible drug effect, Ce_{50} is the Ce associated with 50% of the maximum effect, γ is the steepness of the concentration versus response relation, and ϵ represents additive residual error to the pharmacodynamic observations. Signal delay in the BIS due to epoch generation and smoothing also was set to 15 s.

MOAA/S observations were treated as ordered categorical responses and modeled with a proportional-odds method. The model estimates the cumulative probabilities of MOAA/S scores. Let S denote an observed score, the logits l_x , of the probabilities that $S = 0, S \leq 1, S \leq 2, S \leq 3, S \leq 4$, are:

$$\begin{aligned} l_{S=0} &= b_0 + DEFF \cdot Ce \\ l_{S \leq 1} &= b_0 + b_1 + DEFF \cdot Ce \\ l_{S \leq 2} &= b_0 + b_1 + b_2 + DEFF \cdot Ce \\ l_{S \leq 3} &= b_0 + b_1 + b_2 + b_3 + DEFF \cdot Ce \\ l_{S \leq 4} &= b_0 + b_1 + b_2 + b_3 + b_4 + DEFF \cdot Ce \end{aligned}$$

Where the b_0 is a fixed-effect parameter representing the logit of the probability for score 0 and b_1 through b_4 represent the difference in logits between the scores. $DEFF$ is also a fixed-effect parameter for the model, and C_e represents the effect-site concentration of ABP-700, calculated with a first-order rate constant ($k_{e0,MOAA/S}$) in a similar manner as done for BIS. The corresponding probabilities are given by:

$$PC_x = \frac{e^{I_x}}{(1 + e^{I_x})}$$

The actual probabilities, p_x , of observing a particular score are: $P_{S=0} = PC_{S=0}$, $P_{S=1} = PC_{S=1} - PC_{S=0}$, $P_{S=2} = PC_{S=2} - PC_{S=1}$, $P_{S=3} = PC_{S=3} - PC_{S=2}$, $P_{S=4} = PC_{S=4} - PC_{S=3}$, $P_{S=5} = 1 - PC_{S=4}$.

For BIS and MOAA/S PD model estimation, the individual predicted plasma concentrations were used as the driving force for the effect compartment. This is known as the sequential method,^{8,9} also known as the Individual Pharmacokinetic Parameters (IPP) method. Covariate search was not performed due to the limited variability in age, weight, sex, and BMI in the studied individuals.

Model parameters were assumed to be log-normally distributed or constant across the population. For estimates of logarithmic interindividual variability, we report the estimated variance and the coefficient of variation.

Uncertainty in estimated model parameters was evaluated by estimating the upper and lower 95% confidence limits by spline-interpolation of the likelihood profiles. We determined what increase/decrease in each parameter is required to increase NONMEM objective function by 3.84. To quantify the pharmacokinetic predictive performance for an observation, we calculated the performance error¹⁰ (PE_{PK}) and absolute performance error (APE_{PK}) as follows:

$$PE_{PK} = \frac{C_{observed} - C_{predicted}}{C_{predicted}} \times 100\%$$

$$APE_{PK} = |PE_{PK}|$$

For these measures, the median values are reported. The median PE_{PK} ($MdPE_{PK}$) indicated bias, and median APE_{PK} ($MdAPE_{PK}$) indicates precision.

Clinical Observations

This study was intended to define a MTD for ABP-700. However, it was reasoned that well-tolerated doses would need to be assessed for potential further clinical testing. Therefore, to evaluate the potential clinical utility of every specific dose, we analyzed various relevant clinical observations described by the attending anesthesiologist during the dosing together with a visual inspection of the individual vital signs trends.

IMMs were anticipated with clinical testing of ABP-700 based both on preclinical observations¹ and also by virtue of the parent compound etomidate's known effects on muscle movement in humans.^{11,12} No standardized nomenclature or scoring system was implemented to characterize IMM, as it has not been done for etomidate in the past. Instead, we described the extensiveness of the observed IMMs, rather than their nature. As such, "extensive movements" are movements defined as IMMs that involve the whole body or a considerable part of it. "Few movements" are movements that occur in few body parts, such as both arms or the face.

Breathing was monitored by means of capnography (end-tidal carbon dioxide) and capnography-derived respiratory rate monitoring. Apnea was defined as an absence of breathing for 20 s or more. Tachypnea was defined as a breathing frequency of 20 breaths/min or more. Sinus tachycardia was defined as a heart rate of 100 beats/min or more and if this meant a significant change from baseline (due to possible volunteer stress). Increased blood pressure was defined as a mean arterial pressure greater than 110 mmHg¹³ if this meant a significant change from baseline. Desaturation was defined as a decrease of SpO₂ less than 95%.

Data Recording and Statistics

All vital signs data, MOAA/S, BIS, respiratory function, comments, and a subset of pharmacokinetic sampling time were stored electronically with a dedicated and validated electronic data capturing device (Rugloop II; DEMED, Belgium). Visual and computerized methods of data validation were applied to ensure accurate, consistent, and reliable data for the subsequent statistical analysis.

The sample size was based on previous investigations adequately studying safety, efficacy, clinical effect, and pharmacokinetic–pharmacodynamic behavior of new compounds while minimizing the exposure of volunteers to the compound. Descriptive statistics are displayed as mean ± SD or geometrical mean ± 95% CI unless indicated otherwise.

Results

Subjects

In total, 154 subjects were screened by the contract research organization (QPS, The Netherlands). Of these, 60 subjects were admitted to the study, of whom 50 received ABP-700 and 10 received a placebo. All subjects who were enrolled completed the study; no subject withdrew from the study. Mean height, weight, BMI, and age were similar among the groups, as shown in [table 2-2](#).

Characteristics	Statistic/Category	Placebo (N=10)	ABP-700 (N=50)
Sex (n (%))	Male	10 (100.0)	49 (98.0)
	Female	0 (0.0)	1 (2.0)
Age (yrs)	Mean (SD)	23.4 (3.7)	25.1 (3.06)
Ethnicity (n (%))	Not Hispanic or Latino	10 (100.0)	49 (98.0)
	Hispanic	0 (0.0)	1 (2.0)
Race (n (%))	Asian	1 (10.0)	2 (4.0)
	Black or African American	0 (0.0)	1 (2.0)
	White	9 (90.0)	47 (94.0)
Smoking Status (n (%))	Never	10 (100.0)	58 (96.7)
	Stopped	0 (0.0)	2 (3.3)
- If yes, subject quit smoking > 6 months ago? (n (%))	Yes	0 (0.0)	2 (100.0)
Height (cm)	Mean (SD)	181.64 (6.01)	182.39 (7.18)
Weight (kg)	Mean (SD)	74.23 (7.35)	76.00 (7.13)
BMI (kg/m)	Mean (SD)	22.48 (1.77)	22.85 (1.80)
Alcohol consumption (units/week)	Median (range)	4.5 (0-12)	7.0 (0-14)

■ **Table 2-2.** Demographics

Safety and Tolerability

Treatment-emergent adverse experiences were reported in 41 subjects, with 94 AEs reported in total (**table 2-3**). Of these, three subjects (30%) in the placebo group reported at least one AE. The majority of AEs were of mild intensity (93.7%). The number and severity of reported AEs increased with ascending doses of ABP-700. No volunteers withdrew from the study due to AEs. Of the 41 subjects experiencing at least one AE, all had at least one AE of mild intensity. Three subjects also experienced at least one AE of moderate intensity.

No AEs of severe intensity were reported. AEs that were “possibly” related to the study treatment were reported for 37 subjects. Four subjects had AEs that were unlikely related, and 15 subjects experienced AEs that were unrelated to the study medication.

For each cohort, [figure 2-1](#) presents the hemodynamic and respiratory data. More information on vital signs data are available in the online supplemental materials¹. These are figures plotting the individual data on heart rate, SpO₂, mean arterial blood pressure measured using a noninvasive blood pressure cuff, mean arterial blood pressure using an invasive blood pressure method, respiratory rate, frontal muscles electromyographic activity and BIS measured by the Vista Monitor (Medtronic, Ireland), and end-tidal carbon dioxide for each volunteer receiving ABP-700 or placebo.

Adverse Event	ABP-700 (N=50)	Placebo (N=10)
Muscle twitching	15	0
Apnea	13	0
Hyperventilation ^a	10	0
Sinus tachycardia	9	0
Catheter site related reaction	6	1
Eye disorder ^b	5	1
Blood pressure increased	4	0
Restlessness	4	0
Nausea/Vomiting	2	0
Abnormal respiration ^c	3	0
Decreased oxygen saturation	3	0
Neurological anesthetic complication ^d	3	0
Hiccups	3	0
Injection site pain	2	0
Myoclonus	2	0
Headache	2	0
Presyncope ^e	2	0
Fatigue	2	0
Euphoric mood	2	0

Table 2-3. Adverse Events. Listing of Adverse Events occurring in 2 or more ABP-700 treated subjects:
^aVerbatim term: breathing disorder and paradoxical chest wall movements ^bVerbatim terms: rhythmic movement of eyes, eye lid fluttering and eye lid twitching ^cVerbatim term: sigh ^dVerbatim term: emergence delirium ^eVerbatim term: vasovagal reaction (during placement of arterial line)

* See <https://doi.org/10.1097/ALN.0000000000001662>

Table 2-4 lists the various tolerability parameters. The occurrence and severity of IMM was reported as dose-dependent. IMM was characterized primarily as muscle twitching or myoclonic activity, but movements including clonic, dystonic, and other variants of IMM also were included and reported as IMM. **Figure 2-1** and **table 2-4** show stable hemodynamics without any occurrence of bradycardia or hypotension after bolus injection. In the higher dosing cohorts, an increase in heart rate and mean arterial blood pressure was observed after similar time course as the cerebral drug effect of ABP-700.

Decreased SpO₂ was reported by seven subjects in the highest dose cohorts and was considered mild and self-limiting (**figure 2-1**). Depression in respiratory rate as measured by capnography, mostly related to IMM resulting in short-lasting and self-limiting upper airway obstruction, was found in 13 volunteers and was dose-related (**table 2-4**). Short-lasting chin lift was required in four of these higher dose volunteers to maintain a patent airway. Except in the two highest dosages, where tachypnea was seen, overall respiration rate did not change during the study period, as shown in **figure 2-1**.

mg/kg	Pla- cebo	0,03	0,10	0,175	0,25	0,25 + fen	0,35	0,35 + fen	0,50	0,75	1,00	Total
Involuntary muscle movements												
No movements	10	5	5	1	0	1	0	1	0	0	1	24
Few movements	0	0	0	3	5	3	2	4	0	0	0	17
Extensive movement	0	0	0	1	0	1	3	0	5	5	4	19
Ventilation												
Respiratory depression	0	0	0	1	1	1	0	3	1	2	4	13
Tachypnea	0	0	0	1	0	1	0	1	2	5	3	13
Desaturation	0	0	0	0	1	0	0	2	0	1	3	7
Vital signs												
Hypertension	0	0	0	2	0	0	2	0	0	1	3	8
Hypotension	0	0	0	0	0	0	0	0	0	0	0	0
Tachycardia	0	0	0	2	0	0	2	1	1	3	4	13
Bradycardia	0	0	0	0	0	0	0	0	0	0	0	0
Catheter site related reactions												
Nausea/Vomiting	1	1	0	1	1	0	0	1	0	1	1	7
	0	0	0	0	0	0	0	1	0	1	1	3

Table 2-4. Evaluation of Tolerability of ABP-700 Given to Healthy Volunteers. Values are number of volunteers. ABP-700 = cyclopropyl-methoxycarbonylmetomidate; fen = fentanyl.

Figure 2-1. Vital signs. (A) Heart rate. (B) Oxygen saturation (SpO₂). (C) Mean arterial pressure as measured by no-invasive blood pressure monitoring (NIBP MAP). (D) Mean arterial blood pressure as measured by invasive arterial blood pressure monitoring (INV MAP). (E) Respiration rate. Data are presented as geometrical means per cohort. Fen = fentanyl.

ABP-700 was not tolerated by three subjects because of the occurrence of severe IMM accompanied by hemodynamic disturbances. One of these subjects had received 0.75 mg/kg ABP-700, and the other two subjects had received 1.00 mg/kg ABP-700. Per protocol, midazolam was given in these three subjects. Total dosages of midazolam ranged from 2 to 5 mg. As such, in the group of 1.00 mg/kg ABP-700 the stopping criteria of the study were met, and it was concluded that 1.00 mg/kg of ABP-700 was the MTD.

Serum chemistry, hematology, urinalysis, coagulation, serum methanol concentration, and temperature did not change during the study period (data not shown). In particular, serum methanol levels were not detectable at all doses tested. Arterial blood gas parameters (pH, PaCO_2 , PaO_2 , HCO_3^- , Na^+ , Cl^- , anion gap) did not change significantly between baseline and 15 min postdose (see *supplemental material 2-2*).

On the 12-lead electrocardiogram, a small decrease in the PR interval and QRS interval was observed after bolus injection of ABP-700 of all dosing groups. The changes are not dose-dependent and not clinically significant. A minor increase in the QTcF interval can be seen after bolus injection of ABP-700 (all dosing groups) without dose dependency or clinical significance. ACTH stimulation evoked an adrenal cortisol response in all subjects treated with ABP-700. Plasma cortisol concentrations increased by at least 200 nM/l at 60 or 120 min post-ACTH stimulation, indicating no adrenal suppression (*figure 2-2*). There was no difference observed between placebo and the ABP-700 dose levels, other than differences that would be considered with the normal variability of the test. ACTH testing was not performed for the cohorts that received fentanyl as a pretreatment, because these cohorts were repeats of previous dose levels. Cortisol levels of cohort 10, which received 0.175 mg/kg ABP-700, were not analyzed because an ACTH-stimulation test in previous cohorts that received a higher dose of ABP-700 (0.25, 0.35, 0.50, 0.75, and 1.00 mg/kg) did not reveal any adrenocortical suppression.

Figure 2-2. Human adrenocortical response to adrenocorticotrophic hormone (ACTH) stimulation after bolus administration of cyclopropyl-methoxycarbonylmetomidate (ABP-700) (0.03 to 1.0 mg/kg) or placebo. Adrenocortical response before and after administration of 250 μg synthetic ACTH 1 h after bolus ABP-700 administration. Predose cortisol reference levels were obtained before 9 AM on the day of ABP-700 administration. Values are mean cortisol levels ($\pm$ SD) for ABP-700 and placebo groups. $n = 5$ by ABP-700 dose and $n = 7$ for placebo.

Noncompartmental Pharmacokinetics

For each cohort, the time courses of the arterial and venous concentrations of ABP-700 and of the arterial of CPM-acid are displayed in [figure 2-3](#). The most important noncompartmental pharmacokinetic parameter values for ABP-700 (both arterial and venous) and its metabolite CPM-acid are shown as tables in *supplemental material 2-1*. Overall, pharmacokinetics were dose-proportional. Clear differences between arterial and venous concentrations and pharmacokinetic parameters were observed.

Figure 2-3. Pharmacokinetics of cyclopropyl-methoxycarbonyl metomidate (ABP-700) and its primary metabolite cyclopropyl-methoxycarbonyl acid (CPM-acid). (A) Arterial plasma concentrations of ABP-700. (B) Venous plasma concentrations of ABP-700. (C) Arterial plasma concentrations of the ABP-700 metabolite, CPM-acid. Data are presented as geometrical means $\pm$ 95% CI per cohort. The bolus of ABP-700 was administered at time 0 min.

Compartmental Pharmacokinetics

No significant covariate relationships with age, weight, height, or sex were found. The weight linear scaled model achieved an Akaike information criterion of -2,700.61 with a median absolute percentage error of 1.98 (24.5)%. The summarized equations of the final pharmacokinetic model are as follows:

$$\begin{aligned}
 SIZE &= WGT/70 \\
 V_1(l) &= V_{1,ref} \cdot SIZE \cdot \exp(\eta_1) \\
 V_2(l) &= V_{2,ref} \cdot SIZE \cdot \exp(\eta_2) \\
 V_3(l) &= V_{3,ref} \cdot SIZE \cdot \exp(\eta_3) \\
 CL(l \cdot \min^{-1}) &= CL_{ref} \cdot SIZE \cdot \exp(\eta_4) \\
 Q_2(l \cdot \min^{-1}) &= Q_{2,ref} \cdot SIZE \cdot \exp(\eta_5) \\
 Q_3(l \cdot \min^{-1}) &= Q_{3,ref} \cdot SIZE \cdot \exp(\eta_6)
 \end{aligned}$$

Symbols $V_{1,ref}$, $V_{2,ref}$, $V_{3,ref}$, CL_{ref} , $Q_{2,ref}$, and $Q_{3,ref}$ are the estimated compartmental volumes and clearances for a 70-kg individual, and symbols η_1 to η_6 represent random variances. Estimated parameters are shown in [table 2-5](#). The population and individual predictions versus time and observed ABP-700 arterial concentrations and the likelihood profiles are documented as figures in *supplemental material 3*.

Parameter	Estimated value	95% confidence limits	
		Lower	Upper
V1 _{ref} (L)	0.527	0.427	0.649
V2 _{ref} (L)	3.02	2.47	3.71
V3 _{ref} (L)	5.18	4.59	5.85
CL _{ref} (L/min)	1.66	1.57	1.77
Q2 _{ref} (L/min)	1.14	0.951	1.41
Q3 _{ref} (L/min)	0.336	0.297	0.382
Variance (ω²)		CV(%)	
η1	0.323	61.8	
η2	0.352	64.9	
η3	0.106	33.5	
η4	0.0178	13.4	
η5	0.249	53.2	
η6	0.0804	28.9	
Residual error (SD)			
Arterial observations	0.103		

Table 2-5 Estimated Model Parameters and Population Variances in the Final Pharmacokinetic Model. $V1_{ref}$, $V2_{ref}$, $V3_{ref}$, CL_{ref} , $Q2_{ref}$, and $Q3_{ref}$ are the estimated compartmental volumes and clearances for a 70-kg individual, and symbols η_1 to η_6 represent random variances. CV = coefficient of variation

Clinical Effects and Pharmacodynamics

For each cohort, the time course of the BIS and frontal electromyographic activity are plotted in [figure 2-4](#). At the lowest dose of ABP-700 administered (0.03 mg/kg), there was no observed effect on BIS. Subjects receiving dose levels of 0.1 mg/kg ABP-700 or greater showed a dose-dependent response to the treatment, as indicated by the rapidly decreasing BIS values (within 1 to 2 min). Duration of cerebral drug effect was longer in higher dosing cohorts. Due to increased electromyographic activity,¹⁴ as shown in [figure 2-4](#), the decrease in BIS in the 0.75 mg/kg cohort paradoxically was delayed. For BIS model development, Ce_{50} and $k_{e0,BIS}$ were assumed and log-normally distributed across the population.

Estimating baseline BIS resulted in numerical instability, and its value was fixed to 90. The summarized equations of the final models are as follows:

$$Ce_{50} = Ce_{50,TYP} \cdot \exp(\eta_1)$$

$$k_{e0,BIS} = ke_{0,BIS,TYP} \cdot \exp(\eta_2)$$

Symbols Ce_{50} and $k_{e0,BIS}$ represent estimated model parameters in the individual; $Ce_{50,TYP}$ and $ke_{0,BIS,TYP}$ represent the typical population model parameters; and η_1 and η_2 represent population variances. Estimated parameters are shown in [table 2-6](#). The population and individual predictions versus time and BIS and the likelihood profiles for the model parameters are shown as figures in *supplemental material 2-4*.

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Figure 2-4. Hypnotic effect of cyclopropyl-methoxycarbonylmetomidate. (A) Depth of sedation and anesthesia, as measured by the Bispectral Index and (B) frontal electromyographic activity (EMG). Fen = fentanyl.

Parameter	Estimated value	95% confidence limits	
		Lower	Upper
E_0	90	-	-
E_{max}	0	-	-
$Ce_{50, TYP}$ (ng/mL)	1200	1060	1350
Γ	7.24	6.83	7.66
$k_{e0, BIS, TYP}$ (1/min)	0.156	0.132	0.179
Variance (ω^2)		CV(%)	
η_1	0.139	38.6	
η_2	0.742	64.9	
Residual error (SD)			
BIS observations	6.35	105	

Table 2-6. Estimated Model Parameters and Population Variances in the Final BIS Pharmacodynamic Model. E_0 is the baseline pharmacodynamic measure when no drug is present; E_{max} is the maximum possible drug effect; $Ce_{50, TYP}$ is the typical population model parameter for the effect-site concentration of ABP-700 associated with 50% of the maximum effect; γ is the steepness of the concentration versus response relation; $k_{e0, BIS, TYP}$ is the typical population model parameter for the first-order rate constant between plasma and effect-site compartment; and η_1 and η_2 represent population variances.

BIS = Bispectral Index; CV = coefficient of variation; TYP = population typical value.

Figure 2-5 shows the individual MOAA/S scores. In the placebo and 0.03 mg/kg dose groups, no signs of clinical effect were observed in any subjects. Onset of clinical effect was observed starting with the 0.10 mg/kg dose group. In the 0.175-mg/kg dose group, four subjects (80%) reached clinical effect, and two subjects (40%) reached deep sedation/anesthesia. In the cohorts with a dose of 0.25 mg/kg and higher, both clinical effect and deep sedation/anesthesia was reached in 100% of the subjects. The duration of both the clinical effect and sedation increased with escalating dose. Neither the time to onset of clinical effect nor the time to onset of deep sedation/anesthesia was dose-related.

For MOAA/S PD model development, we assumed log-normally distributed population variability in drug effect.

$$DEFF = DEFF_{TYP} \cdot \exp(\eta_1)$$

$$k_{e0,MOAA/S} = k_{e0,MOAA/S,TYP}$$

Symbols $DEFF$ and $k_{e0,MOAA/S}$ represent estimated model parameters in the individual, $DEFF_{TYP}$ and $k_{e0,MOAA/S,TYP}$ represent the typical population model parameters, and η_1 represents population variance. For the final model, the estimated parameters are shown in [table 2-7](#). The population and individual predictions versus time and MOAA/S and the likelihood profiles for the model parameters are shown as figures in *supplemental material 2-5*.

Parameter	Estimated value	95% confidence limits	
		Lower	Upper
$DEFF_{TYP}$	5.25	4.41	6.23
$k_{e0,MOAA/S,TYP}$	0.488	0.443	0.537
b_0	-10.1	-11.7	-8.77
b_1	0.713	0.459	1.02
b_2	0.518	0.307	0.817
b_3	0.407	0.222	0.670
b_4	1.02	0.735	1.36
Variance (ω^2)		CV(%)	
η_1	0.0472	22.0	

Table 2-7. Estimated Model Parameters and Population Variances in the Final MOAA/S Pharmacodynamic Model. b_0 is a fixed-effect parameter representing the logit of the probability for score 0; b_1 through b_4 represent the difference in logits between the scores; $DEFF$ is also a fixed-effect parameter for the model; $k_{e0,MOAA/S,TYP}$ is the typical population model parameter for the first-order rate constant between plasma and effect-site compartment; and η_1 represents the population variance.

CV = coefficient of variation; MOAA/S = Modified Observers Assessment of Alertness and Sedation; $DEFF_{TYP}$ = typical population parameter for a fixed-effect parameter for the model.

Influence of Fentanyl

After completion of the preplanned cohorts and evaluation for tolerability and potential clinical utility, the 0.25- and 0.35-mg/kg doses of ABP-700 were considered as the two most promising doses and were repeated in the presence of fentanyl pretreatment (1 µg/kg). The administration of fentanyl resulted in decrease in the incidence and extent of IMM and also in less tachycardia when compared with the ABP-700 dose without fentanyl ([table 2-4](#)). Fentanyl pretreatment did not alter the time to onset of clinical effect or onset of sedation, nor did it alter the duration of sedation or clinical effect (data not shown). As shown in [figure 2-4](#), a more pronounced clinical effect of ABP-700 as measured by BIS was observed with fentanyl pretreatment than without, although this did not result in a significant covariate in the pharmacodynamic model parameters. Fentanyl did result in somewhat higher frequency of respiratory depression and desaturation events. The administration of fentanyl did not result in a substantial alteration of the pharmacokinetics of either ABP-700 or its metabolite CPM-acid, as shown by both noncompartmental and compartmental modeling (analysis not shown).

Figure 2-5. Modified Observers Assessment of Alertness and Sedation (MOAA/S) scores by subject. The data are displayed by individual subject who received cyclopropyl-methoxycarbonylmetomidate ($n = 50$) and per cohort (10 cohorts in total). Data for placebo were omitted because no subject was scored less than 5.

Discussion

Cyclopropyl-methoxycarbonyl metomidate, or ABP-700, is a short-acting, soft analog of etomidate and was developed to avoid the adrenocortical suppression while preserving the beneficial dose–response profile of etomidate.⁵ In this first-in-human study, we found that ABP-700 was safe and well-tolerated after single-bolus injections of up to a maximum of 1.0 mg/kg. The two most promising dosages for potential clinical utility as a bolus induction agent were thought to be 0.25 and 0.35 mg/kg.

As expected, based on the preclinical studies,^{2,7,15} ABP-700 demonstrated hypnotic–anesthetic properties. Clinical effect was first seen after a bolus dose of 0.175 mg/kg of ABP-700 (in four of five volunteers). At doses of 0.25 mg/kg and greater, a decrease in MOAA/S score to 0 was observed in all subjects. The time to onset of deep sedation/anesthesia was found to be dose-independent and extremely short, around 30 s after bolus injection, which is somewhat shorter than etomidate and thiopental¹⁶ and significantly shorter than propofol.¹⁷ Duration of deep sedation/anesthesia was dose-dependent. Offset of deep sedation/anesthesia was characterized by an abrupt and rapid return to a MOAA/S score of 5 in most of the volunteers. There were very few recorded instances of MOAA/S 2 or 3 during either induction or emergence from deep sedation/anesthesia. BIS profiles showed a similar time course of drug effect to MOAA/S scores, characterized by a rapid, dose-independent decline, with lowest value between 45 and 55, except for the cohort that received a bolus of 0.75 mg/kg ABP-700, due to possible electromyographic artifacts ([figure 2-4](#)).¹⁴

Pharmacodynamic modeling for both MOAA/S and BIS illustrate the steep relation between the ABP-700 effect-site concentration and the cerebral drug effect. Although possibly influenced by the intermittent nature of MOAA/S scores in this study, [figure 2-6](#) clearly shows the small range in effect-site concentrations between the probabilities for different MOAA/S scores. It is remarkable that the difference in ABP-700 effect-site concentration between 50% probability of MOAA/S score 5 and 0 is only around 500 ng/ml. The steepness also is evident in the large γ found in the BIS sigmoid pharmacodynamic model. The effect-site concentration reaching 50% of drug effect as measured by BIS is found around 1,200 ng/ml and validates the effect-site concentration range found when using MOAA/S score to quantify cerebral drug effect. A small hysteresis effect between plasma and effect site is observed and is characterized by a fast $k_{e0,MOAA/S}$ of 0.488/min. A smaller $k_{e0,BIS}$ was found; however, this value might be biased because of the delay in the BIS monitor during very fast changes in cerebral drug effect.¹⁸

Figure 2-6. Population predictions of Modified Observers Assessment of Alertness and Sedation (MOAA/S) scores versus cyclopropyl-methoxycarbonyl-metomidate (ABP-700) effect-site concentrations. The vertical lines to the x-axis denote the effect-site concentration at a 50% probability for having an MOAA/S score of 5 and 0, respectively.

Clinical observations related to IMM, ventilation, and vital signs were dose-dependent and consistent with the mechanism of action of ABP-700 and its structurally related analog, etomidate.^{5,16} From a dosage of 0.175 mg/kg of ABP-700 upwards, IMM were observed in most of the volunteers. These IMM varied from few muscle movements of a more twitching character to more extensive muscle movements that appeared to be of an intermittent myoclonic nature. In various volunteers, these muscle twitches and myoclonic movements were considered mild and not always considered as an AE by the attending anesthesiologist. In three cases of severe IMM in the highest two dose cohorts, midazolam was administered, which successfully attenuated these movements, consistent with what was seen during preclinical studies.¹ The occurrence of IMM is a common observation among anesthetic agents, such as etomidate,¹⁹ propofol,²⁰ and the novel anesthetic agent AZD-3043.²¹ The etiology of these IMM is not entirely clear. They are not thought to be associated with electroencephalographically measured epileptiform discharges^{16,22} and have been hypothesized to be a subcortically mediated or triggered process.¹¹

ABP-700 did not cause cardiovascular depression, as seen in [figure 2-1](#) and [table 2-4](#). However, increases in heart rate and blood pressure were observed in the higher dosing groups and followed a similar time course as the clinical effect. Increases in heart rate with stable blood pressure have been reported previously for etomidate²³ and also with the inhaled agent desflurane.²⁴ Some short-lasting episodes of increased blood pressures (mean arterial blood pressure greater than 110 mmHg) were found, but without any requirement for treatment.

ABP-700 has a remarkably stable respiratory profile. No centrally induced respiratory depression was recorded. Decreases in amplitude and frequency of respiration rate were induced mostly by the IMM, resulting in a brief and self-limiting upper airway obstruction. Per protocol, brief chin lift was applied in four volunteers after 20 s of airway obstruction to maintain a patent airway. Two of these volunteers had received 0.75 mg/kg ABP-700, and two had received 1.00 mg/kg. Tachypnea was observed in parallel with the hypnotic-anesthetic effect in the two highest dosing groups, receiving 0.75 and 1.00 mg/kg ABP-700 boluses.

Self-limiting changes in SpO_2 were only recorded occasionally. The notion that deep sedation/anesthesia is possible without any appreciable respiratory depression is obviously of potential clinical importance. Etomidate is associated with less respiratory depression than propofol but at deep sedation/anesthesia doses, both decrease medullary respiratory drive and right-shift the hypercarbic ventilator response.^{25,26} Although the stable respiratory rate and arterial carbon dioxide levels seen with ABP-700 are suggestive of preservation of respiratory drive, this MTD dose study was not intended to thoroughly evaluate ABP-700 effects on respiratory function.

Consistent with preclinical findings,^{1,15} ABP-700 did not suppress the physiologic response of the adrenal axis to ACTH stimulation in human volunteers. A significant increase of at least 200 nM at 60 or 120 min poststimulation occurred and was not different from subjects who received a placebo. Although not directly compared in this study, adrenal responsiveness after single-bolus exposure to ABP-700 is superior to the substantial adrenal suppression caused by bolus dosing of etomidate.⁶

Noncompartmental pharmacokinetics were studied for both ABP-700 (arterial and venous) and its metabolite CPM-acid (arterial). The results showed rapid elimination of ABP-700 across all dose regimens. The mean clearance values observed in this study were relatively high compared with hepatic blood flow. Clearance and volume of distribution estimated for the venous blood samples were all higher than those estimated for the arterial blood samples, reflecting the rapid metabolism of ABP-700 by esterases during the arterial to venous transport. ABP-700 plasma exposure increased dose proportionally as the dose increased from 0.03 to 1.00 mg/kg for both arterial and venous blood samples. Dose-proportional arterial plasma exposures of CPM-acid also were observed.

A three-compartment pharmacokinetic model was developed to describe the time course of arterial drug concentration. The final model accurately predicts the arterial concentrations of ABP-700 after a single-bolus injection in the central compartment. Venous samples were not used for model estimation. For soft drugs, it is well known that venous samples are potentially very misleading in describing the clinical behavior of a drug through modeling.²⁷ The values for volumes of distribution and clearance echoes the noncompartmental

kinetics, although one should be aware that a compartmental model based on single-bolus data only is very biased and often characterized by a misspecified central compartmental volume.²⁸ A more realistic model has to be developed, adding data from continuous-infusion investigations, hereby also exploring other than linear models, more accurate size descriptors than weight, and more covariates.

The use of fentanyl as a premedication to dosages of 0.25 mg/kg ABP-700 and 0.35 mg/kg ABP-700 did not alter the onset, duration, and recovery profile of ABP-700 as measured by MOAA/S. Nevertheless, a more pronounced decrease in BIS was seen. Less extensive IMMs were observed with the addition of fentanyl. It is well known IMM due to etomidate can be attenuated by pretreatment with low doses of commonly used drugs during procedural care, such as opioids and benzodiazepines.^{11,12} Although speculative, given the chemical derivation of ABP-700 as an etomidate analog, the origin of IMM seen with ABP-700 may be mechanistically similar to that seen with etomidate.

Consistent with known effects, some respiratory and hemodynamic changes were associated with fentanyl administration before ABP-700. This study was not designed to assess these changes, so it is not possible to comment on their clinical significance. Because ABP-700 is not expected to have any analgesic properties, its use in procedural sedation or anesthesia will require concomitant dosing of opioids.

In general, there were no substantial differences in both noncompartmental and compartmental pharmacokinetic parameters for ABP-700 and CPM-acid after a bolus dose of 0.25 and 0.35 mg/kg of ABP-700 without and with 1 µg/kg fentanyl pretreatment, respectively. However, these comparisons on pharmacokinetics of ABP-700 or CPM-acid between the subjects that have been administered ABP-700 alone or coadministered with fentanyl might be biased and were performed for exploratory purposes. The influence of fentanyl on the pharmacokinetics of ABP-700 and CPM-acid should be studied in greater detail within a properly designed drug–drug interaction study.

As always, studies of this type have limitations. One is that the clinical hypnotic–anesthetic drug effect of ABP-700 was assessed by the MOAA/S scale. A problem with using the MOAA/S scale is that the volunteer is stimulated, which might result in an arousal poststimulus and biasing the hypnotic–anesthetic continuum. Furthermore, clinical observations are made by clinicians and are therefore prone to subjectivity. As there were three attending anesthesiologists involved in this study, some variability in MOAA/S assessments is possible.

Another limitation of this study is that BIS monitoring is not validated for monitoring the depth of anesthesia achieved with ABP-700. However, BIS is an established form of monitoring for other GABA-acting drugs,²⁹ and the BIS pattern follows the MOAA/S pattern closely. Therefore, it can be assumed that the BIS data are valid for ABP-700. In addition, as this is

a safety and tolerability Phase 1 study, findings should not be extrapolated to any clinical practice guidelines yet.

We can conclude that ABP-700 was safe and well tolerated after single-bolus injections of up to a maximum tolerated bolus dose of 1.0 mg/kg. A bolus dose of 0.25 and 0.35 mg/kg was found to have a sufficiently favorable clinical effect versus side-effect profile to be explored in future studies. These dosages of ABP-700 showed dose responsive hypnotic–anesthetic characteristics and a safety and tolerability profile that warrants further investigation and development.

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Supplemental material to Chapter 2

Supplemental material 2-1: Methodology and results for the non-compartmental pharmacokinetic analyses of concentration-time data of plasma ABP-700 (both arterial and venous) and its primary metabolite

Methods:

Non-compartmental pharmacokinetic analyses of concentration-time data of plasma ABP-700 and its primary metabolite (CPM-acid) were conducted using Phoenix®WinNonlin® version 6.3 (Pharsight Corporation, St. Louis, MO). Only plasma PK profiles which contained more than five consecutive data points with a quantifiable concentration value were considered evaluable. Actual elapsed times from dosing were used to estimate all individual plasma PK parameters for evaluable subjects. Observed pre-dose concentrations were set as missing in order to generate C_0 values, which were calculated as the extrapolated concentration at time 0 (computed for parent only). Systemic exposure was calculated using the area under the drug concentration-time curve, AUC_{0-t} , calculated using linear trapezoidal summation from time zero to time t, where t is the time of the last measurable concentration (C_t). Extrapolation for exposure to infinity, AUC_{0-inf} was calculated as the area under the drug concentration-time curve from time zero to infinity, $AUC_{0-inf} = AUC_{0-t} + C_t/k_{el}$, where C_t is the last measurable concentration. The apparent terminal elimination rate constant (k_{el}) was calculated by linear regression of the terminal linear portion of the log concentration versus time curve and the apparent elimination half-life ($t_{1/2}$) was calculated as $\ln(2)/k_{el}$. The k_{el} was determined by at least three data points in the terminal elimination phase for parent and metabolite compounds. Furthermore, the adjusted R^2 value in the linear regression on the terminal linear phase of the semi-logarithmic plots of individual plasma concentration time data had to be greater than 70% and % AUC_{extrap} ($=C_t/k_{el}$) had to be less than 20% for k_{el} determinations. The following parameters were observed: C_{max} being the maximum observed drug concentration, t_{last} being the time of the last measurable concentration. The total plasma clearance (CL) was calculated as $Dose/AUC_{0-inf}$ and was computed for parent drug only. V_z or the volume of distribution during the terminal elimination phase was defined as $[Dose / (k_{el} * AUC_{0-inf})]$ and was computed for parent drug only. Total apparent volume of distribution or V_{ss} following single IV dose administration was calculated as $V_{ss} = MRT_{0-inf} * CL$ (computed for parent only), where MRT_{0-inf} (mean residence time) = $AUMC_{0-inf} / AUC_{0-inf}$ (for IV bolus) and where the area under the moment curve from time 0 to the last measurable concentration (C_t) or $AUMC_{0-inf} = AUMC_{0-t} + [(t_{last} * C_t) / k_{el}] + C_t / (k_{el})^2$

Results: (mean (standard deviation) or median [range])

A) Non-compartmental kinetics of ABP-700 (arterial samples)

Parameter	C _{max} (ng/mL)	t _{max} (min) ^a	AUC _{0-last} (h.ng/mL)	AUC _{0-inf} (h.ng/mL)	k _{el} (1/min)	t _{1/2} (min)	t _{last} (min)	CL (L/h)	V _z (L)	C ₀ (ng/mL)
0.03 mg/kg	856 (293)	0.60 [0.56,0.65]	37 (15)	38 (15)	0.137 (0.0569)	5.6 (1.8)	16.9 (4.4)	71 (40.5)	8.3 (1.4)	3960 (1970)
0.10 mg/kg	2430 (876)	0.58 [0.54,0.60]	116 (37)	118 (37)	0.065 (0.0206)	12.1 (5.9)	36.4 (14.3)	72 (22.2)	22.7 (18.5)	13200 (6380)
0.175 mg/kg	2040 (1660)	0.56 [0.54,1.08]	122 (74)	123 (75)	0.068 (0.0049)	10.3 (0.7)	45.1 (0.0)	129 (51.0)	31.4 (11.6)	9850 (12800)
0.25 mg/kg	5540 (3160)	0.58 [0.54,0.68]	381 (307)	384 (307)	0.054 (0.0105)	13.1 (2.3)	54.2 (8.4)	78 (49.4)	26.0 (18.9)	52700 (61300)
0.25 ^b mg/kg	4300 (2850)	0.60 [0.50,0.65]	215 (126)	217 (127)	0.065 (0.0311)	15.6 (13.9)	60.3 (35.1)	105 (52.2)	29.5 (10.4)	18900 (16800)
0.35 mg/kg	6750 (4790)	0.57 [0.56,0.67]	414 (215)	417 (215)	0.051 (0.0231)	16.2 (7.5)	69.3 (20.3)	80 (48.0)	29.8 (22.0)	44700 (41200)
0.35 ^c mg/kg	4310 (3020)	0.65 [0.54,0.66]	277 (143)	279 (143)	0.055 (0.0094)	13.0 (2.1)	61.9 (14.0)	113 (65.1)	35.0 (19.4)	20400 (19100)
0.50 mg/kg	11800 (5750)	0.57 [0.56,0.58]	643 (336)	645 (337)	0.061 (0.0124)	11.6 (1.9)	57.1 (6.7)	84 (51.5)	22.9 (14.7)	76500 (50500)
0.75 mg/kg	13600 (5180)	0.56 [0.55,0.58]	759 (194)	761 (194)	0.061 (0.0212)	13.2 (7.1)	72.1 (26.8)	84 (22.5)	28.4 (21.8)	71200 (38900)
1.00 mg/kg	15900 (10600)	0.56 [0.56,0.58]	969 (495)	972 (495)	0.046 (0.0216)	18.2 (8.0)	103 (27.8)	98 (47.3)	41.4 (24.1)	80900 (90500)

B) Non-compartmental kinetics of ABP-700: venous samples

Parameter	C _{max} (ng/mL)	t _{max} (min) ^a	AUC _{0-last} (h.ng/mL)	AUC _{0-inf} (h.ng/mL)	k _{el} (1/min)	t _{1/2} (min)	t _{last} (min)	CL (L/h)	V _z (L)	C ₀ (ng/mL)
0.03 mg/kg	NA (NA)	NA [NA,NA]]	NA (NA)	NA (NA)	NA (NA)	NA (NA)	NA (NA)	NA (NA)	NA (NA)	NA (NA)
0.10 mg/kg	164 (48)	1.67 [1.56,3.58]	30 (5)	32 (6)	0.052 (0.0208)	15.4 (8.0)	48 (23)	259 (52)	92.1 (37.4)	203 (105)
0.175 mg/kg	244 (95)	2.57 [1.56,7.17]	67 (24)	74 (27)	0.066 (0.0021)	10.5 (0.4)	35 (< 0.1)	196 (68)	49.9 (18.7)	227 (111)
0.25 mg/kg	459 (180)	1.63 [1.50,3.55]	55 (14)	88 (15)	0.053 (0.0175)	14.4 (5.1)	60 (22)	231 (41)	79.9 (30.5)	648 (402)
0.25 ^b mg/kg	491 (149)	1.75 [1.54,1.93]	83 (18)	88 (22)	0.067 (0.0254)	12.9 (8.5)	43 (18)	213 (59)	59.5 (23.2)	820 (449)
0.35 mg/kg	607 (293)	1.79 [1.57,3.58]	153 (55)	158 (55)	0.056 (0.0208)	13.6 (4.4)	67 (18)	184 (87)	56.1 (20.0)	703 (394)
0.35 ^c mg/kg	920 (220)	1.66 [1.61,3.59]	157 (45)	161 (47)	0.052 (0.0181)	14.4 (3.5)	67 (18)	163 (35)	55.3 (16.7)	1540 (930)
0.50 mg/kg	817 (154)	1.59 [1.55,1.84]	184 (60)	188 (61)	0.058 (0.0236)	13.1 (3.5)	70 (20)	246 (118)	70.1 (10.1)	1010 (311)
0.75 mg/kg	925 (321)	3.56 [1.76,7.21]	269 (53)	275 (51)	0.042 (0.0091)	17.1 (3.7)	85 (21)	227 (39)	93.4 (26.0)	850 (434)
1.00 mg/kg	2530 (2360)	1.61 [1.55,13.08]	466 (161)	469 (162)	0.042 (0.0174)	18.7 (6.7)	118 (45)	174 (42)	75.8 (29.3)	4100 (4690)

C) Non-compartmental kinetics of the metabolite CPM-acid (from arterial samples)

Parameter	$C_{\max}$ (ng/mL)	$t_{\max}$ (min) ^a	$AUC_{0-\text{last}}$ (h.ng/mL)	$AUC_{0-\text{inf}}$ (h.ng/mL)	k_{el} (1/min)	$t_{1/2}$ (min)	t_{last} (min)
0.03 mg/kg	46 (6)	8.07 [8.07,12.08]	15 (3)	NA (NA)	NA (NA)	NA (NA)	27 (5.8)
0.10 mg/kg	126 (27)	8.10 [8.05,12.05]	84 (24)	NA (NA)	NA (NA)	NA (NA)	66.4 (13.2)
0.175 mg/kg	235 (37)	12.08 [4.06,12.14]	167 (23)	192 (27)	0.022 (0.0022)	31.9 (3.8)	104 (15.4)
0.25 mg/kg	305 (47)	12.07 [12.04,12.27]	248 (38)	280 (41)	0.019 (0.0018)	35.9 (3.4)	114 (13.3)
0.25^b mg/kg	366 (112)	12.11 [12.08,12.15]	314 (106)	353 (124)	0.024 (0.0029)	29.1 (3.4)	115 (12.9)
0.35 mg/kg	521 (98)	12.09 [12.07,12.14]	439 (58)	475 (57)	0.020 (0.0030)	35.0 (5.4)	132 (26.7)
0.35^c mg/kg	416 (102)	12.13 [12.04,20.08]	343 (68)	375 (68)	0.019 (0.0048)	38.9 (14.1)	129 (23.6)
0.50 mg/kg	709 (96)	12.04 [8.06,12.06]	618 (184)	646 (186)	0.023 (0.0037)	31.2 (4.8)	145 (32.0)
0.75 mg/kg	1140 (209)	12.09 [12.05,20.08]	1030 (125)	1060 (117)	0.020 (0.0035)	36.2 (6.2)	180 (0.5)
1.00 mg/kg	1170 (115)	12.08 [12.05,20.07]	1050 (62)	1100 (73)	0.019 (0.0023)	37.0 (4.6)	169 (27.2)

Table Legend A-C. The most important non-compartmental arterial (A) and venous (B) PK parameters for ABP-700 and its metabolite CPM-acid (from arterial samples) (C). $C_{\max}$ is the maximum observed drug concentration; $t_{\max}$ is the time of the maximum drug concentration (obtained without interpolation); $AUC_{0-\text{last}}$ is the area under the drug concentration-time curve from time zero to the time of the last measurable concentration; $AUC_{0-\text{inf}}$ is the area under the drug concentration-time curve from time zero to infinity; k_{el} is the apparent terminal elimination rate constant, calculated by linear regression of the terminal linear portion of the log concentration versus the time curve; $t_{1/2}$ is the apparent elimination half-life, calculated as $\ln(2)/k_{\text{el}}$; t_{last} is the time of the last measurable concentration; CL is the total plasma clearance calculated as $[\text{Dose}/AUC_{0-\text{inf}}]$ (computed for parent only); V_z is the volume of distribution during the terminal elimination phase, calculated as $[\text{Dose}/(k_{\text{el}} * AUC_{0-\text{inf}})]$ (computed for parent only); and C_0 is the extrapolated concentration at time 0 (computed for parent only).
a: median (range); b: 0.25 mg/kg ABP-700 with 1 µg/kg fentanyl; c: 0.35 mg/kg ABP-700 with 1 µg/kg fentanyl; NA: Not Applicable

Supplemental material 2-2: Arterial blood gas sampling values at baseline and 15 minutes after drug administration for each ABP-700 dosing cohort and placebo. Data are shown as median [range].

Cohort	Timepoint (min)	pH	PaCO ₂	PaO ₂	HCO ₃	Na ⁺	Cl ⁻	AnionGap
Placebo	0	7.42 [7.39,7.54]	5.58 [3.77,6.22]	13.01 [11.56,15.01]	25.85 [23.7,29.3]	139 [137,141]	103 [101,105]	13.5 [7.0,17.3]
	15	7.40 [7.37,7.54]	5.46 [3.50,6.58]	12.01 [11.69,14.44]	25.2 [21.9,30.2]	140 [137,142]	103 [102,105]	14.55 [8.5,19.6]
0.03 mg/kg	0	7.40 [7.39,7.42]	5.65 [5.38,6.19]	11.81 [11.69,12.68]	26.0 [23.7,27.9]	141 [138,141]	104 [102,104]	14.8 [12.8,17.4]
	15	7.40 [7.40,7.43]	5.32 [5.04,5.95]	13.18 [11.63,13.24]	24.3 [23.8,27.1]	139 [137,140]	103 [102,104]	14.6 [12.8,17.4]
0.10 mg/kg	0	7.41 [7.40,7.44]	5.50 [4.77,5.99]	12.28 [10.06,13.05]	25.8 [22.50,27.60]	140 [138,140]	102 [100,103]	17.8 [16.7,18.0]
	15	7.42 [7.39,7.47]	5.59 [4.29,6.17]	12.11 [11.85,13.70]	25.8 [22.9,29.4]	139 [139,139]	102 [100,104]	16.5 [15.8,16.7]
0.175 mg/kg	0	7.42 [7.40,7.48]	5.57 [4.51,5.88]	12.85 [12.55,14.26]	25.8 [24.2,27.6]	140 [138,142]	104 [101,105]	13.8 [13.1,16.5]
	15	7.42 [7.41,7.47]	5.44 [4.33,5.77]	12.96 [12.50,14.40]	25.0 [22.0,27.4]	139 [137,141]	103 [100,105]	14.7 [14.0,15.9]
0.25 mg/kg	0	7.42 [7.41,7.52]	5.59 [3.98,5.86]	13.07 [11.51,15.04]	26.4 [23.6,28.2]	140 [137,142]	103 [102,105]	15.1 [9.6,16.2]
	15	7.42 [7.40,7.48]	5.23 [4.49,6.00]	12.01 [10.94,14.03]	24.6 [22.7,28.1]	139 [138,142]	102 [102,104]	16.8 [11.2,18.2]
0.25 mg/kg + fentanyl	0	7.41 [7.40,7.43]	5.63 [4.92,6.05]	12.85 [11.92,13.86]	25.6 [24.2,27.9]	139 [138,141]	105 [102,106]	12.4 [12.2,14.5]
	15	7.38 [7.37,7.38]	6.12 [5.77,6.61]	11.97 [11.21,12.78]	26.4 [24.4,28.0]	140 [139,141]	104 [101,105]	13.2 [12.8,14.7]
0.35 mg/kg	0	7.42 [7.42,7.56]	5.31 [3.13,5.75]	13.08 [12.38,15.62]	25.5 [20.7,27.3]	139 [138,140]	103 [101,105]	15.0 [11.6,19.7]
	15	7.43 [7.39,7.52]	5.11 [3.71,5.68]	13.15 [11.52,14.46]	23.4 [22.1,25.9]	139 [138,140]	103 [101,105]	15.1 [14.2,18.8]
0.35 mg/kg + fentanyl	0	7.42 [7.42,7.42]	5.78 [5.64,5.85]	12.57 [10.88,12.69]	27.5 [26.8,27.8]	140 [139,142]	103 [101,104]	15.0 [12.1,15.3]
	15	7.39 [7.36,7.40]	6.25 [5.94,6.41]	12.04 [11.45,12.99]	27.1 [26.1,28.7]	140 [139,141]	103 [100,104]	14.1 [13.0,15.6]
0.50 mg/kg	0	7.41 [7.39,7.44]	5.51 [4.99,6.03]	12.39 [10.95,12.69]	24.9 [22.6,28.2]	140 [137,141]	103 [100,104]	16.4 [15,18.8]
	15	7.42 [7.40,7.43]	5.45 [5.14,5.84]	12.2 [10.96,12.44]	26.2 [24.1,26.7]	140 [138,142]	104 [101,104]	14.6 [14,17.1]
0.75 mg/kg	0	7.41 [7.41,7.47]	5.67 [4.18,5.79]	12.02 [11.20,18.40]	26.2 [22.5,27.4]	140 [137,141]	106 [101,107]	10.2 [7.3,15.2]
	15	7.41 [7.37,7.43]	5.66 [4.89,5.93]	12.53 [11.00,13.34]	26.2 [22.0,27.3]	139 [138,141]	106 [102,106]	13.3 [6.8,14.0]
1.00 mg/kg	0	7.40 [7.39,7.45]	5.63 [4.63,5.98]	13.36 [12.08,13.90]	26.5 [23.4,26.9]	140 [139,141]	104 [103,104]	13.9 [12.4,17.7]
	15	7.40 [7.37,7.505]	5.23 [4.32,5.64]	13.05 [11.36,17.68]	25.0 [21.9,25.4]	140 [139,142]	103 [103,104]	17.3 [14.8,18.8]

Supplemental material 2-3: Population and individual predictions versus time and observed ABP-700 arterial concentrations. The population predictions of the model show some bias for observations after 60 min (top left panel) but these observations are lower than 10 ng/ml (bottom left panel) which is lower than the clinically relevant range. The predictions for each individual show a good match with the observations (right panels).

Likelihood profiles for the estimated PK parameters of ABP-700.

$V1_{ref}$, $V2_{ref}$, $V3_{ref}$, CL_{ref} , $Q2_{ref}$, $Q3_{ref}$ are the estimated compartmental volumes and clearances for a 70 kg individual.

Supplemental material 2-4: ABP-700 population and individual predictions versus time and observed BIS.

■ BIS is the Bispectral Index as measured by the Vista Monitor (Medtronic, Dublin, Ireland)

ABP-700 likelihood profiles for the estimated Bispectral Index (BIS) pharmacodynamic parameters.

$C_{e50,TYP}$ represents the typical population model parameters for the effect-site concentration at 50 % effect as measured by the bispectral index (BIS) and $k_{e0,BIS,TYP}$ represents the typical population model parameters for the first-order rate constant plasma and effect-site. γ represents the steepness of the pharmacodynamic E_{max} curve for BIS effect.

Supplemental material 2-5: ABP-700 population and individual predictions for the versus time and observed Modified Observer's Assessment of Alertness/Sedation (MOAA/S) score.

ABP-700 likelihood profiles for the estimated Modified Observer's Assessment of Alertness/Sedation (MOAA/S) score pharmacodynamic parameters.

2

$DEFF$ is a fixed effect parameter for the model.

$k_{e0,MOAA/S,TYP}$ is the typical population model parameter for the first-order rate constant between plasma and effect-site using MOAA/S as effect parameter.

"logit Pr" stands for the logit probability of the various MOAA/S scores.

CHAPTER 3

Safety and clinical effect of i.v. infusion of cyclopropylmethoxycarbonyl [m]etomidate (ABP-700), a soft analogue of etomidate, in healthy subjects

Modified from *Br.J Anaesth.* 2018 Jun;120(6):1401-1411

Beatrijs I. Valk, Anthony R. Absalom, Peter Meyer, Sascha Meier, Izaak den Daas, Kai van Amsterdam, Jason A. Campagna, Steven P. Sweeney, Michel M. R. F. Struys

Abstract

Background: Cyclopropyl-methoxycarbonyl metomidate, or ABP-700, is a second-generation analogue of etomidate, developed to retain etomidate's beneficial haemodynamic and respiratory profile but diminishing its suppression of the adrenocortical axis. The objective of this study was to characterise the safety and efficacy of 30-min continuous infusions of ABP-700, and to assess its effect on haemodynamics and the adrenocortical response in healthy human volunteers.

Methods: Five cohorts involving 40 subjects received increasing infusion doses of ABP-700, propofol $60 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ or placebo. Safety was evaluated through adverse event (AE) monitoring, safety laboratory tests, and arterial blood gasses. Haemodynamic and respiratory stability were assessed by continuous monitoring. Adrenocortical function was analysed by adrenocorticotrophic hormone (ACTH) stimulation tests. Clinical effect was measured using the modified observer's assessment of alertness/sedation (MOAA/S) and continuous bispectral index monitoring.

Results: No serious AEs were reported. Haemodynamic and respiratory effects included mild dose-dependent tachycardia, slightly elevated blood pressure, and no centrally mediated apnoea. Upon stimulation with ACTH, no adrenocortical depression was observed in any subject. Involuntary muscle movements (IMM) were reported, which were more extensive with higher dosing regimens. Higher dosages of ABP-700 were associated with deeper sedation and increased likelihood of sedation. Time to onset of clinical effect was variable throughout the cohorts and recovery was swift.

Conclusions: Infusions of ABP-700 showed a dose-dependent hypnotic effect, and did not cause severe hypotension, severe respiratory depression, or adrenocortical suppression. The presentation and nature of IMM is a matter of concern.

Cyclopropyl-methoxycarbonyl metomidate (CPMM or ABP-700, The Medicines Company, Parsippany, NJ, USA, currently called MDCO-700) is a novel γ -aminobutyric acid type A (GABA_A) receptor agonist. It is a second generation soft-analogue of etomidate,¹ which was developed for induction and maintenance of general anaesthesia.² In both pre-clinical and clinical research settings, etomidate showed remarkable haemodynamic and respiratory stability,^{3,4} and a quick onset of anaesthesia with a swift recovery.⁵ However, etomidate causes significant suppression of adrenocortical steroid synthesis by inhibiting the cytochrome P450 enzyme 11 β -hydroxylase, causing prolonged adrenal suppression lasting 6-8 h in patients receiving a single bolus of etomidate and more than 24 h after a continuous infusion.⁶⁻¹¹ This suppression of the adrenocortical axis is especially severe in critically ill patients.¹²⁻¹⁴ Nevertheless, etomidate is still used as a single bolus induction agent because of its beneficial cardiovascular and respiratory profile.¹⁵

ABP-700 was created along with a number of other new so-called 'soft-analogues' of etomidate with the aim of retaining its beneficial effect vs side effect profile while diminishing its adrenal suppressive action.^{16,17} Of these soft etomidate analogues, ABP-700 showed the most favourable pharmacology in vitro.^{18,19} Studies in dogs showed that ABP-700 has rapid metabolism and that recovery from the hypnotic effect is quick, and adrenocortical responsiveness was not significantly different from that after a propofol infusion.¹ Recently, a first-in-man, bolus-only study of ABP-700 was conducted,²⁰ in which ABP-700 showed a rapid onset of anaesthesia/sedation with rapid recovery. Stimulation with adrenocorticotrophic hormone (ACTH) caused a physiological cortisol response in all subjects comparable with that seen in placebo subjects. An adverse event (AE) frequently observed in that study was dose-related involuntary muscle movement (36 of 50 volunteers receiving ABP-700). Tachycardia (13 out of 50 volunteers receiving ABP-700) and tachypnoea (13 of 50 volunteers receiving ABP-700) were also frequently observed AEs.²⁰

Here we present the results of a Phase 1 randomised, single-blind, placebo- and propofol-controlled study in healthy human volunteers to assess safety, tolerability, and clinical effect of single ascending, 30 min i.v. infusion doses of ABP-700. Our goal was to characterise the safety and efficacy of ABP-700 and to assess its effect on haemodynamics and the adrenocortical axis. The effects of premedication with fentanyl 1 $\mu\text{g}\cdot\text{kg}^{-1}$, in addition to ABP-700 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, were also studied.

Methods

This was a single-centre, single-blind, randomised, placebo-and propofol-controlled study. A detailed description of the methods can be found in the first-in-man, bolus-only study of ABP-700.²⁰ Here we provide a brief overview of study subjects, study execution, assessments, and data recording.

Study management and registration

This Phase 1 trial was conducted at the QPS Netherlands B.V. early Phase 1 unit, Groningen, The Netherlands in cooperation with the Department of Anesthesiology at the University Medical Center Groningen, The Netherlands in accordance with the Declaration of Helsinki and in compliance with Good Clinical Practice and applicable regulatory requirements. Approval of the responsible ethics committee was received (METc stichting BEBO, NL49894.056.14). Before commencement of the trial, it was registered at the Dutch Trial Register (NTR4735). Written informed consent was obtained from all volunteers before enrolment.

Subjects

Healthy (ASA physical status I), non-smoking men and women aged 18-45 years were eligible for this study. Women were only included if they were of strict non-childbearing potential. Subjects were excluded if they had received ABP-700 in a previous study.

Study execution

Subjects were required to visit the clinical pharmacology unit (CPU) thrice: a screening visit, a 2-day and night admission to the CPU (within 28 days of the screening visit) during which study treatment was administered, and a follow-up visit within 4-6 days after study drug administration.

A consultant anaesthetist was present throughout the conduct of the study until full recovery of the subject. Subjects received O₂ at 5 litre min⁻¹ via nasal cannulae (Microstream®, Medtronic, Dublin, Ireland). Midazolam (Midazolam Actavis, Actavis, Dublin, Ireland) was present as safety medication at an initial 1 mg bolus dose for attenuation of any observed severe involuntary muscle movements (IMM), as was a complete emergency trolley to handle acute cardiopulmonary complications.

Treatment

ABP-700 was administered as a continuous i.v. infusion over 30 min as a sterile solution containing ABP-700 10 mg mL⁻¹ and 10% sulfobutylether- β -cyclodextrin with meglumine, an amino sugar derived from glucose, used as an excipient, in a 20 ml USP Type I clear glass vial closed with a grey 20 mm butyl Flurotec® (West Pharmaceutical Services, Exton, PA, USA) coated rubber stopper and sealed with a 20 mm aluminium overseal. Matching placebo contained the same inactive components as ABP-700 in the same type vials as ABP-700. Propofol was supplied as a 1% sterile emulsion for i.v. injection (Propofol 1% MCT/LCT 'Fresenius', Fresenius Kabi, Bad Homburg, Germany). Fentanyl (Fentanyl-hameln, Hameln Pharmaceuticals, Hameln, Germany) was supplied as an aqueous solution.

Forty subjects were distributed over five cohorts in a single-ascending-dose paradigm from ABP-700 30-60 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. Subjects were randomised using a dedicated randomisation schedule of the CPU in a 5:2:1 ratio for ABP-700, propofol, or placebo, respectively. The starting dose of 30 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ was chosen based on human safety, pharmacokinetic (PK) and pharmacodynamic (PD) data from subjects in the bolus only study.²⁰ PK data from the maximum tolerated dose (MTD) and highest dose level tested in this study (1000 $\mu\text{g}\cdot\text{kg}^{-1}$) were used to determine a starting dose where the total exposure over a 30-min infusion would not exceed the total exposure achieved during a 1 min i.v. bolus of 1000 $\mu\text{g}\cdot\text{kg}^{-1}$. Propofol was administered as an i.v. infusion of 0.15 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for 2-5 min, followed by a maintenance infusion of 60 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, based on the approved label for intended moderate sedation. This cohort served as a positive control for 'moderate sedation' against ABP-700 in terms of sedation scoring and bispectral index (BIS) monitoring.

Fentanyl was given as a pre-treatment in one cohort at 1 $\mu\text{g}\cdot\text{kg}^{-1}$. This dose was selected as it is a representative dose commonly used for anaesthesia care that has been shown to attenuate the severity and incidence of IMM when administered before etomidate.²¹ After each cohort, all available data regarding safety, tolerability, PK, and PD were evaluated by the principal investigator (PI), the sponsor, and the ethics committee before proceeding to the next dose. Stopping criteria for further drug escalation were identical to the single-bolus study:²⁰ Grade 3 or higher dose-limiting toxicity event as defined in the September 2007 Food and Drug Administration Guidance; local injection site reactions that were Grade 2 or higher; bradycardia considered clinically significant; and QTcF (QT interval corrected according to Fridericia) change from baseline 100 ms or total QTcF of >500 ms; SpO₂ <90% not resolved by simple stimulation, jaw thrust, or supplemental oxygen administered via nasal prongs; any serious AEs that were considered by the PI to be related to the study drug; and any clinically significant AEs that the sponsor and PI considered a safety concern.

Assessments

AEs were evaluated according to the Medical Dictionary for Regulatory Activities (MedDRA: version 16.0) throughout the study period. Safety and tolerability were further assessed through physical examinations, safety laboratory tests (serum chemistry, haematology, arterial blood gas, urinalysis, and coagulation assessments) before, during, and after drug dosing, temperature measurements and infusion site reaction monitoring. Intermittent 12-lead ECGs were examined for changes in PR, QRS, and QTcF intervals. Haemodynamic and respiratory stability was monitored continuously through a Philips MP50 monitor (Philips, Eindhoven, The Netherlands) with a high frequency sampling rate of 0.2 Hz. Clinical signs of upper airway obstruction and respiration were observed and documented by the attending anaesthetist.

Arterial blood gas values were obtained before dosing and 30 and 60 min after the start of the infusion.

Adrenal function was assessed by monitoring plasma cortisol before and after an ACTH stimulation test in which synthetic ACTH 250 mg [Cortrosyn® (cosyntropin), Amphastar Pharmaceuticals, Rancho Cucamonga, CA, USA] was administered by i.v. bolus 60 min after stopping ABP-700 infusion. Baseline cortisol concentrations were obtained before 9:00 am and were later measured 60 and 120 min after ACTH stimulation.

MTD was reached if three or more volunteers within one dosing cohort met stopping criteria. Depth of sedation, anaesthesia, or both was evaluated using the modified observer's assessment of alertness/sedation (MOAA/S) scale (see supplemental material 3-1). MOAA/S scoring was performed 1 min before dosing, every 30 s for the first 2 min of infusion of ABP-700, every min until 5 min after the start of the infusion, then every 5 min until termination of the infusion, and finally every 30 s until full recovery of the subject (defined by three consecutive MOAA/S scores of 5). BIS was used to continuously measure drug effect on the bifrontal EEG pattern using the BIS-VISTA™ monitor (Medtronic, Dublin, Ireland) and bilateral BIS® Sensor electrodes. BIS values range from 100 to 0, in which lower values demonstrate more drug effect.²²

Based on pre-clinical observations and initial observations,²⁰ and known properties of the parent compound etomidate, IMM were anticipated. There was no standardised nomenclature or scoring system applied to characterise such movements, as was the case when similar movements were described for etomidate.²³ As such, we performed a post hoc analysis of observed IMM using the same descriptive system used in the previous study of ABP-700 in human volunteers: 'extensive movements' defined as IMM that involve the whole body or a considerable part of it, or 'few movements' defined as IMM that occur in a few body parts, such as the arms bilaterally or the face. Other observations recorded were apnoea (for 20 s or more), tachypnoea (20 rpm or more), sinus tachycardia (heart rate of 100

beats min⁻¹ or more if a clinically significant change from baseline (assessed by the anaesthetist), hypertension (mean arterial pressure of 110 mm Hg or higher), and desaturation (clinically significant decrease of SpO₂ below 95%). All events had to last >20 s to be deemed clinically significant.

Arterial and venous blood samples were also obtained during this study, to analyse the PK of ABP-700; these data will be included in future work on the PK, PD, and PK/PD relationships.

Data recording

A dedicated and validated electronic software package (RUGLOOP II©, Demed, Temse, Belgium) was used to capture data on vital signs, MOAA/S, BIS, comments, and a subset of PK sampling times. Analogue information such as ECG parameters was recorded for further post hoc analysis. Data were validated through visual and computerised methods in order to ensure quality.

Statistics

Sample size was based on previous experience studying safety, efficacy, PD, and PK of new agents. Descriptive statistics are displayed as mean (standard deviation) or geometrical mean (95% confidence interval) unless otherwise indicated.

Results

Subjects

Subjects (233 total) were screened by QPS Netherlands B.V. for participation, and 40 subjects were enrolled. Of the 40 subjects included, 25 received ABP-700, 10 received propofol, and five received placebo. The range of cohort means for weight (75.4-85.3 kg), height (175.8-184.7 cm), and BMI (22.6-26.4 kg⁻¹·m⁻²) were similar between groups. Mean age ranged from 21.6 to 29.2 yr. A detailed patient characteristic analysis can be found in *supplemental material 3-2*.

Safety and tolerability

AEs were experienced by 30 subjects for a total of 93 AEs (*supplemental material 3-3*). The intensity of AEs was mild in 89.2% of subjects, with the remainder of moderate intensity (10.8%). One subject, from the ABP-700 40 µg·kg⁻¹·min⁻¹ cohort, prematurely discontinued the study drug infusion because of extensive IMM. Of the AEs, 75.3% were ‘possibly’ related to the study drug, 3.2% were unlikely to be related, and 21.5% were not related.

Figure 3-1. Haemodynamic data. (A) Systolic arterial pressure, non-invasive (NIBP systolic); (B) systolic arterial pressure, invasive (ART systolic); (C) diastolic arterial pressure, non-invasive (NIBP diastolic); (D) diastolic arterial pressure, invasive (ART diastolic); (E) heart rate. Data are presented as geometrical means per cohort. FEN, fentanyl.

$\mu\text{g kg}^{-1}\text{min}^{-1}$	ABP-700 30 (n=5)	ABP-700 40 (n=5)	ABP-700 50 (n=5)	ABP-700 50 + FEN (n=5)	ABP-700 60 (n=5)	Placebo (n=5)	Propofol 60 (n=10)	Total
Involuntary muscle movements								
No movements	3	0	1	1	0	5	10	20
Few movements	2	3	3	3	3	0	0	14
Extensive movement	0	2	1	1	2	0	0	6
Ventilation								
Respiratory depression	0	0	0	0	2	0	2	4
Tachypnea	0	1	1	0	2	0	0	4
Desaturation	0	0	0	0	1	0	0	1
Vital signs								0
Hypertension	0	1	0	0	2	0	0	3
Hypotension	0	0	0	0	0	0	0	0
Tachycardia	0	3	3	1	3	0	0	10
Bradycardia	0	0	0	0	0	0	0	0
Catheter site related reactions								
Nausea/Vomiting	1	1	0	0	2	0	2	6

Table 3-1. Evaluation of tolerability of ABP-700 given to healthy volunteers. Values presented are number of volunteers. ABP-700, cyclopropyl-methoxycarbonylmetomidate; FEN, $1 \mu\text{g kg}^{-1}$ fentanyl

Increases in systolic and diastolic arterial pressure were observed in all cohorts who received ABP-700 (**Figure 3-1**; for individual data see the online supplemental materials).[†] A small dose-dependent increase in heart rate was observed across the ABP-700 cohorts. The placebo cohort maintained a stable haemodynamic profile, and the propofol cohort showed a slight decrease in heart rate, systolic, and diastolic arterial pressure. After termination of the infusion at 30 min, haemodynamic parameters returned to baseline in all cohorts. An analysis evaluating a possible correlation between hypertension and IMM revealed a positive trend without statistical significance because of the small size of the groups (*supplemental material 3-4*).

Figure 3-2. Respiratory data. (A) Oxygen saturation (SpO₂); (B) ventilatory frequency; (C) PaCO₂. Data are presented as geometrical means per cohort. FEN, fentanyl.

There were no clinically significant differences in ventilatory frequency between cohorts, although ventilatory frequency was slightly higher with higher doses of ABP-700 (Figure 3-2 and Table 3-1). Respiratory pauses were clinically observed in a number of subjects receiving ABP-700, but all such events lasted <20 s and were not deemed clinically significant. Two episodes of true apnoea were recorded in the 60 µg·kg⁻¹·min⁻¹ cohort and two in the propofol cohort. In the 60 µg·kg⁻¹·min⁻¹ cohort, two subjects received airway support, consisting of chin lift and jaw thrust, to maintain a patent airway. Oxygen saturation fell to

<95% in one subject in the 60 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ cohort, who experienced two brief episodes (<5 s) of saturation of 91%. PaCO_2 remained stable in all cohorts.

IMM, initially described as ‘muscle twitching’ or ‘myoclonic activity’, were observed in all cohorts receiving ABP-700 (**Table 3-1**) with higher dosing regimens showing more extensive IMM. We were unable to confirm statistically significant dose dependency as our study was underpowered to address this question. Extensive movements were observed for doses of 40 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and higher. Midazolam was administered to four subjects for attenuation of severe IMM. One of these subjects had received ABP-700 40, one 50, and two 60 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. IMM were not observed in the placebo or propofol cohorts.

No significant changes were seen in serum chemistry, haematology, urinalysis, or coagulation in any cohort. Serum methanol concentration was below the limit of quantification in all cohorts. There were no significant changes in arterial blood gas parameters between baseline, 30 min post-dose, or 60 min post-dose (*supplemental material 3-5*). By 12-lead ECG, there were no apparent effects or a linear relationship between changes in any ECG parameter with arterial or venous ABP-700 concentrations. Stopping criteria for further drug escalation were met for one subject in the 60 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ cohort because of tachycardia, tachypnoea, and hypertension. An MTD was not defined.

Figure 3-3. Human adrenocortical response to adrenocorticotrophic hormone (ACTH) stimulation after a continuous infusion of ABP-700 (30–60 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), propofol (60 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) or placebo. Adrenocortical response before and after administration of 250 mg synthetic ACTH 1 h after the stop of infusion. Pre-dose cortisol reference concentrations were obtained before 09:00 on the day of ABP-700 administration. Values are mean cortisol concentration (standard deviation) for ABP-700 and placebo groups. $n=5$ for ABP-700 and placebo cohorts, and $n=10$ for the propofol cohort.

Upon stimulation with ACTH, plasma cortisol concentrations increased by at least 200 nM at 60 and 120 min after ACTH administration in the ABP-700, propofol, and placebo cohorts, consistent with no adrenal suppression (**Figure 3-3**). ACTH stimulation tests were not carried out in subjects who received fentanyl as a pretreatment to ABP-700 $50 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, as the ABP-700 dose was the same as a previous cohort.

Clinical effect

All cohorts that received ABP-700 showed a rapid decrease in BIS values (**Figure 3-4**). At $40 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and above, BIS fell below 50 within 10 min and reached a stable nadir after 12–15 min. Within 8 min after discontinuation of ABP-700 infusion, BIS returned to baseline values for all cohorts receiving either ABP-700 or propofol. In the propofol cohort, BIS decreased slightly to values around 75. There was no increased EMG activity observed by the BIS monitor in any cohort.

Figure 3-4. Hypnotic effect of ABP-700. (A) Depth of sedation and anaesthesia, as measured by the bispectral index (BIS) and (B) frontal EMG activity. Data are presented as geometrical means per cohort. FEN, fentanyl.

Clinical sedation effect, as measured by the MOAA/S scale, was dose-dependent with higher ABP-700 doses associated with both deeper sedation levels and an increased frequency of sedation events (Figure 3-5). In the placebo cohort, no subject showed sedation. In the propofol group, MOAA/S scores between 5 and 3 were observed except for one observation of 2. Onset of deep sedation or anaesthesia was observed from a dose of ABP-700 $40 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and upwards, albeit not in every subject.

Figure 3-5. Modified observer's assessment of alertness/sedation (MOAA/S) scores by subject. The data are displayed by individual subject who received ABP-700 ($n=25$) and propofol ($n=10$) and per cohort (six cohorts in total). Data for placebo were omitted because no subject was scored below 5.

Influence of fentanyl

After completion of the first four single ascending dose cohorts, the dosage of ABP-700 50 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ was deemed the most useful in terms of clinical effect relative to side effects. Pre-treatment with a bolus of fentanyl 1 $\mu\text{g}\cdot\text{kg}^{-1}$ showed no differences in incidence of IMM or mean haemodynamic and respiratory values compared with the same dose without fentanyl (**Table 3-1**, **Figures 3-1** and **3-2**). Fentanyl pre-treatment resulted in a reduced incidence of tachycardia (**Table 3-1**). Clinical effects of ABP-700 measured by either BIS or MOAA/S were similar with or without use of fentanyl.

Discussion

Continuous, 30-min infusion of ABP-700 ranging from 30 to 60 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ showed a dose-dependent clinical effect measured by BIS and MOAA/S. IMM were detected in all cohorts receiving ABP-700, requiring midazolam treatment in four subjects. IMM in the 60 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ cohort induced short lasting upper airway obstruction and a dose-dependent increase in arterial blood pressure and heart rate. No adrenal depression was found in any subject receiving ABP-700. An MTD for ABP-700 during continuous infusion was not identified.

The haemodynamic profile of a continuous infusion of ABP-700 showed similar characteristics to the first-in man bolus study.²⁰ There was no ABP-700-induced cardiovascular depression, which is reported in propofol induced anaesthesia.²⁴ No decrease in systolic or diastolic arterial pressure was observed in the ABP-700 cohorts, and arterial pressures in the ABP-700 groups were higher than in the propofol and placebo groups.

A dose-dependent increase in heart rate was observed in cohorts receiving ABP-700. An increase in heart rate with preservation of blood pressure stability during induction has been observed with other anaesthetic agents, such as etomidate,²⁵ and volatile anaesthetics desflurane²⁶ and isoflurane.²⁷

ABP-700 produced a slight increase in ventilatory frequency overall, with some short-lasting decreases that did not appear centrally-induced, but rather as a result of IMM causing short-lasting and self-limiting upper airway obstruction. PaCO_2 values indicate that decreases in ventilatory frequency did not cause significant metabolic alterations because of hypoventilation. ABP-700 did not cause suppression of the adrenal axis at any infusion dose, showing a minimum of 200 nM increase in plasma cortisol concentrations 60 min and 120 min after stimulation with ACTH. This is in contrast with etomidate, which is more potent as an adrenocortical inhibitor than as a sedative-hypnotic agent.¹⁵ A typical anaesthetic induction dose of etomidate thus represents a massive dose with respect to

adrenocortical inhibition.¹⁹ ABP-700 is quickly eliminated because of a rapidly hydrolysed ester bond, and because of this, it was hypothesised that ABP-700 does not produce adrenocortical suppression. This hypothesis is supported by pre-clinical studies,^{1,19,28} the first Phase I study in which ABP-700 was administered as a bolus,²⁰ and now when given as a continuous infusion of 30 min. This observation implies that it is safe, in regard to adrenocortical suppression, to use ABP-700 as a continuous infusion, in contrast to etomidate. However, as this was only a 30-min infusion study, no extrapolation of the adrenocortical suppressive action of ABP-700 when administered over longer time periods can be made.

IMM were observed in most subjects who received ABP-700, ranging in severity from a few, twitchy movements in one muscle group to extensive, sometimes myoclonic, movements that involved the whole body. Higher doses showed more extensive IMM. In total, four subjects experienced extensive movements, requiring administration of midazolam to diminish IMM.^{1,21} One of these subjects was discontinued from the study.

IMM is a phenomenon associated with various GABA_A agonists.^{29,30} It is a well-known side effect of ABP-700's parent compound etomidate during induction of anaesthesia.³¹ Early clinical studies of etomidate showed an incidence of myoclonus/IMM in 50-60% of unpremedicated patients who received etomidate.^{32,33} IMM is also observed in the use of propofol^{34,35} and has been observed in novel anaesthetic agents investigated in the past, such as AZD3043³⁶ and eltanolone.³⁷ Although quantifying IMM is difficult, Meinck and colleagues²³ reported 'three types of rhythmic motor hyperactivity: tremor, synchronous jerks and irregular clonus.' When seen with propofol, IMM are described as 'seizure-like phenomena'.³⁵ These descriptions are similar to our observations with ABP-700. It is not the frequency of the movements, but rather the magnitude of IMM that makes such movements undesirable in a clinical setting.

The aetiology of these movements is not clear, but several hypotheses exist. Meinck and colleagues²³ did not show spiking EEG activity indicative of an ictal origin of the myoclonus, which was confirmed by Doenicke and colleagues.²¹ Kugler and colleagues³⁸ hypothesise that IMM are possibly caused 'by an unsynchronised onset of drug action at different sites of action within the central nervous system', resulting in transient disinhibition of subcortical excitatory neuronal circuits, leading to IMM. As benzodiazepines and opioids are known to inhibit subcortical neuronal activity,³⁸ co-administration would cause a more equal rate of depression of subcortical excitatory and cortical inhibitory neuronal activity, thus attenuating IMM. This is consistent with observations in this study and by Doenicke and colleagues.²¹ As ABP-700 is an analogue of etomidate and also a GABA_A receptor agonist, our hypothesis is that the origin of the IMM observed with both drugs is similar. Investigating the potential nature of the IMM in this family of drugs and the influence of opioids and benzodiazepines in attenuating this phenomenon is warranted as unexplained IMM compromises the future development and successful registration for clinical use of this and other hypnotic drugs.

ABP-700 showed a dose-dependent hypnotic-sedative effect. Clinical effect was observed from a dose of ABP-700 $30 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and upwards, with deep sedation or anaesthesia observed from a dose of ABP-700 $40 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ and upwards. However, in every cohort, at least one subject attained an MOAA/S of 5. Time to onset of clinical effect was rapid in general, but large inter-subject variability was observed. Recovery was swift in all subjects, with most subjects being fully recovered within 5 min after termination of infusion. BIS profiles declined steeply and dose-dependently, with lowest values below 40. Subjects who received midazolam to attenuate IMM sometimes had a delayed recovery. In contrast to the bolus only study, MOAA/S patterns did not always follow BIS profiles closely. In two subjects in the $40 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ cohort, two subjects in the $50 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ cohort and one subject in the $60 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ cohort,²⁰ MOAA/S remained 5 throughout the infusion period. However, BIS values in these subjects all declined steeply towards values below 40 and sometimes even below 30, which should be indicative of deep anaesthesia. The cause of this discrepancy between clinically observed and EEG-measured hypnotic effects is unclear. Nevertheless, it seems that some level of responsiveness is still present at lower BIS values for ABP-700, as auditory stimuli are still being perceived and responded to. We hypothesise that a possible explanation of this discrepancy between measured and observed drug effect is an unsynchronised on- and off-set of measures of cerebral drug effect. It is possible that ABP-700 has a cerebral drug effect at different effect sites within the central nervous system at different times because of its seemingly steep PD. This might explain why BIS is low (cerebral drug effect in the frontal lobe) while the volunteer still responds. We did not systematically test for retrograde amnesia, but when we asked volunteers afterwards, they did not remember what had happened during drug infusion. This would be indicative of cerebral drug action at the frontal lobe. However, we measured depth of anaesthesia using only the frontal EEG (BIS), not a full EEG, and as such we do not know the cerebral drug effect at other sites within the central nervous system.

Limitations of this study are mostly similar to those reported in the bolus-only study of ABP-700.²⁰ Assessment of clinical drug effect through the MOAA/S scale is a pragmatic and effective tool, but it does not differentiate between light and deep anaesthesia and is subject to inter-investigator variability.³⁹ In our study, three different anaesthetists carried out the MOAA/S score, and as such this study is also prone to inter-investigator variability. Furthermore, as shown in this study and in contrast to previous studies, BIS patterns sometimes do not correlate with the clinical drug effect. Therefore, the validity of BIS as measuring equipment for depth of anaesthesia achieved with ABP-700 is not certain, although it is validated for other GABA_A-acting drugs.⁴⁰

In conclusion, a 30-min continuous infusion of ABP-700 showed a dose-dependent clinical effect as measured by BIS and MOAA/S, and did not cause severe hypotension or severe respiratory depression. ABP-700 did not cause adrenocortical suppression. ABP-700 caused no serious AEs in the context of this Phase 1 trial. However, the presentation and nature of

the IMM, observed for this and other GABA_A-ergic hypnotic drugs, is a matter of concern to both clinicians and industry. Shortly before submission of this paper, the study sponsor (The Medicines Company, Parsippany, NJ, USA) decided to discontinue its development programme of ABP-700, which had been in early Phase 2 studies in Europe.

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Supplemental material to Chapter 3

Supplemental material 3-1: *Modified Observer's Assessment of Alertness/Sedation (MOAA/S) score*

Responsiveness	Score
Responds readily to name spoken in normal tone	5 (alert)
Lethargic response to name spoken in normal tone	4
Responds only after name is called loudly and/or repeatedly	3
Responds only after mild prodding or shaking	2
Responds only after painful trapezius squeeze	1
Does not respond to painful trapezius squeeze	0

Supplemental material 3-2: *Demographics*

Characteristics	Statistic/Category	Placebo (N=5)	Propofol (N=10)	ABP-700 (N=25)
Sex (n (%))	Male	4 (80.0)	10 (100.0)	25 (100.0)
	Female	1 (20.0)	0 (0.0)	0 (0.0)
Age (yrs)	Range	19-45	19-27	18-45
Ethnicity (n (%))	Not Hispanic or Latino	5 (100.0)	10 (100.0)	25 (100.0)
	Hispanic	0 (0.0)	0 (0.0)	0 (0.0)
Race (n (%))	Asian	0 (0.0)	0 (0.0)	1 (4.0)
	Black or African American	0 (0.0)	0 (0.0)	0 (0.0)
	White	5 (100.0)	10 (100.0)	24 (96.0)
Smoking Status (n (%))	Never	5 (100.0)	10 (100.0)	20 (80.0)
	Stopped	0 (0.0)	0 (0.0)	5 (20.0)
- If yes, subject quit smoking > 6 months ago? (n (%))	Yes			5 (100.0)
Height (cm)	Mean (SD)	175.8 (8.0)	182.7 (6.3)	180.5 (6.5)
Weight (kg)	Mean (SD)	75.9 (7.8)	75.4 (5.8)	81.3 (9.0)
BMI (kg/m)	Mean (SD)	24.6 (2.9)	22.6 (1.6)	24.9 (2.5)
Alcohol consumption (units/week)	Median (range)	4 (0-10.0)	8.5 (1.0-12.0)	5 (0-12.0)

Supplemental material 3-3: Adverse Events

Adverse Event	ABP-700 (N=25)	Propofol (N=10)	Placebo (N=5)
Muscle twitching	16	0	0
Sinus tachycardia	8	0	0
Nausea	5	0	0
Headache	4	1	0
Catheter site related reaction	4	0	0
Restlessness	4	0	0
Myoclonus	3	0	0
Respiratory disorder ^a	3	3	0
Akathisia ^b	2	0	0
Tachypnoea	2	0	0
Yawning	2	0	0
Retching	2	0	0
Vomiting	2	0	0
Blood pressure systolic increased	1	0	0
Respiration abnormal ^c	1	0	0
Cough	1	0	0
Snoring	0	1	0

^a Verbatim term: respiratory pauses ^b Verbatim term: motoric restlessness

^c Verbatim term: sigh

Supplemental material 3-4: Analysis of a correlation between hypertension and IMM.

We divided all subjects who received ABP-700 into our three IMM 'classification' of 'no involuntary muscle movements' (n=5), 'few involuntary muscle movements' (n=14) and 'extensive muscle movements' (n=6) and assessed the difference in invasively measured systolic blood pressure between the groups every 5 seconds

from the start of the ABP-700 infusion. The differences between the blood pressures were plotted against time. We used a technique from De Baerdemaeker and colleagues¹ where individual measurements on a single data point are tested between groups. A difference between groups is significant at times where lower confidence intervals > 0 or when the upper CI < 0 . As you can see in the figure below, there does seem to be a slight difference in blood pressure between groups. However, it is not statistically significant as the 95-% CI included zero. Of course, our groups were not large, which could have influenced the results significantly. With larger groups, differences between groups might be clearer.

¹ De Baerdemaeker, L E C, Struys, M M R F, Jacobs S, et al. Optimization of desflurane administration in morbidly obese patients: A comparison with sevoflurane using an 'inhalation bolus' technique. Br J Anaesth 2003; 91: 638-50.

Supplemental material 3-5: Arterial blood gas sampling values at baseline, 30 minutes and 60 minutes after drug administration for each ABP-700 dosing cohort, placebo and control (propofol). Data are shown as median [range].

Cohort	Timepoint (min)	pH	PaCO ₂ (kPa)	PaO ₂ (kPa)	HCO ₃ (mmol/L)	Na ⁺ (mmol/L)	Cl ⁻ (mmol/L)	AnionGap (mEq/L)
Placebo	0	7.42 [7.40,7.45]	5.1 [4.3,5.9]	13.7 [10.8,22.8]	23.2 [21.7,28.4]	140.2 [135.4,141.4]	105 [103,107]	14.6 [10.6,16.1]
	30	7.41 [7.40,7.46]	5.1 [4.3,6.2]	23.6 [19.6,25.9]	24.1 [22.2,28.2]	140.1 [139.2,141.2]	105 [103,107]	15.9 [11.1,17.7]
	60	7.42 [7.41,7.43]	5.3 [4.6,5.9]	23.9 [19.1,24.0]	25.2 [21.4,27.1]	140.0 [138.7,140.5]	105 [104,108]	13.3 [12.0,17.3]
Propofol	0	7.41 [7.35,7.51]	5.4 [4.3,5.9]	15.5 [11.7,23.9]	24.9 [22.5,28.0]	140.2 [137.7,142.4]	105 [103,107]	14.3 [11.8,16.5]
	30	7.40 [7.34,7.42]	5.8 [5.4,6.9]	22.3 [12.8,29.1]	26.5 [25.0,29.1]	140.1 [138.0,141.4]	104.5 [104,107]	12.8 [10.5,14.0]
	60	7.40 [7.39,7.45]	5.6 [4.6,5.9]	21.7 [12.9,27.5]	25.5 [23.2,27.6]	140.4 [139.1,141.7]	105 [104,107]	13.3 [10.9,16.6]
30 µg kg ⁻¹ min ⁻¹	0	7.43 [7.42,7.47]	5.2 [4.5,5.5]	13.4 [9.7,17.6]	25.3 [24.0,27.4]	141.1 [139.3,141.8]	103 [103,105]	14.9 [14.3,18.3]
	30	7.43 [7.41,7.48]	5.4 [4.8,5.7]	19.7 [18.4,21.4]	25.2 [24.0,27.0]	140.4 [139.7,141.5]	103 [102,105]	15.4 [14.5,18.1]
	60	7.42 [7.38,7.43]	5.3 [4.9,5.9]	21.1 [17.6,22.2]	24.8 [23.7,25.9]	140.2 [139.5,141.7]	104 [103,106]	16.1 [13.8,16.4]
40 µg kg ⁻¹ min ⁻¹	0	7.41 [7.41,7.45]	5.5 [4.8,5.7]	11.6 [9.4,21.2]	25.8 [22.6,26.9]	140.6 [138.3,141.9]	104 [103,106]	14.5 [13.2,17.0]
	30	7.42 [7.40,7.47]	5.1 [4.3,5.6]	17.9 [11.5,23.7]	24.4 [23.2,26.0]	140.3 [138.1,143.2]	104 [102,105]	17.2 [11.8,18.4]
	60	7.41 [7.40,7.42]	5.3 [5.1,5.7]	20.0 [10.8,25.9]	25.3 [23.5,26.7]	140.4 [137.7,141.5]	104 [103,105]	14.6 [11.7,16.7]
50 µg kg ⁻¹ min ⁻¹	0	7.42 [7.41,7.43]	5.6 [5.4,5.9]	19.0 [11.9,21.2]	27.1 [26.1,28.6]	140.3 [138.8,142.0]	104 [102,104]	14.0 [13.0,14.0]
	30	7.43 [7.41,7.45]	5.6 [5.1,5.8]	20.5 [14.9,21.7]	27.5 [25.2,28.5]	140.5 [140.0,141.0]	103 [102,104]	14.0 [13.1,16.4]
	60	7.43 [7.41,7.47]	5.7 [5.3,5.9]	20.7 [16.6,22.4]	28.0 [25.2,28.6]	140.6 [139.2,140.9]	104 [102,104]	12.1 [11.8,16.3]
60 µg kg ⁻¹ min ⁻¹	0	7.42 [7.42,7.53]	5.5 [4.1,5.5]	13.7 [11.4,20.5]	26.0 [22.5,26.7]	139.9 [136.9,142.9]	105 [104,108]	12.8 [9.2,17.5]
	30	7.42 [7.36,7.43]	5.6 [4.4,6.5]	19.2 [13.7,20.2]	26.7 [21.2,27.2]	140.9 [136.5,143.0]	105 [104,106]	14.4 [9.5,15.9]
	60	7.40 [7.39,7.67]	5.4 [2.4,5.6]	20.0 [13.3,27.3]	25.1 [20.2,25.9]	139.6 [136.9,139.9]	105 [104,107]	13.2 [11.1,17.2]
50 µg kg ⁻¹ min ⁻¹ +fentanyl	0	7.42 [7.42,7.43]	5.5 [5.3,6.0]	18.5 [15.5,19.9]	26.4 [25.3,28.2]	140.3 [139.7,143.6]	104 [104,106]	14.0 [12.1,15.7]
	30	7.42 [7.39,7.47]	5.6 [4.6,5.9]	17.8 [16.9,21.4]	26.8 [24.7,27.1]	140.3 [139.8,142.2]	103 [103,105]	14.5 [14.2,16.2]
	60	7.41 [7.39,7.42]	5.7 [5.5,6.3]	18.6 [17.5,25.1]	27.1 [24.8,29.6]	140.4 [139.4,142.0]	104 [103,105]	13.7 [12.0,16.4]

CHAPTER 4

Toxicologic and Inhibitory Receptor Actions of the Etomi- date Analog ABP-700 and Its Metabolite CPM-Acid

Modified from *Anesthesiology*. 2019 Aug. 131(2):287–304

Beatrijs I Valk, Megan McGrath, Dario Lehoux, Brad Zerler, John J.A. Marota,
Douglas E. Raines

Abstract

Background: The etomidate analog ABP-700 produces involuntary muscle movements that could be manifestations of seizures. To define the relationship (if any) between involuntary muscle movements and seizures, electroencephalographic studies were performed in Beagle dogs receiving supra-therapeutic ($\sim 10\times$ clinical) ABP-700 doses. γ -aminobutyric acid type A (GABA_A) and glycine receptor studies were undertaken to test receptor inhibition as the potential mechanism for ABP-700 seizures.

Methods: ABP-700 was administered to 14 dogs (6 mg/kg bolus followed by a 2-h infusion at 1 mg·kg⁻¹·min⁻¹, 1. mg·kg⁻¹·min⁻¹, or 2.3 mg·kg⁻¹·min⁻¹). Involuntary muscle movements were documented, electroencephalograph was recorded, and plasma ABP-700 and CPM-acid concentrations were measured during and after ABP-700 administration. The concentration-dependent modulatory actions of ABP-700 and CPM-acid were defined in oocyte-expressed $\alpha_1\beta_3\gamma_2$ L GABA_A and $\alpha_1\beta$ glycine receptors (n = 5 oocytes/concentration) using electrophysiologic techniques.

Results: ABP-700 produced both involuntary muscle movements (14 of 14 dogs) and seizures (5 of 14 dogs). However, these phenomena were temporally and electroencephalographically distinct. Mean peak plasma concentrations were (from lowest to highest dosed groups) 35 μ M, 45 μ M, and 102 μ M (ABP-700) and 282 μ M, 478 μ M, and 1,110 μ M (CPM-acid). ABP-700 and CPM-acid concentration–GABA_A receptor response curves defined using 6 μ M γ -amino-butyric acid exhibited potentiation at low and/or intermediate concentrations and inhibition at high ones. The half-maximal inhibitory concentrations of ABP-700 and CPM-acid defined using 1 mM γ -aminobutyric acid were 770 μ M (95% CI, 590 to 1,010 μ M) and 1,450 μ M (95% CI, 1,340 to 1,560 μ M), respectively. CPM-acid similarly inhibited glycine receptors activated by 1 mM glycine with a half-maximal inhibitory concentration of 1,290 μ M (95% CI, 1,240 to 1,330 μ M).

Conclusions: High dose ABP-700 infusions produce involuntary muscle movements and seizures in Beagle dogs via distinct mechanisms. CPM-acid inhibits both GABA_A and glycine receptors at the high ($\sim 100\times$ clinical) plasma concentrations achieved during the dog studies, providing a plausible mechanism for the seizures.

ABP-700 is a “soft” analog of etomidate (**figure 4-1**).¹⁻⁴ Similar to other soft drugs such as remifentanyl and esmolol, it contains a metabolically-labile ester moiety that is rapidly hydrolyzed by esterases to a carboxylic acid metabolite (CPM-acid). In developing this and other soft etomidate analogs, our primary goal was to retain the potent sedative-hypnotic activity and benign cardiorespiratory actions of etomidate, while eliminating the prolonged suppression of adrenocortical steroid biosynthesis that plagues etomidate administration. A secondary goal was to produce an agent that would allow rapid anesthetic recovery. Subsequent human studies have confirmed that at clinically relevant doses, ABP-700 minimally affects respiratory and cardiovascular function.^{4,5} However, unlike etomidate, it does not suppress steroid biosynthesis and has a fast recovery profile even after prolonged infusion.^{4,6,7}

Figure 4-1. Chemical structures of (A) ABP-700 and its principal metabolite CPM-acid and (B) etomidate and its principal metabolite etomidate-acid. The elimination half-lives ($t_{1/2}$) of ABP-700 and etomidate in humans are ~15 min and 5.5 h, respectively.^{3,4}

With ABP-700 administration, involuntary muscle movements are commonly observed.⁴⁻⁶ Although such movements are also commonly observed with etomidate administration and not associated with seizures, the corporate licensee of ABP-700 (The Medicines Company, USA) undertook studies in Beagle dogs to determine whether there was any electroencephalographic seizure activity during these movements. During those toxico-

logic studies—which were performed at the direction of the United States Food and Drug Administration with the goal of achieving ABP-700 exposure levels that are 10× higher than the maximum associated with clinical doses—electroencephalographically-confirmed convulsive seizures were sometimes observed. Although no seizures or postictal behaviors were reported in clinical studies, establishing the likely mechanism(s) for these seizures in Beagle dogs and assessing the potential of ABP-700 for causing seizures in humans is critical for the future development of ABP-700 as a therapeutic drug.^{4,5} More broadly, defining the cause of these seizures may be important for guiding the design and development of other novel sedative-hypnotic agents that act by similar receptor mechanisms.

Many general anesthetics are widely believed to produce their behavioral effects—at least in part—by acting on γ -aminobutyric acid type A (GABA_A) receptors in the central nervous system (CNS)⁸. Within the clinically relevant concentration range, these agents enhance (“potentiate”) GABA_A receptor function in a concentration-dependent manner leading to a reduction in CNS excitability that manifests as sedation or hypnosis. This receptor action also accounts for the anti-convulsant activity of many general anesthetic agents.^{9,10} Conversely, at lethally high concentrations and similar to classic convulsants such as pentylenetetrazol and picrotoxin, general anesthetics commonly inhibit GABA_A receptor function.^{11–14} For propofol and etomidate, this inhibitory action is typically evident at concentrations greater than or equal to 30 μ M, which is more than 10-fold higher than their respective sedative-hypnotic concentrations.^{11,14,15} This inhibitory action presumably results from anesthetic binding to a low affinity site(s) on the GABA_A receptor that is distinct from those responsible for potentiation. Consequently, concentration-response curves that define anesthetic actions on GABA_A receptors are typically bell-shaped, exhibiting potentiation at clinically-relevant anesthetic concentrations and inhibition at lethally high ones.^{11,16}

In the current article, we report the results of the dog toxicology studies along with in vitro studies aimed at characterizing the GABA_A and glycine receptor modulatory actions of ABP-700 and CPM-acid. Our goal in undertaking the receptor studies was to test the hypothesis that ABP-700 or its metabolite CPM-acid produced seizures in these dogs because it had reached concentrations sufficient to inhibit the function of these inhibitory receptors, which is a well-established mechanism for producing seizures.^{17,18}

Materials and Methods

Sources of Drugs and Chemicals

ABP-700 for Beagle dog studies was obtained from KABS Laboratories (Canada) as a sterile formulation at 20 mg/ml in 10% sulfobutylether- β -cyclodextrin (pH 2.5) and stored frozen (-20°C) before being brought to room temperature within 6 h of administration. ABP-700 and CPM-acid for GABA_A receptor studies were synthesized by Aberjona Laboratories (USA) using our previously described methods.^{1,19} Etomidate-acid was synthesized as previously described.²⁰ Other chemicals were purchased from Sigma-Aldrich (USA).

Toxicologic Studies of ABP-700 in Beagle Dogs

All Beagle dog studies were performed by CiToxLAB North America (Canada) and conducted in accordance with their institutional animal care and use committee and the principles outlined in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. Dogs ($n = 14$ dogs; 7 males and 7 females; age: 8 to 9 months at the time of ABP-700 dosing) were purchased from Marshall BioResources (USA). Dogs were individually housed in stainless steel cages equipped for telemetry data acquisition and day/night vision cameras for continuous video monitoring. Room temperature was maintained at $21^{\circ} \pm 3^{\circ}\text{C}$ with 12-h light-dark cycles. At least 26 days before ABP-700 dosing, dogs were anesthetized with propofol and isoflurane and surgically instrumented for telemetric monitoring of electroencephalography and electromyography, intraarterial blood pressure, heart rate, body temperature, and physical activity generally as previously described.²¹ Sterile transmitters (D70-EEE and M10; Data Science International, USA) were inserted between the internal abdominal oblique muscle and the aponeurosis of the transverses abdominis and secured with nonabsorbable suture material. The electroencephalographic and electromyographic leads were tunneled to a dorsocervical neck incision. Femoral venous and intraarterial catheters were also inserted at this time. Electroencephalographic electrodes were attached to the cranium to monitor two standard bipolar derivations (CZ-OZ and C4-O2) and the electromyographic electrodes were placed in the temporalis muscle. Both electroencephalographic and electromyographic signals were continuously recorded and analyzed beginning at least 30 min before ABP-700 administration and ending at least 24 h after administration was complete using Dataquest ART software and NeuroScore software with the seizure detection module (Data Sciences International, USA). Electroencephalographic interpretation was completed by a board-certified veterinary neurologist who included a description of the involuntary movements and possible electroencephalographic correlates in a report to the corporate sponsor. That interpretation was peer-reviewed by an independent neuroscientist. A seizure was defined as an electroencephalographic pattern lasting greater than or equal to 10 s and satisfying either of the following criteria: (1) repetitive generalized or focal spikes, sharp-waves, spike-and-wave, or

sharp-and-slow wave complexes at greater than or equal to 3 Hz; or (2) sequential rhythmic, periodic, or quasiperiodic waves at greater than or equal to 1 Hz and unequivocal evolution in frequency (gradual increases/decreases by greater than or equal to 1 Hz), morphology, or location.²² Heart rate, intraarterial blood pressure, and body temperature were acquired and analyzed using DSI Ponemah 6.30 software (Data Sciences International).

Before study, dogs were fasted for 12 to 24 h. Each dog was premedicated with glycopyrrolate (0.02 mg/kg intramuscularly) and then received an ABP-700 bolus (6 mg/kg) immediately followed by a 2-h ABP-700 infusion at 1 mg·kg⁻¹·min⁻¹ (total dose: 126 mg/kg; n = 4 dogs), 1.5 mg·kg⁻¹·min⁻¹ (total dose: 186 mg/kg; n = 6 dogs), or 2.3 mg·kg⁻¹·min⁻¹ (total dose: 282 mg/kg; n = 4 dogs). When sufficiently anesthetized, dogs were placed on a heating pad to maintain core body temperature and endotracheally intubated. Ventilation was mechanically assisted with oxygen (ventilation rate: 7 to 20 breaths/min, inspiratory pressure: 18 to 25 cm H₂O). Hemoglobin oxygen saturation, end-tidal carbon dioxide (target: 25 to 55 mmHg), heart rate, and body temperature (target: 37.2° to 38.8°C) were monitored continuously and maintained at target values. Involuntary muscle movements were identified from video recordings and their time, duration, severity (mild, moderate, or severe), and body locations were noted. The severity of each discrete episode of involuntary muscle movement was judged using two criteria: (1) Were there full muscle contractions affecting at least two different body parts? and (2) Did the movements last more than 3 min? Each individual episode was graded as slight, moderate, or severe if it met neither, one, or both criteria, respectively. When necessary to control severe involuntary muscle movements that risked interfering with electroencephalographic signal acquisition and interpretation (and thus seizure detection) during ABP-700 infusion, rocuronium (0.1 to 2.0 mg/kg, IV) was administered. At the end of ABP-700 infusions, dogs were extubated as they emerged from anesthesia and exhibited adequate respiratory function. Midazolam (0.2 mg/kg, IV) and phenytoin (0.2 mg/kg, IV) were administered after the ABP-700 infusion when needed to control repeated episodes of clonic and/or tonic convulsions.

Blood samples (0.5 ml each) were drawn 5, 15, 30, 45, 60, 90, and 115 min after the start of the ABP-700 infusion and then 2, 10, 20, 60, 120, 240, and 480 min and 24 h after the 2-h ABP-700 infusion was completed. These samples were immediately transferred into pre-chilled tubes containing K₂EDTA and 16 µl NaF, and then centrifuged for 10 min under refrigeration (4°C; 1,500 g) within 30 min of collection. ABP-700 and CPM-acid concentrations were analyzed by Envigo CRS (USA) using a validated liquid chromatography/tandem mass spectroscopic protocol that had lower limits of quantitation of 1.00 and 20.0 ng/ml for ABP-700 and CPM-acid, respectively. A noncompartmental toxicokinetic analysis of the plasma concentration versus time data was performed by CiToxLAB North America using Phoenix WinNonlin 6.3 software (Certara, USA) to define toxicokinetic parameters.

GABA_A Receptor Electrophysiology

Oocytes were harvested from adult female *Xenopus laevis* frogs using procedures that were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee (Boston, Massachusetts) and are in accordance with the principles outlined in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health (Bethesda, Maryland).²⁰ For GABA_A receptor studies, oocytes were injected with 1 ng of messenger RNA (mRNA) encoding the α_1 , β_3 , and γ_{2L} subunits of the human GABA_A receptor at a subunit ratio of 1:1:3. For glycine receptor studies, oocytes were injected with 1 ng of mRNA encoding the α_1 and β subunits of the human glycine receptor at a ratio of 1:1. After injection, oocytes were incubated for 18 to 48 h in ND96 buffer (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, 5 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid, pH=7.4) containing 0.05 mg/ml of gentamicin and then studied using the whole cell two-electrode voltage-clamp technique.²³

Potentiation of 6 μ M GABA-evoked GABA_A Receptor-mediated Currents by ABP-700 and CPM-Acid

Each oocyte was perfused with ND96 buffer containing 6 μ M γ -aminobutyric acid (GABA) alone for 20 s followed immediately by 6 μ M GABA plus test compound (ABP-700 or CPM-acid) at the desired concentration for 60 s and the current response was recorded. This GABA concentration was chosen because it produces a current whose peak amplitude is ~5% of that produced by 1 mM GABA (commonly termed an EC₅ GABA concentration). To account for variable receptor expression among oocytes, all peak current responses were normalized to the peak current response evoked by 1 mM GABA measured in the same oocyte.

Inhibition of Maximally-activated GABA_A and Glycine Receptor-mediated Currents by ABP-700, CPM-Acid, and Etomidate-acid

To maximally activate receptors, each oocyte was perfused for 70 s with ND96 buffer containing 1 mM GABA (for GABA_A receptor experiments) or 1 mM glycine (for glycine receptor experiments). Ten seconds into this activation period, the test compound (ABP-700, CPM-acid, or etomidate-acid) was added for 30 s. The inhibitory effect of the test compound on currents was then quantified from the recorded electrophysiologic traces as the reduction in current amplitude at the end of test compound application. To account for desensitization during test compound application, an interpolated straight line was fit between the pre- and posttest compound phases of the current recording period. That line was then used as the baseline against which the effect of the test compound was quantified.

Voltage-dependence of GABA_A Receptor Inhibition by ABP-700 and CPM-Acid

Oocytes expressing GABA_A receptors were voltage-clamped at the desired transmembrane potential ranging from −110 mV to +10 mV. Receptors were then maximally activated by perfusing each oocyte with 1 mM GABA for 70 s. Ten seconds into this activation period, ABP-700 or CPM-acid was added for 30 s. The inhibitory effect of the test compound on currents was then quantified from the recorded electrophysiologic traces as the reduction in current amplitude at the end of test compound application as described in the previous section.

Impact of CPM-acid on GABA_A and Glycine Receptor Agonist Concentration-response Curves

Each oocyte expressing either GABA_A receptors or glycine receptors was perfused with ND96 buffer containing CPM-acid for 30 s, followed immediately by CPM-acid plus the desired concentration of receptor agonist (GABA or glycine, respectively) for 15 s and the current response was recorded. Control studies were performed in the absence of CPM-acid. The peak current response obtained in the absence or presence of CPM-acid was normalized to the peak current response evoked by 1 mM agonist (without CPM-acid) measured in the same oocyte.

Data Analysis

Concentration-response curves for potentiation were fit using Prism 6.0h software (GraphPad, USA) using its built-in four-parameter equation for stimulation (equation 1):

$$\text{Normalized Peak Current Amplitude} = \text{Minimum} + \frac{\text{Maximum} - \text{Minimum}}{1 + 10^{(\text{LogEC}_{50} - [\text{drug}]) * n}}$$

where minimum is the normalized peak current amplitude in the absence of test compound (ABP-700, CPM-acid, or GABA), maximum is the normalized peak current amplitude at infinitely high test compound concentrations, [test compound] is the test compound concentration, EC₅₀ is the test compound concentration that evokes a peak current amplitude that is half way between the maximum and minimum values, and n is the slope of the relationship.

Concentration-response curves for inhibition of 1 mM GABA-evoked and 1 mM glycine-evoked currents were similarly fit using Prism 6.0h software using its built-in four-parameter equation for inhibition (equation 2):

$$\text{Normalized Peak Current Amplitude} = \text{Minimum} + \frac{\text{Maximum-Minimum}}{1 + 10^{(\text{Log}[C_{50} - [\text{drug}]] * n)}}$$

where maximum is the normalized peak current amplitude in the absence of test compound (ABP-700, CPM-acid, or etomidate-acid), minimum is the normalized peak current amplitude at infinitely high test compound concentrations, [test compound] is the test compound concentration, half-maximal inhibitory concentration is the test compound concentration that evokes a peak current amplitude that is half way between the maximum and minimum values, and n is the slope of the relationship.

Statistical Analysis

In Beagle dog studies, each individual data point represents a single measurement in a dog unless otherwise indicated. In receptor studies, individual data points are reported as the mean $\pm$ SD of five separate oocyte experiments whose results were normally distributed as assessed using the Kolmogorov–Smirnov test with the Dallal-Wilkinson-Lilliefors corrected P value. The extra sum-of-squares F test was used to test whether the agonist EC₅₀ or the at maximum peak current at infinitely high agonist concentrations differed significantly in the presence versus the absence of CPM-acid. The uncertainties in fitted parameters are reported as 95% CI. There was no lost or missing data. To avoid output saturation, oocytes producing 1 mM GABA-evoked peak currents greater than 10 μ A were discarded. Fitting and statistical tests were performed with GraphPad Prism 6.0h (USA). No a priori statistical power calculations were conducted and the sample size was based on previous experience.^{24,25} Statistical significance was assumed for P < 0.05.

Results

Toxicologic Studies of ABP-700 in Beagle Dogs

ABP-700 induced and maintained anesthesia in all 14 Beagle dogs at a depth sufficient to allow endotracheal intubation without a paralytic agent within 5 to 15 min of initiating ABP-700 administration. Involuntary muscle movements were observed in all 14 dogs during 2-h ABP-700 infusions and in 9 of the dogs after the infusions were complete. These movements ranged from slight tongue movements to severe whole body shaking. The total number of recorded episodes of involuntary muscle movements in the 14 dogs was 321 (157 in males and 164 in females), with 278 episodes occurring during ABP-700 infusions and 43 episodes occurring afterward. Only one of the episodes of involuntary muscle movements (0.36%) recorded during ABP-700 infusions coincided with electroencephalographic evidence of seizure activity. This occurred in a dog from the 186 mg/kg group that had a seizure 107.5 min after the start of an ABP-700 infusion (i.e., 12.5 min before the end of

the 2-h infusion and after receiving 167 mg/kg of ABP-700). The percentages of all episodes of involuntary muscle movement characterized as mild, moderate, and severe were 80%, 14%, and 6%, respectively. There was no clear correlation between the ABP-700 dose and the number or severity of these episodes (data not shown). The total dose of rocuronium administered to a dog during ABP-700 infusion to control severe movements ranged from 1.6 to 4.8 mg/kg (126 mg/kg group), 1.6 to 5.6 mg/kg (186 mg/kg group), and 3.2 to 5.6 mg/kg (282 mg/kg group), respectively. Only one dog did not receive any rocuronium because all of its involuntary muscle movements were slight, and no dog received rocuronium during the final 20 min of ABP-700 infusion. Dogs began to right themselves 17 to 58 min after the conclusion of the 2-h ABP-700 infusions.

Heart rate remained within normal values for this species throughout ABP-700 administration (data not shown). However, [figure 4-2](#) shows that mean blood pressures progressively decreased in all dogs during ABP-700 infusions from 115 ± 10 mmHg (mean value in all three dosage groups during the one hour prior to beginning the ABP-700 infusions $\pm$ SD) to mean minimum values of 88 ± 13 mmHg (126 mg/kg group), 76 ± 9 mmHg (186 mg/kg group), and 65 ± 6 mmHg (282 mg/kg group) by the end of the 2-h ABP-700 infusions. Mean blood pressures returned to baseline values within an hour of completing ABP-700 infusions, paralleling recovery from anesthesia.

Figure 4-2. Mean intraarterial blood pressures recorded in Beagle dogs. Blood pressure data is shown for all 14 dogs beginning 1 h prior to ABP-700 administration and ending 2 h after the 2-h infusion was completed. The gray bar highlights the time period when ABP-700 was administered. Dogs received a 6 mg/kg bolus of ABP-700 at time 0, followed by a 2-h continuous ABP-700 infusion of $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (126 mg/kg total dose; black symbols), $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (186 mg/kg total dose; blue symbols) or $2.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (282 mg/kg total dose; red symbols).

Spikes, isolated sharp waves, and repeated sharp waves were identified in the electroencephalographic recordings of all dogs during ABP-700 infusions. Tonic and/or clonic convulsions associated with electroencephalographically-confirmed seizures were observed in 5 of the 14 dogs. A representative electroencephalographic recording of one such seizure is shown in [figure 4-3A](#) to [4-3C](#). By group, there were two dogs (one male and one female) in both the 126 mg/kg and 186 mg/kg groups, and one dog (female) in the 282 mg/kg group that had seizures. In one dog from each of the three groups, the first seizure was preceded by premonitory signs including salivation, chewing, excessive vocalization, tremors, head shaking, and/or muscle twitching or contractions. Seizures first occurred 6.5 and 152 min (126 mg/kg group), 81 min (186 mg/kg group), and 743 min (282 mg/kg group) after completion of ABP-700 infusion ([figure 4-3D](#)). As noted in the first paragraph of the Results section, one dog (186 mg/kg group) had its first (and only) seizure 12.5 min before the end of the 2-h ABP-700 infusion. Of the dogs that had seizures, two dogs had just one seizure and two dogs had three seizures. The fifth dog, which was in the 186 mg/kg group, had a total of 64 seizures over the 24 h that followed ABP-700 infusion. Thus, 71 out of 72 of the electroencephalographic-confirmed seizures occurred during the post-infusion period. These seizures were self-limiting, lasting 10 to 226 s. However, two dogs received midazolam and phenytoin to control convulsive behavior in the post ABP-700 infusion period that the post hoc electroencephalographic review determined were not due to seizures.

Figure 4-3. Electroencephalographic and electromyographic signals recorded from a dog (in the 186 mg/kg group) that had a seizure after receiving a 6 mg/kg ABP-700 bolus immediately followed by a 2-h ABP-700 infusion at $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (A) upon initiation of the seizure 107.5 min after beginning ABP-700 administration (i.e., after receiving 167 mg/kg of ABP-700), (B) during the seizure, and (C) upon spontaneous termination of the seizure 1.4 min after it began. (D) Timing of all seizures in Beagle dogs. Five dogs had electroencephalographically-confirmed seizures. Two dogs received 126 mg/kg ABP-700 (black symbols), two dogs received 186 mg/kg ABP-700 (blue symbols), and one dog received 282 mg/kg ABP-700 (red symbols). The only seizure that occurred during ABP-700 administration is highlighted by a yellow star. In one dog in the 126 mg/kg group, two seizures occurred within 6 min of one another ($T = 13.15$ and 13.24 h). Consequently, their symbols largely overlap. The number of seizures that each dog had during the study period is also numerically indicated. The gray bar highlights the 2-h time period when ABP-700 was administered.

For each of the three dosage groups, **figure 4-4A** and **4-4B** plot the time-dependent changes in plasma ABP-700 and CPM-acid concentrations, respectively. They reveal that the plasma concentrations of both ABP-700 and CPM-acid generally increased during ABP-700 infusions. Plasma concentrations of ABP-700 and CPM-acid were dose-dependent and achieved peak values near the end of the infusions. After completion of the infusions, plasma ABP-700 and CPM-acid concentrations decreased by 90% on approximate timescales of 10 min and 2 h, respectively. For each of the five dogs that had seizures, **figure 4-4A** and **4-4B** also indicate the time of its first seizure. Two dogs (one in each of the 126 mg/kg and 186 mg/kg groups) had their first seizures within minutes of the end of the ABP-700 infusion (6.5 min after and 12.5 min before, respectively) when the plasma concentrations of ABP-700 and CPM-acid were both near their maximum values. The other three dogs had their first seizure 1.4, 2.5, and 12.4 h after both ABP-700 and CPM-acid had reached their peak plasma concentrations.

Figure 4-4. Toxicokinetics of (A) ABP-700 and (B) its principal metabolite CPM-acid in Beagle dogs. T = 0 is the time of ABP-700 bolus administration (6 mg/kg), which was immediately followed by a 2-h ABP-700 infusion at $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (126 mg/kg total dose), $1.5 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (186 mg/kg total dose), or $2.3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (282 mg/kg total dose). The left and right vertical axes report concentrations in units of ng/ml and μM , respectively. In each, individual datasets obtained using a single dog are represented by connected symbols. Data from dogs that had electroencephalographically confirmed seizures are shown as red and blue data points. The time when each of the five dogs had their first seizure is also indicated in each panel by an arrow, which is color-coded to the dog. In each panel, the gray bar highlights the 2-h time period when ABP-700 was administered. Values below the lower limits of quantitation (1.00 ng/ml and 20.0 ng/ml for ABP-700 and CPM-acid, respectively) were not plotted.

Table 4-1 shows the results of noncompartmental analysis of the toxicokinetic data from the three groups for both ABP-700 (**table 4-1A**) and CPM-acid (**table 4-1B**). The toxicokinetic parameters suggest no large differences between male versus female dogs. The mean (combined male and female data) maximum measured plasma concentrations of ABP-700 were 11,025 ng/ml (35 μ M), 14,000 ng/ml (45 μ M), and 32,180 ng/ml (102 μ M) in the 126 mg/kg, 186 mg/kg, and 282 mg/kg groups, respectively. The mean maximum measured plasma concentrations of CPM-acid were an order of magnitude higher than those of ABP-700 with values of 84,180 ng/ml (282 μ M), 142,840 ng/ml (478 μ M), and 331,750 ng/ml (1,110 μ M) in the 126 mg/kg, 186 mg/kg, and 282 mg/kg groups, respectively.

Total Dose		126 mg/kg		186 mg/kg		282 mg/kg	
Sex		M	F	M	F	M	F
Number Animals		2	2	3	3	2	2
C_{max}	(ng/mL)	10,500	11,550	13,770	14,230	26,550	37,800
T_{max}	(h)	1.708	1.916	1.916	1.500	1.708	1.708
$AUC_{(last)}$	(h*ng/mL)	17,780	19,570	23,040	24,290	48,150	60,360
Initial $t_{1/2}$	(h)	0.15	0.22	0.17	0.12	0.18	0.17
Terminal $t_{1/2}$	(h)	1.28	NC	1.30	1.25	0.80	0.86

Table 4-1A. Mean Plasma ABP-700 Toxicokinetic Parameters Following Administration of ABP-700

NC: Not calculated due to non-qualifying parameters (R.adjusted $\leq$ 0.9000)

Total Dose		126 mg/kg		186 mg/kg		282 mg/kg	
Sex		M	F	M	F	M	F
Number Animals		2	2	3	3	2	2
C_{max}	(ng/mL)	85,850	82,500	133,000	152,670	350,500	313,000
T_{max}	(h)	1.98	1.92	2.03	2.03	2.03	2.11
$AUC_{(last)}$	(h*ng/mL)	207,520	194,570	325,550	390,300	921,460	814,160
Initial $t_{1/2}$	(h)	0.55	0.58	0.57	0.62	0.66	0.61
Terminal $t_{1/2}$	(h)	4.63	4.61	5.59	3.48	5.62	5.09

Table 4-1B. Mean Plasma CPM-Acid Toxicokinetic Parameters Following Administration of ABP-700

Potential of 6 μM GABA-evoked GABA_A Receptor-mediated Currents by ABP-700 and CPM-Acid

We characterized the effects of ABP-700 and CPM-acid over a range of concentrations on currents mediated by $\alpha_1\beta_3\gamma_{2L}$ GABA_A receptors expressed in *Xenopus* oocytes and evoked by 6 μM GABA. We chose to use this low, submaximally-activating GABA concentration for our initial receptor studies because such concentrations commonly allow one to detect the GABA_A receptor potentiating and inhibiting actions of test compounds in a single electrophysiologic trace.¹³ **Figure 4-5A** shows representative electrophysiologic traces obtained during ABP-700 studies and demonstrates that the sedative-hypnotic modified 6 μM GABA-evoked currents in a concentration-dependent manner. Within the clinically-relevant ABP-700 concentration range (less than or equal to 10 μM), ABP-700 potentiated GABA-evoked currents. The magnitude of this potentiation progressively increased with ABP-700 concentration before reaching a plateau. However, at high ABP-700 concentrations (greater than or equal to 100 μM), electrophysiologic traces also exhibited “surge” currents upon ABP-700 and GABA wash-out and the peak current amplitude of the GABA response began to decrease with ABP-700 concentration. Together, these phenomena are indicative of GABA_A receptor inhibition.^{13,26} **Figure 4-5B** shows analogous electrophysiologic traces obtained during studies of CPM-acid. At equivalent concentrations, CPM-acid produced consistently less potentiation of 6 μM GABA-evoked currents than ABP-700, although surge currents were still observable in electrophysiologic traces at the very high CPM-acid concentrations where potentiation occurred. **Figure 4-5C** plots the relationship between the mean peak current amplitude of the GABA response and the concentration of ABP-700 or CPM-acid. For both ABP-700 and CPM-acid, this relationship was bell-shaped with the peak current amplitude first increasing before decreasing with concentration. A fit of the rising portions of the ABP-700 and CPM-acid concentration-response relationships to equation 1 yielded respective EC_{50} s for current potentiation of 2.3 μM (95% CI, 1.6 to 3.3 μM) and 350 μM (95% CI, 120 to 970 μM) and respective maximal peak current amplitudes that were 96% (95% CI, 87 to 105%) and 28% (95% CI, 15 to 41%) of that produced by 1 mM GABA.

Figure 4-5. Potentiation by ABP-700 and CPM-acid of peak electrophysiologic currents evoked by 6 μM γ -aminobutyric acid (GABA) and mediated by $\alpha_1\beta_3\gamma_{2L}$ γ -aminobutyric acid type A (GABA_A) receptors. Representative electrophysiologic traces recorded upon perfusing oocytes expressing GABA_A receptors with 6 μM GABA alone for 20 s followed immediately by 6 μM GABA plus either (A) ABP-700 or (B) CPM-acid at the indicated concentrations for 60 s. Surge currents, which are indicated by the arrows and indicate relief from inhibition, were not measured when defining peak current amplitudes because they occur upon agonist and ABP-700/CPM-acid washout. The gray bars highlight the time periods when ABP-700 or CPM-acid was applied. For each compound, all traces were obtained from the same oocyte. (C) ABP-700 and CPM-acid concentration-response curves for potentiation of peak currents evoked by 6 μM GABA. Each symbol is the mean $\pm$ SD derived from five different oocytes. The curves are nonlinear least square fits of the rising portions of the two datasets to equation 1 (see Data Analysis section). As this concentration of GABA is equivalent to a concentration that produces a current whose peak amplitude is $\sim 5\%$ of that produced by 1 mM GABA, the minimum was constrained to 5%. For ABP-700 and CPM-acid, the respective EC_{50} s for potentiation were 2.3 μM (95% CI, 1.6 to 3.3 μM) and 350 μM (95% CI, 120 to 970 μM), the respective maximal peak current amplitudes were 96% (95% CI, 87 to 105%) and 28% (95% CI, 15 to 41%) of that produced by 1 mM GABA, and the respective slopes were 1.2 (95% CI, 0.8 to 1.6) and 1.6 (95% CI, 0 to 3).

Inhibition of 1 mM GABA-Evoked GABA_A Receptor-mediated Currents by ABP-700 and CPM-Acid

To better quantify the GABA_A receptor inhibitory potencies of ABP-700 and CPM-acid, we defined their actions on GABA_A receptor-mediated currents evoked by 1 mM GABA. Under these high agonist conditions, the con-founding effect of simultaneous potentiation on the

inhibitory action is greatly reduced because most (~85%) GABA_A receptors will already have been opened by the agonist prior to the addition of ABP-700 or CPM-acid.^{27–29} This receptor-saturating GABA concentration is also physiologically relevant because it approximates that achieved at synapses.³⁰ **Figure 4-6A** and **4-6B** show representative electrophysiologic traces obtained during such studies. They show that at an intermediate concentration (30 μ M), ABP-700 modestly potentiated 1 mM GABA-evoked currents whereas CPM-acid had no measurable effect. At high concentrations, both ABP-700 and CPM-acid inhibited currents in a concentration-dependent manner. **Figure 4-6C** plots the relationship between the mean amplitude of the current trace recorded during ABP-700 or CPM-acid application and the ABP-700 or CPM-acid concentration. It shows that ABP-700 modestly potentiated 1 mM GABA-evoked currents when applied at low to intermediate concentrations (3 to 30 μ M) but inhibited them at high concentrations (greater than or equal to 300 μ M). In contrast, the only measurable action of CPM-acid was to inhibit 1 mM GABA-evoked currents. A fit of the two datasets to equation 2 yielded half-maximal inhibitory concentrations for current inhibition of 770 μ M (95% CI, 590 to 1010 μ M) for ABP-700 and 1,450 μ M (95% CI, 1,340 to 1,560 μ M) for CPM-acid.

Figure 4-6. Inhibition by ABP-700 and CPM-acid of electrophysiologic currents evoked by 1 mM γ -aminobutyric acid (GABA) and mediated by $\alpha_1\beta_3\gamma_{2L}$ γ -aminobutyric acid type A (GABA_A) receptors. (A and B) Representative electrophysiologic traces recorded upon perfusing oocytes expressing $\alpha_1\beta_3\gamma_{2L}$ GABA_A receptors with 1 mM GABA alone for 70 s. Ten seconds into this activation period, (A) ABP-700 or (B) CPM-acid was added for 30 s. The gray bars highlight the periods when ABP-700 or CPM-acid was applied. For each compound, all traces were obtained from the same oocyte. As an example of how this inhibition was quantified, the trace exemplifying

the inhibitory action of 2000 μM ABP-700 has several overlays. The dotted red line shows the interpolated line used as the baseline against which the effect of ABP-700 was quantified, the solid red line shows the point of maximum inhibition at the end of ABP-700 application, and the intervening red arrow quantifies the inhibitory effect of 2000 μM ABP-700 on the GABA-activated current. (C) ABP-700 and CPM-acid concentration response curves for inhibition of currents evoked by 1 mM GABA. Each symbol is the mean $\pm$ SD derived from five different oocytes. The curves are nonlinear least square fits of the two datasets to equation 2 (see Data Analysis section) with the maximum and minimum values constrained to 100% and 0%, respectively. For ABP-700 and CPM-acid, the respective half-maximal inhibitory concentrations for inhibition were 770 μM (95% CI, 590 to 1010 μM) and 1,450 μM (95% CI, 1,340 to 1,560 μM) with respective slopes of -1.3 (95% CI, -1.7 to -0.8) and -2.0 (95% CI, -2.4 to -1.6).

Voltage-dependence of GABA_A Receptor Inhibition by ABP-700 and CPM-Acid

To determine whether this receptor inhibition was voltage-dependent, we quantified the magnitude of inhibition produced by ABP-700 and CPM-acid at different membrane potentials in receptors activated with 1 mM GABA. **Figure 4-7A** and **4-7B** respectively plot the relationship between the fractional inhibition produced by ABP-700 and CPM-acid (at their approximate respective half-maximal inhibitory concentrations of 770 μM and 1,500 μM) as a function of membrane potential. These figures demonstrate that GABA_A receptor inhibition by ABP-700 was voltage-independent as the slope of this relationship (-0.056 ; 95% CI, -0.15 to 0.04) was not statistically significantly different from zero ($P = 0.247$). In contrast, the magnitude of receptor inhibition produced by CPM-acid was voltage-dependent, increasing as the membrane potential was varied from -110 mV to $+10$ mV. The slope of this relationship was -0.24 (95% CI, -0.38 to -0.09) which was statistically significantly non-zero ($P = 0.002$).

Figure 4-7. Voltage-dependence of γ -aminobutyric acid type A (GABA_A) receptor inhibition by (A) ABP-700 and (B) CPM-acid. Currents were activated with 1 mM γ -aminobutyric acid (GABA). Each symbol is the mean $\pm$ SD derived from five different oocytes. In each panel, the line is a linear least square fit of the data. For ABP-700, the slope of this relationship was -0.056 (95% CI, -0.15 to 0.04) whereas for CPM-acid it was -0.24 (95% CI, -0.38 to -0.09). For CPM-acid (but not ABP-700), this slope was significantly different from zero ($P = 0.002$). Insets show the impact of the drug on electrophysiologic traces obtained with oocytes voltage-clamped at either -110 mV or $+10$ mV as indicated in the figure. In these insets, the amplitudes of all traces were normalized to the peak amplitude produced by 1 mM GABA alone to facilitate visual comparisons and the gray bars highlight the periods when ABP-700 or CPM-acid was applied.

Impact of CPM-Acid on the GABA Concentration—Response Curve

We sought to further define the mechanism of CPM-acid inhibition of GABA_A receptors by assessing its impact on the GABA concentration-response relationship. We chose to focus on CPM-acid rather than ABP-700 because only the former reached plasma concentrations in the dog toxicology studies that were sufficient to measurably inhibit GABA_A receptor function. In addition, almost all of the seizures in dogs occurred in the minutes to hours after terminating the 2-h high-dose ABP-700 infusions suggesting that the rapidly formed (and relatively slowly eliminated) metabolite, rather than the parent compound itself, was the causative agent. Finally, animal studies indicate that as is typical for sedative-hypnotic agents, ABP-700 is an anti-convulsant rather than a convulsant.³¹ Figure 4-8 plots the rela-

relationship between the GABA concentration and the amplitude of the peak current response in the absence and presence of 1,500 μM CPM-acid. A fit of the two datasets to equation 1 yielded essentially identical EC_{50} s of 44 μM (95% CI, 35 to 54 μM) in the absence of CPM-acid and 45 μM (95% CI, 32 to 62 μM) in the presence of CPM-acid ($P = 0.9148$). However, the maximal peak current amplitudes were statistically significantly different with respective values of 104% (95% CI, 99 to 108%) and 64% (95% CI, 60 to 69%) of that produced by 1 mM GABA ($P < 0.0001$).

Figure 4-8. Impact of 1,500 μM CPM-acid on the γ -aminobutyric acid (GABA) concentration-response relationship. Each symbol is the mean $\pm$ SD derived from five different oocytes. The curves are nonlinear least square fits of the two datasets to equation 1 (see Data Analysis section), with the minimum constrained to 0% (i.e., no current in the absence of GABA). These fits yielded GABA EC_{50} s of 44 μM (95% CI, 35 to 54 μM) in the absence of CPM-acid and 45 μM (95% CI, 32 to 62 μM) in the presence of CPM-acid ($P = 0.9148$). The maximal peak current amplitudes were 104% (95% CI, 99 to 108%) in the absence of CPM-acid and 64% (95% CI, 60 to 69%) in the presence of CPM-acid ($P < 0.0001$) with identical slopes of 1.3 (95% CIs, 1.0 to 1.6 and 0.8 to 1.8, respective).

CPM-Acid Also Inhibits the Glycine Receptor

To further evaluate the ways in which CPM-acid might produce seizures in dogs, we assessed whether it could also inhibit the glycine receptor. This is another important inhibitory neurotransmitter receptor in the CNS that is functionally and structurally similar

to the GABA_A receptor, may colocalize at the same neuronal synapses in the brain and spinal cord as the GABA_A receptor thus providing potential redundancy when GABA_A receptors are inhibited, and whose inhibition can also produce seizures.^{32–35} **Figure 4-9A** shows representative electrophysiologic current traces demonstrating the inhibitory effect of CPM-acid on electrophysiologic currents evoked by 1 mM glycine and mediated by $\alpha_1\beta$ glycine receptors. Similar to its effects on GABA_A receptors, CPM-acid inhibited glycine receptors in a concentration-dependent manner. **Figure 4-9B** plots the relationship between the mean amplitude of the current trace during CPM-acid application and the CPM-acid concentration. A fit of the dataset to equation 2 yielded a half-maximal inhibitory concentration for current inhibition of 1,290 μ M (95% CI, 1,240 to 1,330 μ M).

We then studied the effects of CPM-acid on the glycine concentration–response curve for current activation. **Figure 4-9C** plots the relationship between the glycine concentration and the amplitude of the peak current response in the absence and presence of 1,300 μ M CPM-acid, an approximate half-maximal inhibitory concentration in the glycine receptor. A fit of the two datasets to equation 1 yielded EC₅₀s of 115 μ M (95% CI, 100 to 133 μ M) in the absence of CPM-acid and 260 μ M (95% CI, 200 to 340 μ M) in the presence of CPM-acid ($P < 0.0001$) and respective maximal peak current amplitudes of 98% (95% CI, 94 to 102%) and 75% (95% CI, 69 to 81%) of that produced by 1 mM glycine ($P < 0.0001$).

Figure 4-9. Current inhibition by CPM-acid of electrophysiologic currents evoked by 1 mM glycine and mediated by $\alpha_1\beta$ glycine receptors. (A) Representative electrophysiologic traces recorded upon perfusing oocytes expressing glycine receptors with 1 mM glycine alone for 70 s. Ten seconds into this activation period, CPM-acid was added for 30 s. The gray bars highlight the periods when CPM-acid was

applied. All traces were obtained from the same oocyte. (B) CPM-acid concentration response curves for inhibition of currents evoked by 1 mM glycine. Each symbol is the mean $\pm$ SD derived from five different oocytes. The curves are nonlinear least square fits of the dataset to equation 2 (see Data Analysis section) with the maximum and minimum values constrained to 100% and 0%, respectively. This fit yielded a half-maximal inhibitory concentration of 1,290 μ M (95% CI, 1,240 to 1,330 μ M) with a slope of -5.5 (95% CI, -6.4 to -4.6). (C) Impact of 1,300 μ M CPM-acid on the glycine concentration-response relationship. Each symbol is the mean $\pm$ SD derived from five different oocytes. The curves are nonlinear least square fits of the two datasets to equation 1 (see Data Analysis section) with the minimum constrained to 0%. These fits yielded glycine EC₅₀s of 115 μ M (95% CI, 100 to 133 μ M) in the absence of CPM-acid and 260 μ M (95% CI, 200 to 340 μ M) in the presence of CPM-acid, respective maximal peak current amplitudes of 98% (95% CI, 94 to 102%) and 75% (95% CI, 69 to 81%), and slopes of 1.8 (95% CI, 1.4 to 2.2) and 1.4 (95% CI, 1.0 to 1.9).

GABA_A and Glycine Receptor Function Is Also Inhibited by Etomidate-acid

The structural similarity between CPM-acid and etomidate-acid (**figure 4-1**) suggested to us that the latter may also inhibit receptor function at high concentrations. To test this, we defined the ability of etomidate-acid to inhibit GABA_A and glycine receptor function using agonist concentrations of 1 mM GABA and 1 mM glycine, respectively. **Figure 4-10** plots the relationship between the mean amplitude of the current trace during etomidate-acid application and the etomidate-acid concentration. A fit of the two datasets to equation 2 yielded etomidate-acid half-maximal inhibitory concentrations for current inhibition of 7,050 μ M (95% CI, 6,300 to 7,890 μ M) in the GABA_A receptor and 2,930 μ M (95% CI, 2,710 to 3,170 μ M) in the glycine receptor.

Figure 4-10. Current inhibition by etomidate-acid of electrophysiologic currents evoked by 1 mM γ -aminobutyric acid (GABA) or glycine and mediated by $\alpha_1\beta_3\gamma_{2L}$ γ -aminobutyric acid type A (GABA_A) or $\alpha_1\beta$ glycine receptors, respectively. Each symbol is the mean $\pm$ SD derived from five different oocytes. The curves are nonlinear least square fits of the dataset to equation 2 (see Data Analysis section) with the maximum and minimum values constrained to 100% and 0%, respectively. This fit yielded half-maximal inhibitory concentrations of 7,050 μ M (95% CI, 6,300 to 7,890 μ M) in the GABA_A receptor and 2,930 μ M (95% CI, 2,710 to 3,170 μ M) in the glycine

Discussion

The purpose of the Beagle dog toxicologic studies was to determine whether the involuntary muscle movements that commonly occur during ABP-700 administration are manifestations of seizures. They utilized supra-therapeutic ABP-700 doses and achieved plasma concentrations of ABP-700 and CPM-acid that were on the order of 10× and 100× higher, respectively, than those typically reached during Phase 1 studies in humans.⁴ Involuntary muscle movements were observed in all 14 dogs and seizures in 5 dogs. However, these two phenomena were temporally and electroencephalographically distinct. The large majority of involuntary muscle movements (278 episodes, 87% of all recorded episodes) occurred during ABP-700 infusion and essentially all of these (99.6%) occurred in the absence of electroencephalographic evidence of seizure activity. Because rocuronium was given intermittently during (but not after) ABP-700 infusion to eliminate involuntary muscle movements when they risked interfering with electroencephalographic signal acquisition and interpretation, this incidence of involuntary muscle movements almost certainly underestimates that which would have occurred during ABP-700 infusion had the paralytic not been used. In contrast, most seizures (i.e., 80% of first seizures and 99% of all seizures) occurred in the minutes to hours after the 2-h ABP-700 infusions were complete. Only one seizure occurred during ABP-700 infusion, and this occurred during the final 12.5 min of the 2-h infusion. These results indicate that the involuntary muscle movements caused by ABP-700 administration are not of epileptic origin and strongly suggest that the underlying mechanisms responsible for ABP-700-induced involuntary muscle movements and seizures are different.

Electroencephalographic spikes and sharp waves were also recorded in the electroencephalographs of dogs during ABP-700 infusion. These are phenomena that are also frequently found in the electroencephalographs of nonepileptic patients receiving etomidate, sevoflurane, or high doses of opiates.^{36–40} In epileptic patients, numerous sedative-hypnotic agents that act via a variety of different mechanisms produce (or increase the frequency of) spikes and/or sharp waves, including dexmedetomidine, opiates (alfentanil, remifentanil, and fentanyl), methohexital, clonidine, enflurane, and diphenhydramine.^{41–48} The clinical significance of this pharmacologic action is not completely clear; although, it has been used as a tool to help locate epileptiform foci in the brain during neurosurgical resection.^{42,49}

Involuntary muscle movements are also seen upon administration of other anesthetic induction agents, particularly etomidate where the incidence has been reported to be as high as 70 to 87% in unpremedicated patients.^{50–52} Although the etiology of etomidate-induced involuntary muscle movements is unknown, it has been hypothesized to result from either a disequilibrium of the drug and/or differential sensitivity to the drug among its various CNS effect sites, and is not associated with seizures.^{37,53,54} A recent study by our

group showed that when the structure of etomidate is modified to abolish its potent and efficacious GABA_A receptor positive modulatory activity, the ability of the drug to produce myoclonic muscle movements (in rats) is also eliminated.⁵⁵ This supports an important role for GABA_A receptor positive modulation in mediating this side effect.

What, then, caused the seizures in Beagle dogs during the high dose toxicology studies, and why did they sometimes occur hours after the 2-h ABP-700 infusions were complete? We hypothesized that the seizures were caused by inhibition of GABA_A receptors—perhaps together with glycine receptors—by either ABP-700 itself or its principal metabolite, CPM-acid. This hypothesis was based upon the results of previous studies demonstrating that anesthetics and anesthetic-like compounds commonly inhibit GABA_A receptors at very high (typically lethal) concentrations along with the data generated by the industry sponsor and reported in this article revealing that ABP-700 and, in particular, CPM-acid reached very high concentrations in these dogs by the end of these high-dose infusions.^{11–13,56} It was also informed by numerous previous studies showing that inhibition of GABAergic neurotransmission (either chemically or resulting from genetic mutations) causes seizures.^{57,58}

The results of the current studies support this hypothesis for CPM-acid (but not ABP-700) as they reveal that within the plasma concentration range achieved in these dogs, the metabolite inhibits GABA_A receptors. Studies utilizing a range of GABA concentrations showed that CPM-acid reduces the maximal peak current response obtained at high agonist concentrations without affecting the agonist EC₅₀. Such insurmountable antagonism implies that CPM-acid inhibits GABA_A receptors in a noncompetitive manner, a molecular mechanism it shares with the convulsant picrotoxin.^{59,60} The magnitude of this inhibition was voltage-dependent, suggesting direct blockade of the ion channel by the negatively charged metabolite.

In addition, our studies showed that at high concentrations, CPM-acid also inhibits glycine receptors. Similar to its effect on GABA_A receptors, CPM-acid reduced the maximal peak current response obtained at high agonist concentrations. However, it also increased the agonist EC₅₀ of the receptor, suggesting that there may be multiple CPM-acid inhibitory mechanisms (i.e., competitive and noncompetitive) with this receptor. Such multiple mechanisms may explain why inhibition of glycine receptors by CPM-acid is characterized by such a steep concentration-response relationship (slope: −5.5).

We also found that at high concentrations, the principal metabolite of etomidate (i.e., etomidate-acid) similarly inhibits both GABA_A and glycine receptors. However, etomidate-acid is formed *in vivo* at a much slower rate than CPM-acid. Consequently, when their respective parent drugs are administered to dogs at rates that achieve similar levels of sedation/hypnosis, etomidate-acid reaches plasma concentrations that are only one-twentieth that of CPM-acid.⁶ Such concentrations would be far below those required to inhibit either GABA_A or glycine receptors and cause seizures.

In spite of the frequency with which high-dose ABP-700 infusions produced seizures in the 14 Beagle dogs (36%), no seizures or postictal behaviors were observed and reported in Phase 1 clinical studies involving 90 humans who received ABP-700 as boluses or continuous infusions lasting as long as 30 min.^{4,5} In addition, an independent analysis of raw frontal electroencephalographic waveforms from bispectral index monitoring recorded from 52 of these volunteers and a subsequent review of such waveforms from an additional 29 volunteers who received fentanyl premedication prior to ABP-700 administration found no evidence of seizures during or after ABP-700 administration even during episodes of severe involuntary muscle movements (Report to The Medicines Company and Addendum, Brad J. Kolls, M.D., M.M.Ci. and David S. Warner, M.D., September 15, 2015). In these Phase 1 clinical studies, typical peak plasma CPM-acid concentrations reached 1,500 ng/ml (5 μ M) and the highest concentration measured at any time in any individual was only ~3,000 ng/ml.⁴ (Michel Struys, M.D., Ph.D., F.R.C.A., the Department of Anesthesiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands; written communication, April 3, 2018). Such concentrations are just 1% of the peak values achieved in the Beagle dog studies and far below those necessary to inhibit either GABA_A or glycine receptors and produce seizures via these receptor mechanisms ([figure 4-11](#)).

Figure 4-11. Peak plasma concentration ranges of CPM-acid achieved in human clinical studies (blue bar) and Beagle dog toxicology studies (red bar) superimposed on CPM-acid concentration response curves for inhibition of currents evoked by 1 mM γ -aminobutyric acid (GABA) or glycine and mediated by γ -aminobutyric acid type A (GABA_A) receptors or glycine receptors, respectively. Peak CPM-acid plasma concentrations were 100 $\times$ higher in the Beagle dog studies than in the human clinical trials.

An additional potential contributing factor is that Beagle dogs may be relatively susceptible to seizures. A study utilizing a colony of more than 1,200 Beagle dogs determined that 5.7% of all dogs experienced clinically observable spontaneous convulsive seizures resembling epilepsy.⁶¹ This is approximately an order of magnitude higher than the prevalence of epilepsy in humans.^{62,63} In that Beagle study, an analysis of the pedigree data along with the seizure rates showed a nonrandom incidence that led the authors to conclude that there was a genetic basis for this seizure-susceptibility. Such genetic susceptibility differences may explain why some of our Beagle dogs seized while others did not. Finally (and perhaps most relevantly), Beagle dogs may be relatively sensitive to GABA_A receptor-inhibiting compounds as they convulse with lower doses of pentylenetetrazol than Sprague–Dawley rats or cynomolgus monkeys, two other preclinical models commonly used during drug development.²¹ Such high sensitivity is mirrored in *in vitro* studies as hippocampal slices from Beagle dogs are more sensitive to pentylenetetrazol-induced changes in population spike area and number than slices from rats, minipigs, and cynomolgus monkeys.⁶⁴

The U.S. Food and Drug Administration has issued non-binding guidance for establishing the safety of drug metabolites that provides useful context for the present studies.⁶⁵ It advocates for the safety testing of drug metabolites prior to initiating large scale human trials if such metabolites reach plasma concentrations that are greater than 10% of total parent drug-related exposure at steady-state. Such concentrations are routinely achieved when administering soft drugs because they are specifically designed to be metabolized rapidly.^{66,67} Safety testing may include the use of animal models, provided that the chosen model produces adequate metabolite exposure levels. Such levels are defined as being at least as high as those encountered in humans. In the current dog studies, however, plasma metabolite concentrations reached levels that are 100× greater than those achieved in human studies, so high that they inhibited both GABA_A and glycine receptors. Thus, it seems highly unlikely that inhibition of these receptors with resultant seizure production is relevant for ABP-700 administration to humans, particularly when such administration is brief.

A limitation to this study is that we cannot directly relate the concentrations of CPM-acid that we found inhibit GABA_A and glycine receptors with those present in the CNS of the Beagle dogs because the latter were not measured. We used peak venous plasma concentrations as a surrogate, but peak CNS concentrations may have been higher and occurred later in time. In particular, if ABP-700 was metabolized within the CNS of these dogs, then CPM-acid—which is negatively charged at physiologic pH—could have become trapped within their CNS by the blood brain barrier. With prolonged ABP-700 infusions, this could have led to peak CPM-acid concentrations in the CNS that were even higher than those measured in peripheral venous blood. This is a phenomenon that we previously observed in rats that received 30-min infusions of methoxycarbonyl etomidate, the lead compound for the development of ABP-700 and other soft etomidate analogs.⁷ In that study, peak

metabolite concentrations measured in the cerebrospinal fluid were twice those measured in peripheral blood. Such trapping would cause CPM-acid to accumulate within the CNS during ABP-700 infusion, followed by its slow release from the CNS into the peripheral blood after the ABP-700 infusion was complete. Such a release might explain the slow component of the biphasic decay evident in the CPM-acid toxicokinetic plot that has a half-life of ~5 h (**figure 4-4B**). The resulting disequilibrium between CPM-acid concentrations in the CNS and plasma could explain why seizures sometimes occurred in dogs hours after plasma CPM-acid concentrations had reached their maximum values. A similar disequilibrium has been described for tranexamic acid, a carboxylic acid antifibrinolytic that also inhibits GABA_A and glycine receptors, has poor blood–brain barrier permeability, and produces seizures approximately 5 to 10 h after administration has ceased and blood concentrations have peaked.^{33,68,69} Such a large disequilibrium cannot occur with ABP-700 because it is uncharged, highly hydrophobic, and crosses the blood–brain barrier readily to allow rapid anesthetic induction and emergence.

In summary, high doses of ABP-700 produced both involuntary muscle movements and seizures in Beagle dogs. However, these two phenomena were temporally and electroencephalographically distinct. These results indicate that ABP-700-induced involuntary muscle movements are not of epileptic origin and strongly suggest that the underlying mechanisms responsible for ABP-700-induced involuntary muscle movements and seizures are different. The principal metabolite of ABP-700 (i.e., CPM-acid) reached plasma concentrations during these toxicology studies that are sufficient to inhibit the function of both GABA_A and glycine receptors. Such inhibition provides a plausible mechanism for the seizures that occurred in this seizure-prone animal model. It occurs at CPM-acid concentrations that are approximately 100× higher than those achieved in clinical studies of ABP-700 and during which no seizures were observed, strongly suggesting that these actions are irrelevant to the administration of therapeutic ABP-700 doses to humans.

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CHAPTER 5

Modeling the Effect of Excitation on Depth of Anesthesia Monitoring in γ -Aminobutyric Acid Type A Receptor Agonist ABP-700

Modified from *Anesthesiology*. 2021 Jan 1;134(1):35-51.

Beatrijs I. Valk*, Douglas J. Eleveld*, Peter Meyer, Sascha Meier, Izaak den Daas, Kai van Amsterdam, Jason A. Campagna, Steven P. Sweeney, Anthony R. Absalom, Michel M. R. F. Struys

**both authors contributed equally to this article and should both be considered as first authors.*

Abstract

Background: γ -Aminobutyric acid type A (GABA_A) receptor agonists are known to cause involuntary muscle movements. The mechanism of these movements is not known, and its relationship to depth of anesthesia monitoring is unclear. We have explored the effect of involuntary muscle movement on the pharmacokinetic-pharmacodynamic model for the GABA_A receptor agonist ABP-700 and its effects on the Bispectral Index (BIS) as well as the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scores.

Methods: Observations from 350 individuals (220 men, 130 women) were analyzed, comprising 6,312 ABP-700 concentrations, 5,658 ABP-700 metabolite (CPM-acid) concentrations, 25,745 filtered BIS values, and 6,249 MOAA/S scores, and a recirculatory model developed. Various subject covariates and pretreatment with an opioid or a benzodiazepine were explored as covariates. Relationships between BIS and MOAA/S models and involuntary muscle movements were examined.

Results: The final model shows that the pharmacokinetics of ABP-700 are characterized by small compartmental volumes and rapid clearance. The BIS model incorporates an effect-site for BIS suppression and a secondary excitatory/disinhibitory effect-site associated with a risk of involuntary muscle movements. The secondary effect-site has a threshold that decreases with age. The MOAA/S model did not show excitatory effects.

Conclusions: The GABA_A receptor agonist ABP-700 shows the expected suppressive effects for BIS and MOAA/S, but also disinhibitory effects for BIS associated with involuntary muscle movements and reduced by pretreatment. Our model provides information about involuntary muscle movements that may be useful to improve depth of anesthesia monitoring for GABA_A receptor agonists.

Involuntary muscle movements are frequently observed after administration of γ -amino-butyric acid type A (GABA_A) receptor agonists such as the widely used sedative and general anesthetic agents etomidate¹ and propofol^{2,3} but also novel anesthetic agents such as AZD3043⁴ and ABP-700.⁵ The presentation and nature of the involuntary muscle movements are diverse, ranging from isolated twitching of a finger to whole-body movements that may mimic tonic-clonic seizure activity, and given the unknown origin of these movements, they can be alarming for clinicians. Furthermore, subjects receiving ABP-700⁵ and AZD3043⁶ who exhibited involuntary muscle movements sometimes showed elevated Bispectral Index (BIS) values, although they were deeply anesthetized as judged clinically using the Modified Observer's Assessment of Alertness/ Sedation (MOAA/S) scale. This discrepancy between processed electroencephalogram and clinical monitoring signs and scores remains unexplained for GABA_A receptor agonists and complicates the assessment of the cerebral drug effect.

Cyclopropyl-methoxycarbonyl metomidate (CPMM or ABP-700) is a GABA_A receptor agonist and a second-generation analog of etomidate. It is under development for use as an induction agent for general anesthesia.⁷ First-in-human studies showed that ABP-700 has a rapid onset of action and recovery from sedation and deep general anesthesia.⁵ This clinical profile is attributed to an ester bond that undergoes rapid hydrolysis by nonspecific tissue esterases and generates a metabolite, CPM-acid, that is approximately 1,000-fold less potent than ABP-700 as a GABA_A receptor agonist. A preliminary three-compartmental pharmacokinetic-pharmacodynamic model was previously published as a part of the article on the first-in-human, bolus only study on ABP-700. Of note was the steep relationship between effect-site concentration of ABP-700 and the cerebral drug effect as measured by both BIS and MOAA/S.⁵

Notable side effects of ABP-700 include the occurrence of excitatory phenomena such as involuntary muscle movements, tachycardia, and tachypnea.^{5,8} Although recent preclinical studies showed that CPM-acid can inhibit the GABA_A receptor, a potential mechanism for seizure induction,⁹ this effect was only seen at concentrations two orders of magnitude greater than those achieved clinically.

To better understand the relationship between clinical anesthesia pharmacodynamic measurements and involuntary muscle movements for GABA_A receptor agonists, and to better characterize the pharmacokinetics of ABP-700, we developed a recirculatory pharmacokinetic-pharmacodynamic model of ABP-700 as a prototype GABA_A receptor agonist based on data from five phase 1, single-center studies. We developed a recirculatory model because it allows arterial and venous concentrations of both ABP-700 and CPM-acid to inform pharmacokinetic model development. Addition of a pharmacodynamic component to model the relationships between plasma ABP-700 concentrations and BIS and MOAA/S

observations allows for inferences of the physiologic mechanisms underlying these measures of drug effect.

Materials and Methods

In total, data from 350 individuals (220 men, 130 women) were analyzed. The data of three subjects were removed due to dosing errors, such as an erroneous subcutaneous infusion, or a faulty cannula. Weight range was from 51 to 108 kg, age range was from 18 to 55 yr, and height range was from 151 to 200 cm. For ABP-700, 6,312 observations (3,310 arterial, 3,002 venous) were analyzed. For the development of the CPM-acid, BIS, and MOAA/S models, we only used data from studies in which individualized arterial concentration ABP-700 profiles were available. Hence, CPM-acid pharmacokinetic model development and pharmacodynamic model development were performed using data from 266 individuals. For the CPM-acid pharmacokinetic model, 5,658 observations (3,199 arterial, 2,459 venous) were analyzed. For BIS, 25,745 (median filtered) observations were analyzed, and for MOAA/S, 6,249 observations were analyzed.

Study Execution

This analysis involves data from five studies of the safety and tolerability of ABP-700 (see *supplemental material 5-1*): ANVN-01⁵ (bolus-only dose escalation study), ANVN-02⁸ (infusion dose escalation study), ANVN-03 (bolus-only dose optimization study, venous samples only), ANVN-04 (infusion dose optimization study), and ANVN-05 (induction dose study). In various cohorts, pretreatment in the form of an opioid agent or benzodiazepine was administered before administration of ABP-700. The pretreatments fentanyl (ranging from 0.5 to 2 $\mu\text{g}\cdot\text{kg}^{-1}$), sufentanil (0.2 $\mu\text{g}\cdot\text{kg}^{-1}$), and midazolam (ranging from 15 to 30 $\mu\text{g}\cdot\text{kg}^{-1}$) were administered as a bolus 5 min before administration of ABP-700. Remifentanyl (ranging from 0.05 to 0.25 $\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) was administered as a continuous infusion, starting 5 min before the administration of ABP-700 and ending when the administration of ABP-700 was halted.

These studies were performed at the QPS Early Phase I unit, Groningen, The Netherlands, in cooperation with the Department of Anesthesiology at the University Medical Center Groningen, University of Groningen, Groningen, The Netherlands, in accordance with the Declaration of Helsinki, in compliance with Good Clinical Practice and applicable regulatory requirements. They were approved by the ethics committee (Medical Ethics Review Committee, Bebo Foundation, Assen, The Netherlands, NL48312.056.14) and were registered at the Dutch Trial Register (NTR4545, NTR4735, NTR5173, NTR5231, and NTR5442).

Healthy subjects aged between 18 and 55 yr were eligible for these studies. Inclusion criteria were an American Society of Anesthesiologists (Schaumburg, Illinois) physical status score of I and no risk of a difficult airway (modified Mallampati score I or II). Exclusion criteria were a body mass index less than 17.5 or above 30.0, significant medical history, chronic use of medication (oral contraceptives excluded), alcohol, drugs, or tobacco, and having previously received ABP-700. For the first two studies, women had to be of nonchild-bearing potential (i.e., to have been sterilized at least 6 months before the first dose, or to have been postmenopausal with amenorrhea for at least 1 yr before the first dose with a measured follicle-stimulating hormone level less than 30 U/l). During the study execution, an attending anesthesiologist was present throughout the conduct of the study until the full recovery of the subject.

Safety and tolerability of ABP-700 were assessed as described by Struys et al.⁵ and Valk et al.,⁸ by assessment of adverse events, safety laboratory tests, intermittent 12-lead electrocardiograms, temperature, and infusion site reaction monitoring. Continuous monitoring of three-lead electrocardiogram, heart rate, pulse oximetry, noninvasive blood pressure (every minute), continuous arterial blood pressure, respiration rate and pattern, and end-tidal carbon dioxide were monitored in all subjects using a Philips MP50 monitor (Philips, The Netherlands). As the investigators had not fully anticipated the extent of the involuntary muscle movements, no standardized scoring system was implemented a priori in the study protocol. Instead, the responsible clinicians recorded their observations on the character and extent of the involuntary muscle movements electronically. Involuntary muscle movement severity was evaluated by post hoc analysis of the recorded observations as described by Struys et al.⁵ and Valk et al.⁸ Involuntary muscle movements were defined as “extensive” when they involved the whole body or a considerable part of it. Involuntary muscle movements were defined as “few movements” when they were observed in a few body parts, such as in both arms or both legs.

All BIS and MOAA/S observations and blood sampling times, as well as data on vital signs and manually written comments, were stored electronically using a dedicated and validated electronic data capturing device (RUGLOOP II, DEMED, Belgium).

Blood Sampling

For blood sampling times, see *supplemental material 5-2*. Blood samples were collected in pre-chilled EDTA vacutainers. Samples were stored on wet ice for no longer than 30 min until centrifuged at 5°C for 7 min at 1,800g to separate the plasma. Plasma was transferred using disposable pipettes into cryovials and placed immediately on dry ice until transferred to a -80°C freezer within a total allotted time of 60 min. The analysis process was identical to the methods described in a previous publication on ABP-700.⁵

In brief, high-performance liquid chromatography was used to measure plasma concentrations of ABP-700 with tandem mass spectrometric detection using a SCIEX API4000 instrument, including a Turbo Ion Spray interface in positive mode at QPS Netherlands B.V. (The Netherlands). Deuterium-labeled D5-ABP-700 and D5-CPM-acid were used as internal standards. The internal standard solution and acetonitrile were added to 0.05 ml of plasma. The supernatant was then mixed and centrifuged, and partly transferred onto the Ostro Protein Precipitation & Phospholipid Removal Plate, 25 mg (Waters Chromatography B.V., The Netherlands). The supernatant was eluted using positive pressure and consecutively collected in a 96-well plate. A dilution step with Milli-Q ultrapure water (Merck Millipore, The Netherlands) and acetonitrile was applied before analysis. On a C18 column (ACE 3 μ m column, 50 $\times$ 3.0 mm, Advanced Chromatography Technologies, Aberdeen, United Kingdom), mounted in line with a 4 $\times$ 3.0 mm C18 guard column (Advanced Chromatography Technologies, United Kingdom) on an Agilent 1100/1200 LC system (Agilent Technologies, USA), liquid chromatography was performed, with the column temperature maintained at 50°C. The mobile phase A was 0.1% formic acid in water, and the mobile phase B was 0.1% formic acid in 50% aceto-nitrile. A gradient of 30 to 95% B was applied over a period of 1 min, at a flow rate of 1 ml·min⁻¹ for sample elution. The approximate elution time for ABP-700 was 2.1 min. The nominal mass transitions monitored had mass-to-charge-ratios of 315.2 to 211.1. The method was validated over a concentration range of 5 to 1,250 ng·ml⁻¹ for ABP-700 and 25.0 to 6,250 ng/ml for CPM-acid. Both within a single run of 6 aliquots (within-run for repeatability) and between different runs (between-run for reproducibility) distributed over at least 2 days, precision and accuracy were demonstrated for the validation samples. Validation samples were prepared in blank human NaF/NaEDTA plasma by spiking known concentrations of ABP-700 and CPM-acid. The acceptance criterion for precision, the percentage of the coefficient of variation (CV%) not to exceed 20.0% at the lower limit of quantification or 15% at all other levels, was met for all samples. The acceptance criterion for accuracy of the percentage of the relative error (%RE). not to exceed 20.0% at the lower limit of quantification or 15% at all other levels, was also met for all samples. The lower limits of quantification for ABP-700 and CPM-acid were determined at 5 ng·ml⁻¹ and 25 ng·ml⁻¹, respectively.

Model Development

For pharmacokinetic and pharmacodynamic model development, NONMEM version 7.4 (Icon Development Solutions, USA) was used for model estimation and simulations, and R version 2.14.1¹⁰ (available at <https://www.R-project.org/>, accessed January 2020) was used for performance estimation and generation of diagnostic plots. Model parameters were calculated relative to a reference individual,¹¹ a 70-kg, 35-yr-old, 170-cm man. Interindividual variability in model parameters was assumed to be log-normally distributed across the population. The ABP-700 pharmacokinetic model was developed first, and afterward the pharmacodynamic and CPM-acid models were developed using the individual predictions from the final ABP-700 pharmacokinetic model. This is known as the “sequential method.”^{12,13}

Initial models assumed the theoretical allometric scaling exponents,¹⁴ volumes scaling linearly with weight, clearances to the three-fourths power, and rate constants to negative one-fourth power. Compartmental allometry^{15–17} was applied to peripheral compartments, which includes interindividual variability of peripheral compartment volume as a contributor to variability in the associated compartmental clearance. This arises when a peripheral compartmental clearance is considered a property of the associated volume as well as the body as a whole. Observations lower than the reported limit-of-quantification of an assay were not used. Individual variability (η) values obtained from NONMEM estimation were examined for potential covariate relationships, which were subsequently tested in the model. Inclusion of a parameter in the model required an improvement of at least 9.21 in the corrected Akaike Information Criterion (AIC). For estimates of logarithmic interindividual variability, we report the estimated variance and the coefficient of variation using the equation

$$CV(\%) = \sqrt{e^{\omega^2} - 1} \cdot 100\%$$

where ω^2 is the estimated parameter population variance. For the sake of brevity, we do not describe all models tested during model development, and report only those that led to the final models.

The pharmacokinetic model development focused on a mammillary recirculatory structural model for ABP-700 and CPM-acid, similar to that used for another drug, AZD3043.⁶ We used a recirculatory pharmacokinetic model structure because it is a classical pharmacometrical approach,¹⁸ which allows simultaneous fitting of arterial and venous concentrations with a recognizable mechanistic basis. From dosing, drug initially appears in a depot compartment and is then transported to arterial and then to venous compartments via peripheral compartments and a nondistributive pathway. Drug transport through the central circulation was a flow representing the rate of venous-arterial circulation of drug mass in the model. We refer to this as the apparent cardiac output because of its structural similarity to cardiac output in circulation models. Apparent cardiac output is estimated from the data and does not represent blood pumped by the heart over time as we did not have measurements of cardiac output.

A similar arterial-venous-peripheral compartmental model structure was used for the CPM-acid. All of the drug transport determined by ABP-700 elimination clearance was assumed to result in CPM-acid production. Residual observation error (ϵ) was assumed to be proportional to the predicted concentrations, separately for ABP-700 and CPM-acid.

BIS Model Development

Initially, BIS values were modeled as a sigmoidal E_{max} function of effect-site compartment concentrations (C_e), which in turn were driven by concentration in the arterial compartment $V1$ (C_{V1}) and a first-order rate constant ($k_{e0,BIS}$). The equation of the initial pharmacodynamic model was

$$\begin{aligned} k_{e0,BIS} &= k_{e0,BIS,ref} \cdot \exp(\eta_1) \\ EC_{50,BIS} &= EC_{50,BIS,ref} \cdot \exp(\eta_2) \\ \frac{\delta C_e}{\delta t} &= k_{e0,BIS} \cdot (C_{V1} - C_e) \\ BIS_{observed} &= (BIS_{baseline} + \eta_3) \cdot \left(1 - EC^\gamma / (EC^\gamma + EC_{50}^\gamma)\right) + \varepsilon \end{aligned}$$

where $BIS_{baseline}$ is the baseline pharmacodynamic measure when no drug is present, $EC_{50,BIS}$ is the effect-site concentration associated with 50% of the maximum drug effect, γ is the steepness of the concentration versus response relation, and ε represents additive residual error. During model development, we also considered a second sigmoidal function causing increased BIS values. The equations are detailed in the Results section. To reduce estimation times, we median-filtered BIS observations every 30 s with a filter width of 30 s. Due to the large variability in pretreatment agents and their dosing regimens, we considered the absence or presence of pretreatment as a binary variable when assessing the influence of pretreatment on predicted BIS. As such, we did not analyze their effect according to individual pretreatment drug and/or dosing regimen. This binary approach has been successfully applied previously for other drugs such as propofol.¹⁹

5

Modified Observer's Assessment of Alertness/Sedation Model Development

MOAA/S observations were treated as ordered categorical responses and modeled using a proportional-odds method. Based on the predicted effect-site concentration, the model estimates the cumulative probabilities of MOAA/S scores. Let S denote an observed score, the logits l_x , of the probabilities that $S = 0, S \leq 1, S \leq 2, S \leq 3, S \leq 4$, are

$$\begin{aligned} l_{S=0} &= b_0 + DEFF \cdot C_e \\ l_{S \leq 1} &= b_0 + b_1 + DEFF \cdot C_e \\ l_{S \leq 2} &= b_0 + b_1 + b_2 + DEFF \cdot C_e \\ l_{S \leq 3} &= b_0 + b_1 + b_2 + b_3 + DEFF \cdot C_e \\ l_{S \leq 4} &= b_0 + b_1 + b_2 + b_3 + b_4 + DEFF \cdot C_e \end{aligned}$$

where b_0 is a fixed effect parameter representing the logit of the probability for score 0, and b_1 through b_4 represent the differences in logits between the scores. The parameter for drug effect ($DEFF$) is a fixed effect for the model, and EC represents the effect-site concentration

of ABP-700, calculated using a first-order rate constant ($k_{e0,MOAA/S}$) in a similar manner as done for BIS. The corresponding probabilities are given by

$$PC_x = \frac{e^{l_x}}{(1 + e^{l_x})}$$

The actual probabilities, p_x , of observing a particular score are $P_{S=0} = PC_{S=0}$, $P_{S=1} = PC_{S \leq 1} - PC_{S=0}$, $P_{S=2} = PC_{S \leq 2} - PC_{S \leq 1}$, $P_{S=3} = PC_{S \leq 3} - PC_{S \leq 2}$, $P_{S=4} = PC_{S \leq 4} - PC_{S \leq 3}$, $P_{S=5} = 1 - PC_{S \leq 4}$.

Simulations

To illustrate the characteristics of the final pharmacokinetic model, as well as the BIS and MOAA/S models, we performed simulations of a short infusion for illustrative individuals to demonstrate expected drug concentrations and the influence of pretreatment, age, weight, and infusion rate on predicted BIS and MOAA/S.

Results

Pharmacokinetic Model Development

First, the ABP-700 pharmacokinetic model was developed, and incorporating two peripheral compartments performed better than one ($\Delta AIC = -464.08$). Decreasing clearance with age improved the model ($\Delta AIC = -12.46$). Removing compartmental allometry from the model does not change the number of estimated model parameters and caused poorer model fit ($\Delta AIC = -31.90$), so compartmental allometry was retained in the model. To obtain sufficient estimation stability, interindividual variability was fixed to 0 for the depot compartment volume, peripheral compartment clearances, and apparent cardiac output. Doing so obtains the expected smooth parabolic shape of the likelihood profiles, indicating reliable estimates for parameters and their confidence limits. The equations for the final ABP-700 pharmacokinetic model are as follows:

$$\begin{aligned} CL_{CPM-A} &= CL_{CPM-A,ref} \cdot Fsize^{0.75} \cdot \exp(\theta_1 \cdot (AGE - 35)) \cdot \exp(\eta_1) \\ V_{CPM-A,arterial} &= V_{CPM-A,arterial,ref} \cdot Fsize \cdot \exp(\eta_2) \\ V_{CPM-A,venous} &= V_{CPM-A,venous,ref} \cdot Fsize \cdot \exp(\eta_3) \\ V_{CPM-A,P} &= V_{CPM-A,P,ref} \cdot Fsize \cdot \exp(\eta_4) \\ Q_{CPM-A,ND} &= Q_{CPM-A,ND,ref} \cdot Fsize^{0.75} \cdot \exp(\eta_5) \\ Q_{CPM-A,P} &= Q_{CPM-A,P1,ref} \cdot \left(V_{CPM-A,P} / V_{CPM-A,P,ref} \right)^{0.75} \\ K_{CPM-A,depot} &= K_{CPM-A,depot,ref} \cdot Fsize^{-0.25} \end{aligned}$$

Symbols with the subscript *ref* and θ represent estimated parameters and η estimated variances, WGT is weight in kg, and AGE is age in years.

Subsequently, the CPM-acid pharmacokinetic model was developed. All of the ABP-700 eliminated was assumed to produce CPM-acid, appearing in the CPM-acid depot compartment. To reduce the number of parameters, the apparent cardiac output from the ABP-700 pharmacokinetic model was transferred to the CPM-acid model, and interindividual variability of the depot compartment time constant and intercompartmental clearance were fixed to zero as in the ABP-700 pharmacokinetic model. Using a single peripheral compartment did not show bias in the diagnostic plots, and a second peripheral compartment was not tested. Decreasing apparent clearance of CPM-acid with age improved model fit ($\Delta \text{AIC} = -9.53$). The equations for the final CPM-acid pharmacokinetic model are as follows:

$$\begin{aligned}
 Fsize &= WGT/70 \\
 CL &= CL_{ref} \cdot Fsize^{0.75} \cdot \exp(\theta_1 \cdot (AGE - 35)) \cdot \exp(\eta_1) \\
 V_{arterial} &= V_{arterial,ref} \cdot Fsize \cdot \exp(\eta_2) \\
 V_{venous} &= V_{venous,ref} \cdot Fsize \cdot \exp(\eta_3) \\
 V_{p1} &= V_{p1,ref} \cdot Fsize \cdot \exp(\eta_4) \\
 V_{p2} &= V_{p2,ref} \cdot Fsize \cdot \exp(\eta_5) \\
 Q_{ND} &= Q_{ND,ref} \cdot Fsize^{0.75} \cdot \exp(\eta_6) \\
 Q_{CO} &= Q_{CO,ref} \cdot Fsize^{0.75} \\
 Q_{p1} &= Q_{p1,ref} \cdot \left(V_{p1}/V_{p1,ref} \right)^{0.75} \\
 Q_{p2} &= Q_{p2,ref} \cdot \left(V_{p2}/V_{p2,ref} \right)^{0.75} \\
 V_{depot} &= V_{depot,ref} \cdot Fsize
 \end{aligned}$$

The estimated parameters of the final ABP-700 and CPM-acid pharmacokinetic models are shown in [table 5-1](#). NONMEM codes for the model differential equations are available in the online supplemental materials.*

* See: <https://doi.org/10.1097/ALN.0000000000003590>

Parameter	Estimated	Confidence limits		Variability		
		Lower 1%	Upper 99%		Variance (σ²)	Coefficient of Variation(%)
ABP-700						
CL_{ref} (l·min ⁻¹)	1.95	1.86	2.05	η1	0.0443	21.3
$V_{arterial,ref}$ (l)	4.54	3.70	5.28	η2	0.652	95.9
$V_{venous,ref}$ (l)	7.01	5.64	8.25	η3	0.222	49.9
$V_{P1,ref}$ (l)	3.43	3.02	3.94	η4	0.115	34.9
$V_{P2,ref}$ (l)	6.10	5.06	7.43	η5	0.563	86.9
$Q_{ND,ref}$ (l·min ⁻¹)	1.12	0.933	1.27	η6	0.0824	29.3
$Q_{CO,ref}$ (l·min ⁻¹)	2.41	2.25	2.55			
$Q_{P1,ref}$ (l·min ⁻¹)	0.372	0.259	0.495			
$Q_{P2,ref}$ (l·min ⁻¹)	0.104	0.0775	0.130			
$V_{depot,ref}$ (l)	0.463	0.342	0.568			
Θ ₁	-0.00568	-0.00975	-0.00187			
Residual error				ε	0.0627	25.4
CPM-acid						
$CL_{CPM-A,ref}$ (l·min ⁻¹)	0.769	0.732	0.808	η1	0.0466	21.8
$V_{CPM-A,arterial,ref}$ (l)	3.47	3.00	4.00	η2	0.423	72.6
$V_{CPM-A,venous,ref}$ (l)	2.50	2.18	2.86	η3	0.493	79.9
$V_{CPM-A,VP,ref}$ (l)	17.0	16.0	18.1	η4	0.115	34.9
$Q_{CPM-A,ND,ref}$ (l·min ⁻¹)	2.23	2.19	2.28	η6	0.00622	7.9
$Q_{CPM-A,P1,ref}$ (l·min ⁻¹)	0.525	0.489	0.562			
$K_{CPM-A,depot,ref}$ (min ⁻¹)	0.0760	0.0720	0.0803			
Θ ₁	-0.00575	-0.00987	-0.00163			
Residual error				ε	0.0209	14.5

Table 5-1. Model parameters for the final PK model for ABP-700 and CPM-acid
The role of each parameter in the model structure is shown in figure 5-5.
(CL = clearance; V= volume of distribution; Q_{ND} = non-distributive flow; Q_{CO} = apparent cardiac output; Q_p = peripheral flow; K = rate constant)

Diagnostic plots for the ABP-700 model are shown in **figure 5-1** (arterial) and **figure 5-2** (venous), and for the CPM-acid are shown in **figure 5-3** (arterial) and **figure 5-4** (venous). The structure of the final ABP-700 and CPM-acid pharmacokinetic models is shown in **figure 5-5**. The best, typical, and worst fit of arterial and venous ABP-700 predictions to the data are included in *supplemental material 5-3*. Similarly, for CPM-acid predictions, these are included in *supplemental material 5-4*. An expanded view of the initial arterial and venous concentration predictions for bolus dosing (ANVN-01) and infusion (ANVN-02) are shown in *supplemental material 5-5*. The final model for ABP-700 shows some overprediction for early arterial and venous samples and underprediction for late venous observations. Misfit in the early samples shows limitations of the model for front-end kinetics. Misfit in the late venous samples suggests limitations of the model for how the peripheral compartments interact with the venous compartments.

Figure 5-1. Diagnostic plots for ABP-700 arterial samples. Red line: Smoother. (A) Population-observed/-predicted arterial plasma ABP-700 concentrations versus time. (B) Post hoc individual-observed/-predicted arterial plasma ABP-700 concentrations versus time. (C) Population-observed versus population-predicted arterial plasma ABP-700 concentrations. (D) Post hoc individual-observed versus individual-predicted arterial plasma ABP-700 concentrations.

Figure 5-2. Diagnostic plots for ABP-700 venous samples. *Red line:* Smoother. (A) Population-observed/-predicted venous plasma ABP-700 concentrations *versus* time. (B) *Post hoc* individual-observed/-predicted venous plasma ABP-700 concentrations *versus* time. (C) Population-observed *versus* population-predicted venous plasma ABP-700 concentrations. (D) *Post hoc* individual-observed *versus* individual-predicted venous plasma ABP-700 concentrations.

Figure 5-3. Diagnostic plots for CPM-acid arterial samples. *Red line:* Smoother. (A) Population-observed/-predicted arterial plasma CPM-acid concentrations *versus* time. (B) *Post hoc* individual-observed/-predicted arterial plasma CPM-acid concentrations *versus* time. (C) Population-observed *versus* population-predicted arterial plasma CPM-acid concentrations. (D) *Post hoc* individual-observed *versus* individual-predicted arterial plasma CPM-acid concentrations.

Figure 5-4. Diagnostic plots for CPM-acid venous samples. *Red line:* Smoother. (A) Population-observed/-predicted venous plasma CPM-acid concentrations *versus* time. (B) *Post hoc* individual-observed/-predicted venous plasma CPM-acid concentrations *versus* time. (C) Population-observed *versus* population-predicted venous plasma CPM-acid concentrations. (D) *Post hoc* individual-observed *versus* individual-predicted venous plasma CPM-acid concentrations.

Figure 5-5. Structural model for the recirculatory pharmacokinetic model for ABP-700 and CPM-acid and the pharmacodynamic models for the Bispectral Index and the Modified Observer's Assessment of Alertness/Sedation. The estimated value for each model parameter is shown in [tables 5-1](#) and [5-2](#). BIS, bispectral index; CL, clearance; $K_{transit}$, transit rate constant; $k_{e0,excite}$, effect-site rate constant for BIS disinhibition; $k_{e0,MOAA/S}$, effect-site rate constant for MOAA/S; $k_{e0,suppress}$, effect-site rate constant for BIS suppression; MOAA/S, Modified Observer's Assessment of Alertness/Sedation; QCO, apparent cardiac output; QP, peripheral flow; QND, non-distributive flow.

BIS Model Development

The initial BIS pharmacodynamic model varied $EC_{50,BIS}$, $k_{e0,BIS}$, and $BIS_{baseline}$ across individuals with $k_{e0,BIS}$ scaling to its theoretical allometric exponent (-0.25) and removing allometric scaling of $k_{e0,BIS}$ resulted in slightly worse model fit ($\Delta AIC = 2.72$). For the model, periods of high drug concentration were poorly predicted. Adding a nonzero limit to the BIS pharmacodynamic model (lower limit to BIS value) showed considerable improvement to model fit. However, there was insufficient model stability for reliable parameter estimates. We also considered a secondary pharmacodynamic effect-site that functions in opposition to the primary effect-site, i.e., reducing predicted drug effect as concentrations increased. If the primary effect-site is interpreted as drug effect causing suppression of the electroencephalogram processes underlying the BIS value, i.e., BIS suppression, then the secondary effect-site would be interpreted as drug effect causing the apparent excitation of said electroencephalogram processes, leading to increased BIS values, i.e., disinhibition of BIS. The equations of this pharmacodynamic model were

$$\begin{aligned}
 EC_{50,suppress} &= EC_{50,suppress,ref} \cdot \exp(\eta_1) \\
 k_{e0,suppress} &= k_{e0,suppress,ref} \cdot Fsize^{-0.25} \\
 \delta Ce_1 / \delta t &= k_{e0,suppress} \cdot (C_{Varterial} - Ce_1) \\
 Suppression &= EC_1^{\lambda_{suppress}} / (EC_1^{\lambda_{suppress}} + EC_{50,suppress}^{\lambda_{suppress}}) \\
 EC_{50,excite} &= EC_{50,excite,ref} \cdot \exp(\eta_2) \\
 k_{e0,excite} &= k_{e0,excite,ref} \cdot Fsize^{-0.25} \\
 \delta Ce_2 / \delta t &= k_{e0,excite} \cdot (C_{Varterial} - Ce_2) \\
 Excitation &= EC_2^{\lambda_{excite}} / (EC_2^{\lambda_{excite}} + EC_{50,excite}^{\lambda_{excite}}) \\
 BIS_{obs} &= (BIS_{baseline} + \eta_3) \cdot (1 - (1 - Excitation) \cdot Suppression) + e
 \end{aligned}$$

where EC_1 , $EC_{50,suppress}$, $k_{e0,suppress}$, and $\lambda_{suppress}$ represent effect-site concentration, concentration for 50% effect, effect-site time constant, and sigmoidicity parameters, respectively, for an effect-site that represents suppression of BIS. Parameters EC_2 , $EC_{50,excite}$, $k_{e0,excite}$, and λ_{excite} represent equivalent parameters for a secondary effect-site that causes increases in BIS values. An increase in excitation in the presence of constant suppression effects would be associated with an increase in observed BIS values. The balance of these suppression and disinhibitory effects determines the overall BIS value.

Inclusion of this secondary excitatory pharmacodynamic effect resulted in a very large improvement in model fit ($\Delta AIC = -6,176.39$). However, interindividual variability could not be reliably estimated for the k_{e0} values, and these were fixed to zero. Estimating a shared k_{e0} for both suppression and excitation resulted in a poorer fit ($\Delta AIC = 39.62$). Higher $EC_{50,excite}$ values were found in the presence of pretreatment, and incorporating this into the model resulted in improved model fit ($\Delta AIC = -34.62$). Lower individual $EC_{50,excite}$ values were

estimated with increasing age and adding an age covariate for $EC_{50,excite}$ improved model fit ($\Delta AIC = -13.58$). The final equation for $EC_{50,excite}$ was

$$EC_{50,excite} = EC_{50,excite,ref} \cdot \exp(\eta_2) \cdot \begin{cases} 1, & \text{no pretreatment} \\ \theta_1, & \text{pretreatment} \end{cases} \cdot \exp(\theta_2 \cdot (AGE - 35))$$

The estimated model parameters are shown in [table 5-2](#), and diagnostic plots are shown in [figure 5-6](#). Interestingly, low $EC_{50,excite}$ values were associated with involuntary muscle movements scored as “extensive” ($P = 1.95e-6$), while increased $EC_{50,excite}$ values were associated with involuntary muscle movements scored as “few” ($P = 8.58e-4$). No role of CPM-acid was found in the BIS model.

Figure 5-6. Diagnostic plots for the Bispectral Index (BIS) model. Red line: Smoother. (A) Population-observed/-predicted BIS values versus time. (B) *Post hoc* individual-observed/-predicted BIS values versus time. (C) Population-observed versus population-predicted BIS values. (D) *Post hoc* individual-observed versus individual-predicted BIS values.

Parameter	Estimated	Confidence limits		Variability		
		Lower 1%	Upper 99%		Variance (σ²)	Coefficient of Variation(%)
BIS						
EC _{50,suppress,ref} (ng·ml ⁻¹)	1041	986	1100	η1	0.0995	32.3
k _{e0,suppress,ref} (1 min ⁻¹)	0.844	0.823	0.865			
λ _{suppress}	4.45	4.27	4.63			
EC _{50,excite,ref} (ng·ml ⁻¹)	1230	943	1590	η2	0.560	86.6
k _{e0,excite,ref} (1·min ⁻¹)	1.03	0.934	1.14			
λ _{excite}	1.12	1.00	1.25			
BIS _{Baseline}	88.3	87.6	89.1	η3	20.7	-
Θ ₁	2.00	1.52	2.67			
Θ ₂	-0.0226	-0.0376	-0.00798			
Residual error				ε	42.3	-
Modified Observer's Assessment of Alertness/Sedation						
DEFF	3.35	3.17	3.53			
k _{e0,MOAA/S}	0.848	0.795	0.907			
b0	-5.38	-5.65	-5.12			
b1	0.186	0.145	0.234			
b2	0.190	0.148	0.238			
b3	0.385	0.326	0.449			
b4	0.941	0.856	1.03			
Θ ₁	0.00846	0.00657	0.0103			

Table 5-2. Model parameters for the final BIS and Modified Observer's Assessment of Alertness/Sedation model

The role of each parameter in the model structure is shown in figure 5-5.

BIS, bispectral index; DEFF, drug effect scaling constant; $EC_{50, excite}$, effect-site concentration producing 50% of maximal BIS suppression; $EC_{50, suppress}$, effect site concentration producing 50% of maximal BIS suppression; $k_{e0, excite}$, effect-site rate constant for BIS disinhibition; $k_{e0, MOAA/S}$, effect-site rate constant for the Modified Observer's Assessment of Alertness/ Sedation (MOAA/S); $k_{e0, suppress}$, effect-site rate constant for BIS suppression; λ_{excite} , slope for BIS disinhibition; $\lambda_{suppress}$, slope for BIS suppression.

Modified Observer's Assessment of Alertness/Sedation Model Development

The initial MOAA/S pharmacodynamic model scales $k_{e0MOAA/S}$ by its theoretical exponent of -0.25 , and removing this scaling did not improve model fit ($\Delta AIC = -0.05$). An age relationship was found between age and the DEFF parameter, improving model fit ($\Delta AIC = -123.41$). The equation for DEFF for the final MOAA/S model was

$$DEFF = DEFF_{ref} \cdot \exp(\theta_1 \cdot (AGE - 35))$$

The final model parameters are shown in [table 5-2](#). No role of the CPM-acid was found in MOAA/S model.

Simulation of Pharmacokinetic and Pharmacodynamic Model Characteristics

To explore the characteristics of the final models, we performed simulations of a 7-min infusion at $120 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ for a 70-kg, 35-yr-old individual. The dose was based on simulations from preliminary pharmacokinetic-pharmacodynamic models,⁵ and the results are shown in [figure 5-7](#). Arterial and venous ABP-700 concentrations rise and fall rapidly when starting and stopping the infusion. The predicted BIS shows the interaction of the suppression and disinhibition component effects of the BIS. At low effect-site concentrations, suppressive effects dominate, lowering BIS values from the baseline. As effect-site concentrations increase further, disinhibitory effects cause BIS to reverse its downward trend and return toward baseline. Time to peak effect for BIS suppressive effects is 1.6 min, and for BIS disinhibition effects is 1.5 min. The presence of pre-treatment reduced disinhibitory effects and thus lowers the obtained BIS values for the same dose. These opposing disinhibitory effects are not evident in the time course of the MOAA/S score, where the MOAA/S score decreases monotonically with increasing effect-site concentrations.

Figure 5-7. Simulation of a 70-kg, 35-yr-old individual with and without pretreatment receiving $120 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ABP-700 for 7 min. (A) Plasma concentrations, arterial and venous, of ABP-700 and CPM-acid. (B) Time course of Bispectral Index (BIS) in the absence of pretreatment. (C) Time course of BIS in the absence versus in the presence of pretreatment. (D) Time course of the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scores weighted by the predicted probability of each score.

To explore the influence of patient covariates and infusion rate for the final models, we performed simulations shown in **figure 5-8**. The overall influence of age on BIS and MOAA/S appear to be opposing. With advancing age, BIS values decrease less from baseline whereas the MOAA/S shows more rapid, deeper, and longer-duration effects. For weight, the covariate relationships are more canonical, with both BIS and MOAA/S showing slightly less drug effect and shorter duration for smaller individuals when dosed on a per-kilogram basis. For different infusion rates, BIS again shows paradoxical characteristics. Higher infusion rates result in more rapid initial onset but may stabilize at higher BIS values than lower infusion rates do. The MOAA/S shows more canonical monotonic dose-response characteristics, with higher infusion rates resulting in more rapid, greater, and longer duration decreases in the MOAA/S.

Figure 5-8. The influence of age (A and B), weight (C and D), and infusion rate (E and F) for a 70-kg, 35-yr-old individual receiving ABP-700 without pretreatment on Bispectral Index (BIS; A, C, and E) and Modified Observer's Assessment of Alertness/Sedation (MOAA/S) (B, D, and F).

Discussion

We present a recirculatory mammillary pharmacokinetic-pharmacodynamic model to predict arterial and venous concentrations of ABP-700 and its metabolite, CPM-acid, as well as BIS and the MOAA/S. The recirculatory ABP-700 pharmacokinetic model's characteristics are roughly similar to a previously developed three-compartmental model after bolus administration⁵ with an elimination clearance of 1.95 versus 1.66 l·min⁻¹. The small distribution volumes, indicating a small amount of body distribution and accumulation of the drug, and rapid clearance result in drug concentrations that stabilize rapidly after a change in drug administration rate and decrease rapidly after the cessation of infusions. The CPM-acid estimated apparent clearance of 0.769 l·min⁻¹ is low compared to that of ABP-700, so high CPM-acid plasma concentrations may occur with prolonged infusions. As reported by Valk et al.⁹ very high concentrations of CPM-acid can cause inhibition of the GABA_A receptor, potentially resulting in epileptic seizure activity. However, the maximum CPM-acid concentration observed during all phase 1 studies of ABP-700 in any individual was about 3,000 ng·ml⁻¹. This is approximately two orders of magnitude lower than the CPM-acid concentration associated with the GABA_A current inhibition *in vitro*, and with occurrence of seizures in dogs,⁹ rendering it unlikely that the involuntary muscle movements observed in humans during the phase 1 studies were of convulsive etiology. A simulation of a 5-h infusion of 120 mg·kg⁻¹·min⁻¹ shows that CPM-acid concentrations reach a steady-state plasma concentration after about 3 to 4 h at a concentration around 10,000 ng·ml⁻¹ (30 μM; supplemental material 5-6). This is also well below the concentration of CPM-acid that would cause GABA_A receptor inhibition,⁹ and thus, it is not expected that this is a mechanism that could cause seizure activity in humans receiving ABP-700. However, this simulation is a considerable extrapolation from the kind of data we had available for model development, and as such, interpretation of these data should be done with caution.

Compared to the previous investigation of ABP-700, more informative covariate relationships could also be discerned because of the larger and more diverse dataset. We found that clearance decreases with age, and thus, older individuals require lower maintenance infusion rates to achieve the same drug concentrations. A similar decrease of apparent clearance of CPM-acid with age was found.

As we developed the BIS model, we found that the initial model did not have a satisfactory fit. Based on our observation that increased BIS values were reported in subjects experiencing severe involuntary muscle movements,^{5,8} we then decided to separate the BIS effect into a suppression and a disinhibition, or excitation, component. The final BIS model thus contains effect-sites for both suppression and disinhibition as pharmacodynamic endpoints of a GABA_A receptor agonist. We found lower values of $EC_{50,excite}$ in individuals where involuntary muscle movement was noted as extensive. This conforms with the idea that involuntary muscle movement interferes with BIS as a method to monitor depth of

anesthesia. The threshold for disinhibition decreases with increasing age, and this results in apparently paradoxical BIS behavior; the pharmacodynamic effect of ABP-700 appears to diminish with increasing age and plateau for high effect-site concentrations, resulting in high BIS values. For our model, the threshold for disinhibition is increased in the presence of pretreatment, reflecting the apparently reduced disinhibitory drug effect pathways for a given drug concentration.

Consistent with our observations during the clinical studies of ABP-700, our pharmacodynamic model shows a very fast-acting drug, with an “on-off switch”-like action on clinical effect. This is illustrated by the quite steep slope of the sigmoid model for BIS suppression, having a $\gamma_{\text{suppression}}$ value of 4.45. This is steeper than another hypnotic drug, propofol, for which $\gamma_{\text{suppression}}$ values between 1.86 and 2.17^{19–21} were found. The $k_{\text{e0, suppression}}$ is also quite fast at 0.844 min⁻¹, considerably faster than that of other hypnotic drugs.

It is not clear whether the disinhibitory submodel influences the actual depth of hypnosis in the clinical sense. A possible explanation is that in the presence of involuntary muscle movements, the BIS monitor is reporting a higher BIS value than would be expected based on drug effect as measured by the MOAA/S, because of the interference of electromyography frequencies, which are misinterpreted by the BIS algorithm,²² thus masking the presence of sufficient anesthetic depth. If this mechanism is correct, then individuals receiving ABP-700 may show BIS values higher than clinically acceptable, but still have sufficient suppressive cerebral drug effect. This separation of hypnotic effects and clinically excitatory effects is partially supported by the developed pharmacodynamic model for the clinical MOAA/S score. Our model shows a high probability of a MOAA/S value of 0 at high effect-site concentrations, and we did not find a biphasic relationship in the MOAA/S with a “return of responsiveness” at higher concentrations. This suggests that cerebral drug effect as measured by the MOAA/S is not affected when involuntary muscle movement is present. If BIS values can increase while the MOAA/S score remains unchanged, then this would potentially reduce the utility of BIS monitoring for detecting insufficient anesthetic states for GABA_A receptor agonists such as ABP-700 in the presence of involuntary muscle movements.

For most anesthetic drugs, elderly patients experience a greater drug effect, e.g., there is more BIS suppression for a given dose and achieved drug concentration. **Figure 5-8** shows that the influence of age, weight, and dose on predicted BIS is not straightforward: BIS does not always diminish with increasing age and dose. Elevated BIS values could be caused by (1) inadequate ABP-700 concentrations resulting in insufficient hypnosis or (2) excessively high ABP-700 concentrations resulting in disinhibition of BIS and excessive clinical excitation. This discrepancy could potentially cause a dilemma in clinical practice: If clinicians focus on achieving BIS values in the usual hypnotic range of 40 to 60, they may tend to increase drug administration when BIS values above this range are observed. In the case

of ABP-700, this could result in further disinhibition, further increasing BIS values, and a failure to achieve the target BIS values. It is unknown whether this applies to other GABA_A receptor-modulating agents.

We found lower values of $EC_{50,excite}$ in individuals where involuntary muscle movement was scored as extensive. In other words, there seems to be an intrinsic factor that makes some individuals more prone to disinhibition, clinically presenting as involuntary muscle movements. Our model, combined with our observations from the clinical studies, in which there was a notable interindividual variability in severity of involuntary muscle movements within dosing cohorts,⁵ suggests that there is considerable interindividual susceptibility for this pharmacodynamic effect. We can only speculate what might cause this interindividual susceptibility. As it is probable that involuntary muscle movements are modulated by the GABA_A receptor, various GABA_A receptor subtypes might have a slightly different effect on disinhibition of BIS, and thus, a different distribution of GABA_A receptor subtypes might cause a higher susceptibility to involuntary muscle movements. There is also interindividual variability in speed and extent of drug distribution, which may be most evident for rapid-onset drugs, such as GABA_A receptor agonists ABP-700, etomidate, and, albeit to a smaller extent, propofol. This variability may result in unsynchronized onset of drug action at different sites in the central nervous system resulting in transient disinhibition of the subcortical excitatory neuronal circuits and involuntary muscle movements.²³ This hypothesis is supported to an extent by the $k_{e0,excitation}$ (1.03) and the $k_{e0,suppression}$ (0.844), showing that the onset of excitation/disinhibition is faster than the onset of BIS suppression, potentially resulting in a temporary disequilibrium in which clinical excitation has the upper hand.

The limitations of the study are similar to the ones reported in previous work by Struys et al.⁵ and Valk et al.⁸ While the covariate range for age and weight is broader than in those studies, it remains rather limited, and thus, the ability of the study to identify relevant covariate relationships is not as strong as it would have been had the study considered broader, stratified covariate ranges. While the recirculatory model does address arterial-venous differences, it does not fully address front-end kinetics. Especially for drugs with such a rapid onset and offset of effect, the front-end kinetics may play a considerable role in the pharmacologic action of the drug. Our blood sampling schedule did not include intense early sampling, so the front-end-kinetics structural model is rather simple, consisting of only a single depot compartment. Pharmacokinetic models developed with more extensive early sampling typically include more complex structural models incorporating multiple depot compartments.^{6,24} So, our pharmacokinetic model only provides limited information on drug concentration prediction in the very early phase of drug distribution. During the study design, we did not consider the possibility of dual pharmacodynamic effects causing both BIS suppression and excitation. If this had been suspected a priori, we would have considered changes to the study design, such as measuring raw electroencephalographic

waves using a full-montage electroencephalogram, to determine if differences in electroencephalographic activity can be observed between subjects experiencing clinical excitation versus subjects not experiencing clinical excitation.

Conclusions

Our model shows that, in a recirculatory mammillary pharmacokinetic-pharmacodynamic model, ABP-700 pharmacokinetics are characterized by a small amount of body distribution and rapid clearance. A secondary disinhibitory effect-site for BIS was associated with clinical excitation and involuntary muscle movements. This disinhibition functions in opposition to drug effects underlying suppression of the BIS signal and thus affects BIS monitoring. It is not known whether the presence of this disinhibition indicates insufficient actual anesthetic depth, or whether it is a separate signal, only interfering with values produced the BIS monitor. Our MOAA/S model does not indicate a relationship between disinhibition and clinical hypnosis. There are potential implications for the reliability of the BIS monitor, and indeed of other electroencephalogram-based monitors of depth of anesthesia, for ABP-700 and other GABA_A-ergic anesthetic agents. An interindividual susceptibility to excitation seems to exist, and the threshold for experiencing involuntary muscle movements decreases with increasing age. The mechanisms for this variability are unclear and warrant further investigation, as they might provide insight into the nature of the phenomenon of involuntary muscle movements and may improve the predictability of depth of anesthesia monitoring during excitation in GABA_A receptor agonists.

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Supplemental material to Chapter 5

Supplemental material 5-1: Number of subjects per ABP-700 dosing regimen and allocated premedication. Blue: ANVN-01, Green: ANVN-03, Maroon: ANVN-05, Orange: ANVN-02, Yellow: ANVN-04

	No premed	Fentanyl	Remi- fentanyl	Mida- zolam	Sufen- tanil	Fentanyl- Midazolam
Bolus						
30 µg/kg	5					
100 µg/kg	5					
175 µg/kg	5					
250 µg/kg	5	5				
350 µg/kg	5	5				
500 µg/kg	5					
750 µg/kg	5					
1000 µg/kg	5					
250 µg/kg		8	4	16	8	8
350 µg/kg				16		8
Slow Bolus- 175 µg/kg/min for 4 minutes		16				
7 min infusion						
100 µg/kg/min	7	8	8	8		
120 µg/kg/min	8	8		8		
140 µg/kg/min	8	8	8	16		
160 µg/kg/min	8					
140 µg/kg/min for 2 minutes - 100 µg/kg/min for 2 minutes- 90 µg/kg/min for 3 minutes		7	8			
30 min infusion (µg/kg/min)						
30 µg/kg/min	5					
40 µg/kg/min	5					
50 µg/kg/min	5	5				
60 µg/kg/min	5					
350 µg/kg bolus followed immediately by 50 µg/kg/min infusion		5				
two 250 µg/kg bolus, separated by 2 min followed immediately by 50 µg/kg/min infusion		5				
100 µg/kg/min for 3 min, 75 µg/kg/min for 6 min, 58 µg/kg/min for 21 min	4	4				
25 µg/kg/min ABP-700 for 10 minutes followed by 20 µg/kg/min for 20 minutes		4				
50 µg/kg/min ABP-700 for 5 minutes followed by 30 µg/kg/min for 25 minutes		8				
70 µg/kg/min ABP-700 for 3 minutes followed by 40 µg/kg/min for 27 minutes		8	7			
80 µg/kg/min ABP-700 for 3 minutes followed by 45 µg/kg/min for 27 minutes		8	8			
90 µg/kg/min ABP-700 for 3 minutes followed by 50 µg/kg/min for 27 minutes		4				
Titrate to effect: adjustment infusion rate if MOAA/S ≤ 4						
80 µg/kg/min ABP-700 for 1-3 minutes followed by 45 µg/kg/min for 29-27 minutes			1			
80 µg/kg/min ABP-700 for 3-4 minutes followed by 50 µg/kg/min for 27-26 minutes			2			
80 µg/kg/min ABP-700 for 4-5 minutes followed by 60 µg/kg/min for 26-25 minutes			5			

Supplemental material 5-2: Blood Sampling times for all 5 studies;

BL = Baseline

Study	Arterial Blood Sampling times (min)	Venous Blood Sampling times (min)
ANVN-01	BL, 0.5, 1, 2, 3, 4, 8, 12, 20, 30, 45, 60, 90, 120, 180	BL, 1.5, 3.5, 7, 13, 21, 35, 75, 125, 185, 240, 360, 480, 720
ANVN-02	BL, 0.5, 1, 3, 5, 10, 15, 20, 30, 30.5, 31, 33, 34, 38, 42, 45, 60	BL, 1.5, 3.5, 6, 18, 28, 31.5, 35, 40, 50, 75, 90, 180, 255, 480, 750
ANVN-03	NO ARTERIAL SAMPLES TAKEN	BL, 0.5, 1.5, 2.5, 3.5, 7, 13, 21, 35, 75, 125, 185
ANVN-04	BL, 0.5, 1, 3, 5, 10, 15, 20, 31, 38, 42, 45, 60, 90, 120	BL, 1.5, 3.5, 6, 18, 28, 31.5, 35, 40, 50, 75, 90, 180, 255
ANVN-05	BL, 0.5, 1, 3, 5, 7, 9, 11, 13, 15, 20, 30, 45, 60	BL, 0.5, 1.5, 2.5, 3.5, 7, 13, 21, 35, 60, 75, 120, 180

Supplemental material 5-3: The best, typical, and worst fit of arterial and venous ABP-700 predictions to the data

ABP-700, venous samples

Supplemental material 5-4: The best, typical, and worst fit of arterial and venous CPM-acid predictions to the data

CPM-acid, venous samples

Supplemental material 5-5: Expanded view of the initial arterial and venous concentration predictions for bolus dosing (ANVN-01) and infusion (ANVN-02)

Supplemental material 5-6: A simulation of a 5-h infusion of 120 mg·kg⁻¹·min⁻¹ for a 70-kg, 35-yr-old individual. (A) Plasma concentrations, arterial and venous, of ABP-700 and CPM-acid. (B) Time course of Bispectral Index (BIS) in the absence of pretreatment. (C) Time course of BIS in the absence versus in the presence of pretreatment (D) Time course of the Modified Observer's Assessment of Alertness/Sedation (MOAA/S) scores weighted by the predicted probability of each score.

CHAPTER 6

Summary, discussion and future perspectives

Partially modified from *Etomidate and its analogues: A Review of Pharmacokinetics and Pharmacodynamics*, Clinical Pharmacokinetics, 2021, online ahead of print

Beatrijs I. Valk, Michel M.R.F. Struys

In this thesis, a part of the road of the development of novel anesthetic agent ABP-700 is described. Several pharmacological approaches are applied in order to understand the action of the molecule at both a receptor level, and on a systemic level. This thesis can be roughly divided into two parts. The first part reports the basic safety and efficacy of a single potential novel hypnotic agent. The second part dives deeper into the observations made in the first part and uses the molecule to shed a little more light on the mechanism of anesthesia through GABA_A receptor modulation.

Chapter 1 provides background information on the current state of drug development in intravenous anesthesia. The working mechanism of the GABA_A receptor, the receptor on which most anesthetic agents act, is explained. An overview is given of the intravenous anesthetic agents that are currently used in daily anesthetic practice, and recent developments in anesthetic drug discovery are described. **Chapter 1** also introduces ABP-700, the GABA_A receptor agonist that is investigated in this thesis. It explains the rationale behind the design of ABP-700 and comparable compounds and describes the pre-clinical process of development of ABP-700. Furthermore, the process of drug development in general is explained to put the developmental process of ABP-700 into context. In **chapter 1** of this thesis the properties of an ideal anesthetic are presented: a rapid onset and cessation of hypnotic action, minimal circulatory and respiratory effects, and a minimum amount of side effects.

In **chapter 2** and **chapter 3**, the extent to which ABP-700 approaches this ideal intravenous anesthetic agent is investigated, by assessing the general safety, efficacy and tolerability of various dosing regimens of ABP-700.

Chapter 2 describes the first in-human study of ABP-700. It was a double-blind and placebo-controlled study. Healthy volunteers were given a bolus dose of either ABP-700 or a placebo with each cohort receiving a higher dose of study drug than the previous cohort. ABP-700 doses ranged from 0.03 mg/kg to 1.0 mg/kg. Cerebral drug effect as defined by the bispectral index (BIS) or Modified Observers Assessment of Alertness/Sedation (MOAA/S) started to occur at a dose of 0.175 mg/kg. At doses of 0.25 mg/kg and higher, all subjects reached a MOAA/S of 0, indicating that they were unresponsive to noxious stimuli. Higher doses produced a generally longer lasting clinical effect, although time to onset of clinical effect was very rapid through all effective doses, at under 30 seconds after administration. The course of the BIS profiles showed a similar drug effect. From a dose of 0.175 mg/kg and up, decline in BIS was rapid and time to BIS recovery was also dose-dependent. In the study of **chapter 3**, the volunteers received a continuous infusion of either ABP-700, propofol or a placebo. In this case, propofol acted as an active control. Here, ABP-700 infusion doses ranged from 30 µg/kg/min to 60 µg/kg/min. Clinical effect was observed throughout all dose-regimens, with values indicative of deep sedation or anesthesia being observed from a dose of 40 µg/kg/min and upwards. Of note is that sometimes BIS values and MOAA/S

observations did not correspond. For example, a subject who was judged as deeply anesthetized by the MOAA/S scale, showed elevated BIS values.

In **chapter 2**, a noncompartmental pharmacokinetic and a preliminary three-compartmental pharmacokinetic-pharmacodynamic model of ABP-700 is presented. These analyses showed that ABP-700 is rapidly eliminated throughout all dosing regimens. Additionally, pharmacodynamic modelling for both BIS and MOAA/S show a steep relation between the ABP-700 effect-site concentration and drug effect, indicating an “on-off switch” like effect of ABP-700 at the GABA_A receptor. This is illustrated by the MOAA/S model, in which the difference in an effect-site concentration between the 50% probability of maximum and minimum MOAA/S scores is very small. This indicates that there is almost no in-between in MOAA/S score. This observation is confirmed by the sigmoid E_{max} BIS-model, which had a steep slope.

These results show that ABP-700 possesses the first two characteristics of an ideal anesthetic agent: a very rapid onset of action after administration, with clinical effect occurring within 30 seconds, and a rapid cessation of clinical effect. They are both favorable properties for an intravenous anesthetic, because it allows the anesthesiologist to control the drug effect closely.

In both **chapter 2** and **chapter 3** it is demonstrated that, like its parent compound etomidate, and unlike other intravenous anesthetic agents propofol and the barbiturates, ABP-700 does not cause hemodynamic depression. What is more, a dose-related increase in heart rate and systolic blood pressure was observed with both a bolus dose and a continuous infusion dose. The time course of these effects runs parallel to the time course of clinical effect.

As it is an analogue of etomidate, ABP-700 likely affects the cardiovascular system in a similar way. Etomidate maintains hemodynamic stability because it does not significantly inhibit sympathetic tone and it preserves autonomic reflexes, such as the baroreflex.¹ This can potentially be explained by an agonistic action at the α_2 -adrenoreceptors, in particular the α_{2B} – adrenoreceptor responsible for the peripheral vasoconstrictive response to hypotensive effects.² Due to the preservation of sympathetic tone and autonomic reflexes and the lack of analgesic action, responses to laryngoscopy and endotracheal intubation are not blunted. In order to satisfactorily blunt the sympathetic response, opioid co-administration with an adequate dose is needed.³ In the studies of **chapter 2** and **chapter 3** tachycardia and systolic hypertension occurred without laryngoscopy or endotracheal intubation triggers and as such, it is possible that these measures could increase the incidence and severity of the tachycardia and systolic hypertension produced by ABP-700. Higher ABP-700 dosages were also associated with ‘excitatory’ phenomena such as involuntary muscle movements.

As such, it is possible that this cardiovascular hyperdynamic state is caused by a general excitatory state.

ABP-700 has remarkable respiratory stability, similar to etomidate. It does not produce any centrally induced apnea, contrary to several other intravenous anesthetic agents, and no significant desaturation occurred in any subject. In the volunteers that received ABP-700, only short-lasting episodes of apnea occurred and none of them were clinically relevant. Ventilatory frequency was higher in subjects receiving ABP-700 compared to control groups receiving placebo or propofol. However, PaCO₂ did not change significantly.

This means that ABP-700 largely fulfills the third and the fourth requirement of an ideal anesthetic agent, namely that of minimal cardiovascular and respiratory effect. The increase in heart rate and systolic blood pressure might not be ideal but preferable to a depression in cardiovascular tone. Furthermore, in clinical practice the molecule would be co-administered with an opioid agent which would further blunt the sympathetic nervous system and decrease heart rate and systolic blood pressure.

Contrary to etomidate, ABP-700 did not cause suppression of the adrenal axis, either after any bolus dose or after any infusion dose. Values of plasma cortisol after stimulation with ACTH, a hormone that stimulates cortisol production, were not significantly different in subjects who had received ABP-700 than in subjects who had received placebo or propofol. This is consistent with pre-clinical findings. A side effect that was frequently observed in subjects receiving ABP-700, was the occurrence of involuntary muscle movements (IMM). In the bolus study of **chapter 2**, 36 out of 50 subjects receiving ABP-700 (72%) experienced IMM, in the continuous infusion study of **chapter 3** 20 out of 25 (80%) subjects receiving ABP-700. These movements ranged from a subtle twitch in a single finger to myoclonic movements of the whole body. The incidence and severity of the IMM increased with higher doses and they occurred in combination with other 'excitatory' phenomena such as tachycardia, hypertension and tachypnea.

Etomidate is associated with postoperative nausea and vomiting (PONV)^{4,5} with incidences reported to be as high as 40%. However, studies comparing the lipid emulsion of etomidate to propofol found no significant difference in incidence of PONV. This suggests that the emetogenicity of etomidate lies in the formulation, rather than the anesthetic itself.⁶ ABP-700 too shows emetogenic properties, although the incidence is relatively moderate. During the bolus study, 2 out of 50 subjects (4%) experienced PONV, whereas during the continuous infusion, 6 out of 25 subjects (24%) experienced PONV. As ABP-700 is not formulated in a lipid emulsion, this might explain the smaller incidence of PONV compared to etomidate. A side effect that was relatively scarce with ABP-700 was pain on injection, a phenomenon observed with several anesthetics such as propofol and also etomidate.

All major side effects that ABP-700 produces, including the occurrence of involuntary muscle movements, are also observed with etomidate, its parent-compound. No adrenal suppression occurs after ABP-700 administration, which means that ABP-700 technically improves upon etomidate in the side-effect department.

In **chapter 2** and **chapter 3**, the effect of the use of 1.0 mg/kg fentanyl as a premedication is studied, in addition to the effect of ABP-700 without premedication. Fentanyl was added as a premedication to the doses of ABP-700 that had the most beneficial effect-versus-side effect profile. For the bolus this was 0.25 mg/kg and 0.35 mg/kg, and for the continuous infusion this was 50 µg/kg/min. The addition of fentanyl did not significantly change the onset, duration and recovery of the hypnotic effect of ABP-700, but did produce a more pronounced decrease in BIS values. Fentanyl also reduced the incidence and severity of the IMM.

ABP-700 causes rapid on- and offset of hypnotic effect, it does not cause cardiovascular or respiratory depression and has side-effects similar to etomidate, bar the adrenocortical suppression. Its cardiovascular profile could make ABP-700 a suitable anesthetic induction agent for patients who are hemodynamically unstable. However, the major concerning side effect of ABP-700 is the occurrence of involuntary muscle movements. Besides the unknown origin of these movements - which will be discussed later -, the impact of this effect on clinical practice could be considerable if not accurately controllable by co-administered opiates or benzodiazepines. As discussed previously, co-administration of these agents can considerably decrease the incidence and severity of the movements. However, these agents also affect respiratory drive and the duration of hypnotic action. Of course, the use of muscle relaxants would prevent the occurrence of involuntary muscle movements altogether. However, patients would need to be intubated, cancelling out the advantage of the preservation of respiratory drive that ABP-700 has. Furthermore, the “on-off-switch” like characteristics of ABP-700 could pose a challenge in daily clinical practice. The onset and particularly the offset of drug effect should not be too fast. If, for example, an intravenous drug is used as an induction agent, the anesthesiologist should have enough time to switch to inhalational anesthesia for maintenance and not worry that his patient might wake up during endotracheal intubation. Where exactly on this spectrum ABP-700 is, cannot be said for certain, and further studies examining for example tolerance of laryngoscopy should be conducted to answer this question. In such studies, a clinical dosage of an opioid agent should be administered alongside ABP-700 to accurately simulate daily clinical practice.

In conclusion, ABP-700 is a hypnotic that has favorable characteristics. Its added value in daily clinical practice compared to the established anesthetic agents is not yet clear.

In **chapter 4** and **chapter 5**, the etiology of the aforementioned involuntary muscle movements and the potential mechanism behind this phenomenon are investigated and

explored by various pharmacological approaches: in vitro and in vivo pharmacological techniques in **chapter 4**, and population pharmacokinetic-pharmacodynamic modeling in **chapter 5**.

In **chapter 4**, it is investigated if the IMM are of a convulsive nature. To this end, supra-therapeutic doses of ABP-700 were administered to fourteen beagle dogs over the course of two hours. IMM were observed in all fourteen dogs. In five out of the fourteen dogs, seizures occurred. However, these phenomena occurred at distinct time points and the electroencephalographic pattern was different. Where the IMM mostly occurred during the infusion, the seizures took place in the minutes and hours after the infusion had stopped. This led us to hypothesize, that the etiology of the IMM and the seizures was different and that the seizures were not produced by ABP-700, but by high plasma concentrations of its metabolite, CPM-acid. To test this hypothesis, electrophysiologic experiments were performed. In *Xenopus laevis* oocytes, the $\alpha_1\beta_3\gamma_{2L}$ GABA_A receptor and the $\alpha_1\beta$ glycine receptor, two receptors important for the production of anesthesia, were brought to expression. In these, concentration-dependent modulatory effects of ABP-700 and CPM-acid were analyzed. These experiments showed that high concentrations of both ABP-700 and CPM-acid were able to cause inhibition of the GABA_A receptor and the glycine-receptor, known mechanisms of seizures. Concentrations of CPM-acid high enough to produce receptor inhibition were reached in the beagle dogs, but these CPM-acid concentrations were about 100 times higher than the highest concentration reached in the human trials.

Because in subjects who experienced IMM, BIS values and MOAA/S observations regularly did not correspond, the relationship between involuntary muscle movements and measurements of clinical effect was further investigated in **chapter 5**. To this end, a recirculatory pharmacokinetic-pharmacodynamic model of ABP-700 was developed, incorporating the data of 266 subjects across five phase 1 studies. The pharmacokinetics of ABP-700 were similar to those reported in the preliminary three-compartmental model in **chapter 2**. They are characterized by small compartmental volumes and a rapid clearance. It was found that for the BIS-model, a secondary effect-site had to be incorporated in order to have a good model: besides an effect-site of suppression, an excitatory effect-site was added. This secondary effect-site is associated with the occurrence of involuntary muscle movements and its threshold appears to decrease with increasing age. Additionally, pretreatment with an opioid or a benzodiazepine could reduce the excitatory, or disinhibitory, effect.

With etomidate, several strategies have been studied to reduce the incidence of these movements. They can be reduced or prevented by pre-medication of the patients receiving etomidate with central nervous system depressant effects. These include opiates (fentanyl, remifentanyl),⁷⁻¹⁰ benzodiazepines,^{11,12} dexmedetomidine,^{13,14} thiopental,¹³ lidocaine¹⁵ and magnesium.¹⁶ Another strategy is a split-dose infusion of etomidate as a 'primer-dose'.^{17,18}

As ABP-700 is an analogue of etomidate, it can be assumed that both hypnotics cause movements by the same mechanism. This mechanism, however is not yet known; it is nonetheless unlikely that the movements are the result of epileptogenic activity.¹⁷ Several studies have recorded the electroencephalogram during administration of etomidate and have found that involuntary muscle movements do not coincide with epileptiform paroxysms.^{17,19–21} For ABP-700, no clinical “full-montage” EEG studies have been performed so far. However, based on the results of **chapter 4**, it is extremely unlikely that the healthy volunteers in clinical studies experienced seizures.

Doenicke et al. and Kugler et al. hypothesized that the origin of the involuntary muscle movements observed with etomidate lies in a temporary disequilibrium of the drug at effect sites within the central nervous system.¹⁷ This hypothesis postulates that low concentrations of anesthetic drug depress inhibitory neuronal circuits earlier than the excitatory neuronal circuits. Possible explanations for this disequilibrium are differences in local cerebral blood flow or differences in affinity²⁰. This is supported by the observations of several studies that CNS-depressing pre-treatment reduces the incidence of involuntary muscle movements (see before) and that higher dosages of etomidate and ABP-700 produce more involuntary muscle movements.

In the PK-PD model of ABP-700 presented in **chapter 5**, the secondary effect site mentioned previously was associated with a risk of occurrence of IMM. Lower values of EC_{50} of this disinhibitory effect-site were observed in individuals who also experienced more severe involuntary muscle movements. The EC_{50} for this effect-site was higher in individuals who received pre-medication with opioids or benzodiazepines. This observation supports the hypothesis by Doenicke and Kugler that an unsynchronized onset of drug effect at different effect-sites within the CNS exists.

What then, in turn, might be the cause of this disequilibrium in drug effect? It is likely that on a molecular level, the involuntary muscle movements are modulated by the $GABA_A$ receptor. McGrath et al. demonstrated that when the structure of etomidate is modified to eliminate its $GABA_A$ positive modulatory activity, involuntary muscle movements are no longer observed in rats.²² Note that in the PK-PD model of **chapter 5** there also seems to be an interindividual variability in susceptibility to IMM. There are several explanations for this observation that might also explain the disequilibrium in drug effect. One is that there is a difference in distribution of $GABA_A$ receptor subtypes.²³ Because etomidate, and by extension ABP-700, has a very specific binding site on the $GABA_A$ receptor, different distribution of subtypes within the CNS might cause higher susceptibility to IMM. Propofol, for example, binds less specifically to the $GABA_A$ receptor, which could be why, although observed, the incidence and severity of IMM is lower with propofol than with etomidate and ABP-700. Another explanation could be that as etomidate and ABP-700 are rapid-onset drugs, there might be an interindividual variability in drug distribution and/or metabolism.

Conclusions and future perspectives:

In the first part of this thesis, the novel anesthetic agent ABP-700 is investigated and assessed to what extent it approaches the ideal anesthetic agent. ABP-700 seems to have beneficial properties, especially concerning hemodynamic and respiratory stability, but also has a major side effect that has proved to be a concern for several other GABA_A receptor agonists in development: the occurrence of involuntary muscle movements. Although these movements do not seem to have an epileptic origin, they are still undesirable and the added value of ABP-700 as a potential established drug needs to be further assessed. After the conclusion of these five phase I studies, a phase II trial was initiated to assess ABP-700 in a sedation setting. Unfortunately, this study was discontinued because the sponsor of the time, the Medicines Company, chose not to further invest in the molecule. The decision of pharmaceutical companies to discontinue a drug development program is multifaceted. Financial issues often play a large part, especially if a molecule does not seem to be promising at first glance. In some instances, the intellectual property of the molecule is sold to another pharmaceutical company or a holding which can choose to revive the development of a molecule. In anesthesia, development of several molecules was discontinued by their initial sponsors, only to be revived by other companies and successfully make it to the market. Examples of this are sevoflurane²⁴ and remimazolam.^{25,26} So even if the development of ABP-700 is halted at present, it does not mean that it will not get picked up again.

The second part of this thesis focusses on exploring the origin behind the involuntary muscle movements observed with the use of ABP-700. This is an issue with several GABA_A receptor agonists, such as propofol, but also developmental anesthetic molecules such as AZD3043.²⁷ In this thesis, it could be concluded that the IMM most likely are not of an epileptic nature and that it has likely to do with a disequilibrium that occurs in the central nervous system during induction of anesthesia. This is further supported by the PK-PD-model presented in **chapter 5**, in which it was necessary to add a secondary effect-site to the model, to account for excitation effects on the BIS. As IMM prove to be an increasingly bigger stumbling block for novel GABA_A receptor agonists, it is important to further study the mechanism behind this phenomenon in order to assist the development of future GABA_A receptor agonists.

During the design of the phase I studies of ABP-700, the incidence and the severity of involuntary muscle movements had not been anticipated. Had they been so, full electroencephalographic measurements would have been implemented in the study protocol. For future studies, it is essential to include EEG measurements. Firstly, in order to confirm that also in humans, the origin of the involuntary muscle movements is not of epileptic origin and secondly, to which end fMRI studies could also provide more insight, to observe if in some parts of the brain, onset of drug effect is faster than in other parts. This would support the hypothesis that involuntary muscle movements are caused by a disequilibrium in drug

action and that suppressive areas of the brain are affected before the excitatory parts of the brain.

In the end, this thesis might provide insight for future drug developmental programs of novel anesthetic agents, specifically GABA_A receptor agonists. It hopes to provide investigators seeking to embark on their long and winding road of developing potential novel anesthetic agents with a partial roadmap to their final destination: approval of regulatory bodies and distribution into daily clinical practice.

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CHAPTER 7

Summary, discussion and future perspectives in Dutch/ Nederlandse samenvatting, discussie en een visie voor de toekomst

Deels bewerkt uit *Etomidate and its analogues: A Review of Pharmacokinetics and Pharmacodynamics*, Clinical Pharmacokinetics, 2021, online ahead of print

Beatrijs I. Valk, Michel M.R.F. Struys

Dit proefschrift beschrijft een deel van het geneesmiddelenonderzoekstraject van ABP-700, een nieuw anestheticum. Een aantal farmacologische benaderingen wordt toegepast om de werking van deze molecule te begrijpen op zowel receptor- als op een systemisch niveau. Dit proefschrift kan ruwweg in tweeën worden verdeeld. In het eerste deel ligt de nadruk op het onderzoeken van de werking en de veiligheid van deze specifieke nieuwe molecule. In het tweede deel wordt de molecule gebruikt om observaties uit het eerste deel nader te kunnen verklaren en het mechanisme van anesthesie door GABA_A-receptor modulatie te onderzoeken.

Hoofdstuk 1 bevat achtergrondinformatie over de huidige stand van zaken binnen de ontwikkeling van nieuwe intraveneuze anesthetica. De functie en het werkingsmechanisme van de GABA_A-receptor, de receptor waar de meeste intraveneuze anesthetica op werken, worden beschreven. Er wordt aandacht besteed aan de hedendaags veelgebruikte intraveneuze anesthetica, maar ook aan de meest recente ontwikkelingen in geneesmiddelenonderzoek van anesthetica. **Hoofdstuk 1** introduceert ABP-700, de molecule die centraal staat in dit proefschrift. De gedachte achter het ontwerp van ABP-700 en vergelijkbare stoffen, evenals de preklinische fase van de ontwikkeling van ABP-700 worden samengevat. Tevens wordt het proces van geneesmiddelenonderzoek kort weergegeven om de ontwikkeling van ABP-700 in perspectief te plaatsen. In **hoofdstuk 1** worden de belangrijkste eigenschappen van een ideaal anestheticum weergegeven: een snelle intrede en snelle afname van hypnotisch effect, minimaal effect op de hemodynamische en respiratoire systemen en een minimaal aantal aan bijwerkingen.

In **hoofdstuk 2** en **hoofdstuk 3** wordt onderzocht in hoeverre ABP-700 deze eigenschappen van een ideaal anestheticum benadert. Hiervoor worden de algemene veiligheid, werkzaamheid en verdraagzaamheid van verschillende doseerschemata van ABP-700 onder de loep genomen.

Hoofdstuk 2 beschrijft de eerste klinische studie van ABP-700. Dit was een dubbelblinde en placebogecontroleerde studie. Een bolus ABP-700 of placebo werd toegediend aan gezonde vrijwilligers, waarbij elk cohort een hogere dosis toegediend kreeg dan het voorgaande cohort. 0,03 mg/kg was de laagst toegediende dosis ABP-700 en 1,0 mg/kg de hoogste. Een duidelijk klinisch hypnotisch effect, gedefinieerd door de bispectral index (BIS) en de Modified Observers Assessment of Alertness/Sedation (MOAA/S), was voor het eerst zichtbaar bij een dosis van 0,175 mg/kg. Een MOAA/S van 0, waarbij een proefpersoon niet meer reageert op een pijnprikkel, werd vastgesteld in elke cohort met een dosis van 0,25 mg/kg en hoger. Hogere doseringen leidden over het algemeen tot een langer durend klinisch effect. De duur tot het optreden van een klinisch effect was echter zeer kort bij alle doseringen, namelijk korter dan 30 seconden na toediening. Het verloop van de BIS, een afgeleide van het elektro-encefalogram (EEG), liet een vergelijkbaar patroon zien. Vanaf een dosis van 0,175 mg/kg en hoger trad er een snelle daling in BIS-waarden op en een dosis-

afhankelijk herstel. In het onderzoek beschreven in **hoofdstuk 3** kregen de proefpersonen een continu infuus van ofwel ABP-700, ofwel propofol, ofwel een placebo toegediend. In dit geval werd propofol gebruikt als een actieve controle. In deze studie begonnen de ABP-700 doseringen bij 30 µg/kg/min en eindigden bij 60 µg/kg/min. Het klinisch effect werd ook hier beoordeeld middels de MOAA/S-score en BIS-waarde. Waardes passend bij diepe sedatie of narcose werden gezien vanaf een dosis van 40 µg/kg/min en hoger. Opvallend was dat er af en toe een discrepantie werd waargenomen tussen de BIS-waarden en de MOAA/S-scores, waarbij er een duidelijk hogere BIS-waarde werd gezien dan verwacht kon worden op grond van de MOAA/S-score.

In **hoofdstuk 2** is er een non-compartmenteel farmacokinetiek verricht en een eerste, voorlopig drie-compartmenteel farmacokinetisch-farmacodynamisch model van ABP-700 ontwikkeld. Deze analyses lieten zien dat ABP-700 snel geëlimineerd wordt bij alle doseringen. Verder toonden de farmacodynamische modellen voor zowel BIS als MOAA/S dat ABP-700 een soort “aan-uitschakelaar”-achtig effect heeft op de GABA_A-receptor: bij een minimaal verschil in concentratie kan er een groot verschil in klinisch effect optreden. Dit wordt geïllustreerd door het MOAA/S-model, waarin het verschil in EC₅₀, de concentratie waarop er een 50% kans is dat een bepaald klinisch effect optreedt, voor de maximale en minimale MOAA/S-score heel erg klein is. Dit leidt ertoe dat een MOAA/S-score die ergens in het midden ligt nauwelijks optreedt. Deze observatie wordt bevestigd door het sigmoïd concentratie-effect model van de BIS, die een hele steile helling laat zien.

Deze resultaten laten zien dat ABP-700 de eerste beide eigenschappen van een ideaal anestheticum tot op zeker mate bezit: snelle intrede, namelijk 30 seconden, en snelle afname van hypnotisch effect. Dit zijn gewenste eigenschappen voor een anestheticum omdat het de anesthesioloog in staat stelt om het klinisch effect strak te controleren.

Hoofdstuk 2 en **hoofdstuk 3** laten tevens zien dat ABP-700, net zoals zijn stam-molecule etomidaat, geen hemodynamische depressie veroorzaakt in tegenstelling tot andere intraveneuze anesthetica zoals propofol en de barbituraten. Sterker nog, een dosis-gerelateerde toename in hartslagfrequentie en systolische bloeddruk werd gezien bij zowel een bolus dosis als een continu infuus van ABP-700. Het verloop van deze effecten is parallel aan het klinisch, hypnotische effect.

Omdat ABP-700 een analoog is van etomidaat, is het aannemelijk dat ABP-700 een vergelijkbaar effect heeft op het cardiovasculaire systeem. Etomidaat zorgt voor hemodynamische stabiliteit omdat het de sympathicus niet significant remt en het autonome reflexen zoals de baroreflex behoudt.¹ Dit effect kan deels verklaard worden door een agonistisch effect op de α₂-adrenoceptoren, en dan met name de α_{2B} – adrenoceptor die verantwoordelijk is voor de perifere vasoconstrictieve respons op hypotensieve effecten.² Doordat de sympathicus niet geremd wordt, autonome reflexen behouden worden en etomidaat geen

analgetische werking heeft, wordt de respons op laryngoscopie en endotracheale intubatie echter niet geremd.³ Om de sympathicus voldoende te dempen is derhalve gelijktijdige toediening van een adequate dosis opiaten noodzakelijk.

In de studies uit **hoofdstuk 2** en **hoofdstuk 3** traden tachycardie en systolische hypertensie al op zonder triggers zoals laryngoscopie of intubatie. Het is daarom aannemelijk dat deze triggers kunnen leiden tot een hoger aantal episodes van tachycardie en systolische hypertensie veroorzaakt door ABP-700, en de ernst hiervan. Hogere doses van ABP-700 werden ook geassocieerd met andere effecten gerelateerd aan 'excitatie', zoals onwillekeurige spierbewegingen. Het is dus in principe mogelijk dat de tachycardie en hypertensie veroorzaakt worden door een algemene staat van excitatie.

ABP-700 heeft nauwelijks effect op het ademhalingsstelsel. In tegenstelling tot een aantal andere intraveneuze anesthetica, maar vergelijkbaar met etomidaat, veroorzaakt het geen centrale ademhalingsdepressies. In bovenstaande studies werden geen significante desaturaties of klinisch relevante apneu gezien. Wel werd een dosis-afhankelijke tachypneu gezien. Proefpersonen die ABP-700 toegediend kregen hadden over het algemeen een hogere ademfrequentie dan proefpersonen die propofol of placebo kregen. Desondanks was er geen significant verschil in PaCO_2 .

Dit betekent dat ABP-700 voor een deel ook de derde eigenschap van een ideaal anestheticum bezit, namelijk die van een minimaal effect op de hemodynamisch en respiratoire systemen. De toename van hartslagfrequentie en systolische bloeddruk zijn weliswaar niet ideaal, maar worden eerder geaccepteerd dan een hypotensie. Daarnaast worden anesthetica in de klinische praktijk meestal samen met een opiaat gegeven, waardoor de sympathicus weer geremd wordt en de hartslagfrequentie en bloeddruk omlaag gebracht worden.

In tegenstelling tot etomidaat veroorzaakten zowel een bolus als een continu infuus van ABP-700 geen suppressie van de bijnier-as. Zoals verwacht na preklinische studies, verschilden plasma cortisol waarden van proefpersonen die ABP-700 toegediend hadden gekregen na stimulatie met ACTH niet significant van de waarden van proefpersonen die propofol of placebo hadden gekregen. Het optreden van onwillekeurige spierbewegingen was een bijwerking die regelmatig werd gezien bij proefpersonen die ABP-700 hadden gekregen. In de bolus-studie in **hoofdstuk 2** werd dit gezien in 36 van de 50 proefpersonen (72%), en in de continu infuus studie in **hoofdstuk 3** in 20 van de 25 proefpersonen (80%) die ABP-700 toegediend kregen. Deze bewegingen varieerden van een enkele subtiele spiertrekking in een enkele vinger tot myoklonieën in het hele lichaam. Deze bewegingen traden vaker en uitgebreider op bij hogere doseringen en worden vaak gezien in combinatie met andere 'excitatie' verschijnselen, zoals tachycardie, hypertensie en tachypneu.

Etomidaat is geassocieerd met postoperatieve misselijkheid en braken (PONV).^{4,5} De incidentie loopt daarbij soms op tot 40%. Studies ontdekten echter dat dit waarschijnlijk vooral te wijten is aan de formulatie van etomidaat in een vet-oplossing, nadat geen significant verschil in incidentie van PONV vastgesteld kon worden bij een vergelijking van etomidaat en propofol.⁶ Ook in de studies naar ABP-700 werd PONV gerapporteerd door een aantal proefpersonen. De incidentie was echter relatief klein. In de bolus studie gaven 2 van de 50 vrijwilligers (4%) en in de continu infuus-studie 6 van de 25 (24%) aan misselijk te zijn geweest of over te hebben gegeven. Deze lagere incidentie in vergelijking met etomidaat kan verklaard worden doordat ABP-700 niet in een vetemulsie wordt gegeven. Een bijwerking die relatief zelden werd gemeld bij ABP-700 was pijn bij injectie, een fenomeen dat regelmatig optreedt bij toediening van propofol, en ook bij etomidaat.

Alle belangrijke en opvallende bijwerkingen die gezien worden bij ABP-700 worden ook gezien bij etomidaat, de 'stam-molecule'. Depressie van de bijnier-as kon niet worden vastgesteld, waaruit geconcludeerd mag worden dat ABP-700 superieur is in vergelijking met etomidaat als het gaat om bijwerkingen. De belangrijkste, en klinisch meest zorgwekkende, bijwerking van ABP-700 is het voorkomen van onwillekeurige spierbewegingen. De oorsprong van dit fenomeen kon niet definitief worden vastgesteld middels de fase I studies in **hoofdstukken 2 en 3**.

In **hoofdstuk 2** en **hoofdstuk 3** wordt ook het effect van 1,0 mg/kg fentanyl als premedicatie bestudeerd. Fentanyl werd toegevoegd als premedicatie aan de doses ABP-700 die het meest gunstige effect-versus-bijwerking profiel hadden. Voor de bolus was dit 0,25 mg/kg en 0,35 mg/kg en voor het continue infuus was dit 50 µg/kg/min. De toevoeging van fentanyl had geen significant effect op de tijd tot intreden van het klinisch effect, de duur en het herstel van het klinisch effect van ABP-700. Wel was de snelle daling in BIS-waarden meer uitgesproken. Fentanyl verminderde ook de ernst en het aantal gevallen van onwillekeurige spierbewegingen.

ABP-700 heeft dus gunstige eigenschappen voor een intraveneus anestheticum: het veroorzaakt snel optreden en afname van het klinisch hypnotisch effect, het veroorzaakt geen depressie van de hemodynamische en respiratoire systemen en heeft bijwerkingen die vergelijkbaar zijn met die van etomidaat, met uitzondering van de suppressie van de bijnier-as. Het cardiovasculaire profiel van ABP-700 maakt het een potentieel inductiemiddel bij hemodynamisch instabiele patiënten. Het vóórkomen van onwillekeurige spierbewegingen is echter zorgelijk. Naast dat de oorsprong van deze bewegingen onbekend is -zie ook later in dit hoofdstuk-, kan de impact van deze bijwerking op de dagelijkse kliniek aanzienlijk zijn indien deze niet adequaat onder controle gehouden kan worden middels opiaten of benzodiazepines. Zoals eerder vermeld kan gelijktijdige toediening van deze middelen de incidentie en de ernst van de bewegingen aanzienlijk verminderen. Deze middelen hebben echter ook een impact op bijvoorbeeld de ademhaling en de duur van het klinisch hypno-

tische effect. Het gebruik van spierverslappers zou het optreden van deze bewegingen in zijn geheel kunnen voorkomen. In dit geval zouden patiënten echter geïntubeerd moeten worden, wat het voordeel van minimaal effect op de ademhaling van ABP-700 tenietdoet. Een andere potentiële uitdaging van dit middel in de kliniek is de snelheid waarmee het klinisch effect optreedt, maar ook weer afneemt. Het is weliswaar wenselijk dat het effect van een anestheticum snel weer afneemt, maar te snel is in de praktijk ook niet handig. Als een intraveneus anestheticum bijvoorbeeld als een inductiemiddel wordt gebruikt, en dus als een bolus gegeven wordt, moet de anesthesioloog voldoende tijd hebben om over te stappen op inhalatieanesthesie, zonder bang te hoeven zijn dat de patiënt weer wakker wordt tijdens de intubatie. Waar ABP-700 precies ligt op dit spectrum is nog onduidelijk, en om deze vraag te beantwoorden zijn studies nodig die bijvoorbeeld de verdraagzaamheid van laryngoscopie onderzoeken. In zulke studies zou een klinische dosis opiaat gelijktijdig toegediend moeten worden om de dagelijkse praktijk zo goed mogelijk te simuleren.

Concluderend is ABP-700 een intraveneus anestheticum met een aantal wenselijke eigenschappen. De toegevoegde waarde van dit middel ten opzichte van de gevestigde intraveneuze anesthetica is nog onduidelijk.

In **hoofdstuk 4** en **hoofdstuk 5** worden de etiologie van de eerder vermelde onwillekeurige spierbewegingen en hun mogelijke mechanisme onderzocht en verkend met behulp van verschillende farmacologische technieken: in vitro en in vivo technieken in **hoofdstuk 4**, en door middel van een populatie farmacokinetisch-farmacodynamisch model in **hoofdstuk 5**.

In **hoofdstuk 4** wordt onderzocht of een convulsieve oorzaak ten grondslag ligt aan de spierbewegingen. Hiervoor werden suprathérapeutische doses van ABP-700 toegediend aan 14 Beagle honden gedurende twee uur. Onwillekeurige spierbewegingen werden gezien in alle veertien honden. Bij vijf van de veertien honden werden ook epileptische insulten vastgesteld. Deze verschijnselen traden echter op verschillende tijdstippen op en ook het EEG-patroon kwam niet overeen. Terwijl de spierbewegingen vooral geobserveerd werden terwijl ABP-700 nog werd toegediend, vonden de insulten met name plaats tijdens de minuten en uren nadat de toediening gestopt was. Dit leidde tot onze hypothese dat de etiologie van de spierbewegingen een andere moest zijn dan die van de epileptische insulten, en dat de insulten niet veroorzaakt konden worden door ABP-700, maar waarschijnlijk veroorzaakt werden door diens metabooliet, CPM-acid. Om deze hypothese te testen werden elektrofysiologische experimenten uitgevoerd. In de celmembranen van *Xenopus laevis* oocyten (eicellen van de *Xenopus laevis* kikker) werden $\alpha_1\beta_3\gamma_{2L}$ GABA_A en $\alpha_1\beta$ glycine receptoren tot expressie gebracht. Deze twee receptoren zijn belangrijk in het veroorzaken van narcose. In deze cellen werden de concentratie-afhankelijke modulerende effecten van ABP-700 en CPM-acid geanalyseerd. Deze experimenten lieten zien dat zowel hoge concentraties ABP-700 en CPM-acid de GABA_A-receptor en de glycine receptor

blokkeerden, een bekend mechanisme dat ten grondslag ligt aan epileptische insulten. Dusdanig hoge concentraties van CPM-acid waren eerder vastgesteld bij de Beagle honden, maar deze concentraties waren ongeveer honderdmaal hoger dan de hoogste concentratie van CPM-acid in de klinische studies.

In proefpersonen die onwillekeurige spierbewegingen doormaakten bestond er regelmatig een discrepantie tussen de BIS-waarden en de MOAA/S-waarden. De relatie tussen de onwillekeurige spierbewegingen en de manieren om anesthesie diepte te monitoren wordt daarom verder verkend in **hoofdstuk 5**. Dit is gedaan door een recirculatoir farmacokinetisch-farmacodynamisch model van ABP-700 te ontwikkelen, waarin de data van 266 proefpersonen uit vijf fase I studies verwerkt zijn. De farmacokinetische eigenschappen van ABP-700 in dit model waren vergelijkbaar met die uit het driecompartimentele model uit **hoofdstuk 2**: kleine verdelingsvolumes en een snelle klaring. Opvallend in het farmacodynamische model was dat voor het BIS-model er een secundaire effect-site geïntegreerd moest worden om een goed model te kunnen maken. Hiervoor werd naast een remmende effect-site ook een exciterende effect-site toegevoegd. Deze secundaire effect-site is geassocieerd met het optreden van onwillekeurige spierbewegingen en het lijkt erop dat de drempel voor deze effect-site lager wordt bij toenemende leeftijd. Verder konden premedicatie met een opiaat of een benzodiazepine dit exciterende, of ontremmende effect verminderen.

Onwillekeurige spierbewegingen worden ook gezien bij etomidaat en er zijn verschillende strategieën onderzocht om het optreden van deze bewegingen te reduceren of te voorkomen. Middelen met een remmend effect op het centrale zenuwstelsel zoals opiaten,⁷⁻¹⁰ benzodiazepines,^{11,12} dexmedetomidine,^{13,14} thiopental,¹³ lidocaine¹⁵ en magnesium¹⁶ lijken dit te bewerkstelligen, evenals een gefractioneerde toediening van etomidaat.^{17,18}

Het daadwerkelijke mechanisme dat verantwoordelijk is voor het ontstaan van deze bewegingen is nog niet bekend. Het lijkt echter onwaarschijnlijk dat deze het gevolg zijn van epileptogene activiteit.¹⁷ Meerdere studies hebben een elektro-encefalogram opgenomen tijdens toediening van etomidaat en hebben geen epileptiforme paroxysmen gevonden die tegelijkertijd optraden met de onwillekeurige spierbewegingen.^{17,19-21} Er werd echter geen epileptiforme activiteit gezien op het EEG tijdens het optreden van de spierbewegingen. Ook traden er geen onwillekeurige spierbewegingen op tijdens de epileptische insulten. Bij ABP-700 is er geen volledig EEG afgenomen tijdens de klinische studies. Gebaseerd op de resultaten uit **hoofdstuk 4** is het echter zeer onwaarschijnlijk dat de gezonde vrijwilligers in deze klinische studies epileptische insulten hebben doorgemaakt.

Doenicke et al. en Kugler et al. formuleerden de hypothese dat de oorzaak van de onwillekeurige spierbewegingen die optreden bij etomidaat ligt in een tijdelijke disbalans van de werking van het anestheticum op verschillende plekken binnen het centrale zenuwstelsel.¹⁷

Deze hypothese postuleert dat lage concentraties van het anestheticum remmende neuronale circuits eerder onderdrukken dan de exciterende neuronale circuits. Mogelijke verklaringen voor deze disbalans zijn verschillen in lokale cerebrale bloedstroming of verschillen in affiniteit.²⁰ Deze verklaringen worden ondersteund door de observatie uit verschillende studies die laten zien dat middelen die het centrale zenuwstelsel dempen de incidentie van onwillekeurige spierbewegingen reduceren (zie hierboven) en dat hogere doses van etomidaat en ABP-700 meer spierbewegingen veroorzaken.

In het PKPD-model uit **hoofdstuk 5** is de secundaire effect-site die nodig was voor een goed passend model geassocieerd met een verhoogd risico op onwillekeurige spierbewegingen. Lagere waarden van de EC_{50} van deze ontremmende effect-site werden vastgesteld bij proefpersonen die ook vermeerderde spierbewegingen vertoonden. De EC_{50} voor deze effect-site was hoger bij proefpersonen die gepremediceerd werden met opiaten of benzo-diazepines. Deze observatie ondersteunt de hypothese van Doenicke en Kugler dat er een niet gesynchroniseerd begin van klinisch effect bestaat op verschillende plekken in het centrale zenuwstelsel.

De vraag is dan wat deze disbalans veroorzaakt. Het is aannemelijk dat de onwillekeurige spierbewegingen op moleculair niveau gemoduleerd worden door de $GABA_A$ -receptor. McGrath et al. stelden eerder vast dat als de moleculaire structuur van etomidaat dusdanig aangepast wordt dat de molecule de $GABA_A$ -receptor niet meer moduleert, er geen spierbewegingen meer voorkomen bij ratten.²² Merk op dat in **hoofdstuk 5** er ook een interindividuele variabiliteit bestaat voor vatbaarheid voor onwillekeurige spierbewegingen. Er zijn verschillende verklaringen voor deze observatie die tegelijkertijd ook de disbalans in effect van het anestheticum kunnen verklaren. Een mogelijke verklaring is dat er een verschil zou kunnen bestaan in de verdeling van verschillende subtypen van de $GABA_A$ -receptor.²³ Omdat etomidaat, en daarmee ook ABP-700, een zeer specifieke aanhechtingsplaats heeft op de $GABA_A$ -receptor, zou een verschil in verdeling van subtypes binnen het CZS een hogere vatbaarheid voor spierbewegingen kunnen veroorzaken. Propofol bindt bijvoorbeeld minder specifiek op de $GABA_A$ -receptor, wat zou kunnen verklaren waarom de incidentie en de ernst van spierbewegingen minder zijn bij propofol dan bij etomidaat en ABP-700. Een andere verklaring zou kunnen zijn dat aangezien ABP-700 en etomidaat een zeer snelle werking vertonen, er een interindividuele variabiliteit in verdeling en/of metabolisme van het anestheticum bestaat.

Conclusies en perspectieven voor de toekomst:

In het eerste deel van dit proefschrift is het nieuwe anestheticum ABP-700 onderzocht en is er gekeken in hoeverre deze het ideale anestheticum benadert. ABP-700 lijkt positieve eigenschappen te hebben, zeker wat betreft hemodynamische en respiratoire stabiliteit. Het vertoont echter ook een ongewenste bijwerking die al zeer zorgelijk bleek voor andere GABA_A-receptor agonisten in ontwikkeling: het optreden van onwillekeurige spierbewegingen. Hoewel deze bewegingen niet van epileptiforme origine lijken te zijn, zijn ze desalniettemin onwenselijk voor de klinische praktijk en moet de toegevoegde waarde van ABP-700 als een praktisch toepasbaar anestheticum kritisch worden bekeken. Na het afronden van de vijf fase I studies beschreven in dit proefschrift, werd er een fase II studie opgestart om ABP-700 te onderzoeken in een sedatie setting. Helaas werd deze studie voortijdig gestopt omdat de toenmalige sponsor er voor koos om niet meer in dit middel te investeren. Een farmaceutisch bedrijf kan op verschillende gronden besluiten om het geneesmiddelenonderzoek van een bepaald middel te stop te zetten. Financiële aspecten spelen vaak een grote rol, vooral als een molecule op het eerste gezicht niet veelbelovend lijkt. In sommige gevallen wordt het intellectuele eigendom van de molecule verkocht aan een ander farmaceutisch bedrijf of een holding die ervoor kan kiezen om het onderzoek nieuw leven in te blazen. Binnen de anesthesie zijn er verschillende voorbeelden van molecules die werden afgeschreven door hun eerdere sponsors, maar vervolgens werden opgepakt door andere bedrijven die het succesvol op de markt brachten. Voorbeelden hiervan zijn sevofluraan²⁴ en remimazolam^{2,5,26}. Dus zelfs als de ontwikkeling van ABP-700 op dit moment gestopt is, betekent dat niet dat het niet opnieuw wordt opgepakt.

Het tweede deel van dit proefschrift is meer gericht op het ontdekken van de origine van de onwillekeurige spierbewegingen die veelvuldig werden gezien bij ABP-700. Dit fenomeen is een probleem voor verschillende GABA_A-receptor agonisten zoals propofol, maar ook experimentele anesthetica zoals AZD3043.²⁷ In dit proefschrift kon worden geconcludeerd dat de spierbewegingen hoogstwaarschijnlijk niet van epileptische origine zijn en dat het eerder te maken heeft met een disbalans in het centrale zenuwstelsel tijdens inductie van narcose. Dit wordt verder ondersteund door het PKPD-model uit **hoofdstuk 5** waarin het nodig was om een secundaire effect-site toe te voegen aan het model om te corrigeren voor de excitatie effecten op de BIS. Aangezien de spierbewegingen een toenemend struikelblok blijken voor nieuwe GABA_A-receptor agonisten, is het belangrijk om het mechanisme achter dit fenomeen verder te bestuderen en te begrijpen.

Tijdens de voorbereidingen op de fase I studies van ABP-700 werden de hoge incidentie en ernst van onwillekeurige spierbewegingen niet verwacht. Als dat wel zo was geweest, hadden we een complete afname van het EEG in het protocol geïmplementeerd. Voor toekomstige studies is het essentieel dat EEG-metingen worden opgenomen in het protocol. Ten eerste om daadwerkelijk te bevestigen dat de oorzaak van onwillekeurige spierbewe-

gingen niet in de epileptiforme hoek ligt en ten tweede, waarvoor ook fMRI studies van belang zouden kunnen zijn, om te bestuderen of in sommige delen van het brein er eerder een effect van het anestheticum zichtbaar is dan in andere delen. Dit zou de hypothese dat onwillekeurige spierbewegingen het gevolg zijn van een disbalans binnen het centrale zenuwstelsel ondersteunen.

Met dit proefschrift wordt getracht handvatten te geven voor toekomstige ontwikkelingsprogramma's voor nieuwe anesthetica in het algemeen, en GABA_A-receptor agonisten in het bijzonder. Hopelijk dient het als een wegwijzer voor onderzoekers die zich met hun potentiële, nieuwe anestheticum vol goede moed op hun lange en kronkelende weg begeven naar hun beoogde eindbestemming: goedkeuring van de benodigde instanties en toepassing in de klinische praktijk.

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CHAPTER 8

Summary, discussion and future perspectives in German/Deutsche Zusammenfassung, Diskussion und Zukunftsperspektiven

Teilweise adaptiert aus: Etomidate and its analogues: *A Review of Pharmacokinetics and Pharmacodynamics*, Clinical Pharmacokinetics, 2021, online ahead of print

Beatrijs I. Valk, Michel M.R.F. Struys

In dieser Dissertation wird ein Teil der Entwicklung des neuen Anästhetikums ABP-700 beschrieben. Verschiedene pharmakologische Herangehensweisen werden angewandt, um die Wirkung des Moleküls sowohl auf Rezeptor-Niveau als auch auf systemischem Niveau zu verstehen. Diese Arbeit kann grob in zwei Teile aufgeteilt werden. Der erste Teil beschreibt die grundsätzliche Sicherheit und Wirksamkeit eines experimentellen Anästhetikums. Im zweiten Teil werden Beobachtungen aus dem ersten Teil anhand dieses Moleküls in Hinsicht auf die Wirkung von Anästhetika durch Modulation am GABA_A Rezeptor näher erläutert.

Kapitel 1 enthält Hintergrundinformationen über den heutigen Stand der Medikamentenforschung in der intravenösen Anästhesie. Der Wirkungsmechanismus des GABA_A Rezeptors, des Rezeptors an dem die meisten intravenösen Anästhetika andocken, wird erklärt. Es werden die intravenösen Anästhetika, die heutzutage am häufigsten verwendet werden und die aktuellsten Entwicklungen in der Erforschung neuer Anästhetika beschrieben. **Kapitel 1** stellt ABP-700 vor, den GABA_A Rezeptoragonist, der in dieser Arbeit untersucht wird. Die Gründe und Gedankengänge hinter der Entwicklung von ABP-700 und vergleichbaren Molekülen sowie der präklinische Erforschungsprozess werden erläutert. Außerdem wird der generelle Prozess der Medikamentenforschung kurz erklärt um die Entwicklung von ABP-700 einzuordnen. In **Kapitel 1** werden die Eigenschaften eines „idealen“ Anästhetikums umrissen: ein schneller Beginn der Wirkung, ein ebenso schnelles Ende des hypnotischen Effektes, minimale Beeinflussung von Hämodynamik und Spontanatmung sowie ein Minimum an Nebenwirkungen.

In **Kapitel 2** und **Kapitel 3** wird untersucht wie nahe ABP-700 diesem idealen intravenösen Anästhetikum kommt. Hierzu werden die allgemeine Sicherheit, Wirksamkeit und Verträglichkeit verschiedener Dosisschemata von ABP-700 betrachtet.

Kapitel 2 beschreibt die erste Studie von ABP-700 am Menschen. Hierbei handelte es sich um eine doppelblinde und Placebo-kontrollierte Studie. Gesunden Probanden wurde ein Bolus von entweder ABP-700 oder einem Placebo verabreicht, wobei jede Kohorte eine höhere Dosis erhielt als die vorherige. Die niedrigste ABP-700 Dosis, die verwendet wurde, war 0,03 mg/kg Körpergewicht (KG) und die höchste 1,0 mg/kg KG. Ab einer Dosis von 0,175 mg/kg KG trat ein klinischer, hypnotischer Effekt auf, definiert durch den Bispectral Index (BIS) und das sogenannte „Modified Observers Assessment of Alertness/Sedation“ (MOAA/S). Ab einer Dosis von 0,25 mg/kg KG wurde ein MOAA/S-Wert von 0 erreicht. Dieser Wert gibt an, dass der Proband nicht mehr auf Schmerzreize reagiert. Höhere Dosierungen führten generell zu einer längeren Wirkungsdauer. Die Zeit bis zum klinischen Effekt lag bei allen Dosierungen unter 30 Sekunden. Der Abfall des Bispectral Index (BIS) zeigte einen ähnlichen Verlauf; ab einer Dosierung von 0,175 mg/kg KG war der BIS-Abfall rasch und die Zeit bis zur Erholung dosisabhängig. In der in **Kapitel 3** beschriebenen Studie wurde den Probanden eine kontinuierliche Infusion von entweder ABP-700, Propofol oder einem

Placebo verabreicht. Propofol diene in diesem Fall als aktive Kontrolle. ABP-700 Dosierungen reichten von 30 µg/kg KG/min bis zu 60 µg/kg KG/min. Ein klinischer Effekt wurde innerhalb des gesamten Dosierspektrums festgestellt, wobei einer tiefen Sedierung oder Narkose entsprechende Werte ab einer Dosis von 40 µg/kg KG/min beobachtet wurden. Auffällig war, dass die BIS und MOAA/S Werte nicht in allen Fällen miteinander korrespondierten. Es wurde zum Beispiel ein Proband mittels des MOAA/S Wertes als tief sediert eingestuft, während seine BIS-Werte erhöht waren.

In **Kapitel 2** wird ein non-kompartimentelles pharmakokinetisches und ein vorläufiges dreikompartimentelles pharmakokinetisch-pharmakodynamisches Modell beschrieben. Die Analysen zeigten, dass ABP-700 in allen Dosisschemata schnell abgebaut wird. Außerdem zeigte das pharmakodynamische Modell sowohl für den BIS als auch für MOAA/S einen starken Zusammenhang zwischen der Effectsite-Konzentration und dem klinischen Effekt von ABP-700, was auf einen Schalter-ähnlichen Effekt bei ABP-700 am GABA_A Rezeptor schließen lässt. Diese Beobachtung wird unterstützt durch das MOAA/S Modell, in dem der Abstand zwischen den Effect-Site-Konzentrationen mit 50 prozentiger Wahrscheinlichkeit eines maximalen und minimalen MOAA/S Wertes sehr klein ist. Dies wird ebenfalls bestätigt durch das sigmoidale E_{max} BIS-Modell, das eine steile Kurve zeigte.

Diese Resultate zeigen, dass ABP-700 die ersten beiden Merkmale eines „idealen“ Anästhetikums besitzt: ein sehr schnelles Einsetzen der hypnotischen Wirkung binnen 30 Sekunden nach Infusionsstart, und ein schnelles Abklingen. Diese sind wünschenswerte Eigenschaften für ein intravenöses Anästhetikum, die es dem Anästhesisten erlauben die Wirkung engmaschig zu steuern.

In **Kapitel 2** und **Kapitel 3** wird gezeigt, dass ABP-700, ähnlich wie sein „Stamm-Molekül“ Etomidat und im Gegensatz zu anderen intravenösen Anästhetika wie Propofol und den Barbituraten, keine hämodynamische Beeinträchtigung verursacht. Tatsächlich war sowohl bei einer Bolus- als auch einer kontinuierlichen Gabe eher ein dosisabhängiger Anstieg der Herzfrequenz und des systolischen Blutdrucks zu beobachten. Diese Effekte verlaufen zeitlich parallel zur hypnotischen Wirkung.

Da ABP-700 von Etomidat abstammt, ist es sehr wahrscheinlich, dass es das kardiovaskuläre System auf ähnliche Weise beeinflusst. Etomidat hält die Hämodynamik stabil, da es keine sympatolytische Reaktion erzeugt und gleichzeitig autonome Reflexe wie den Baroreflex aufrechterhält.¹ Dies ist durch eine agonistische Wirkung an den α₂-Adrenorezeptoren zu erklären, vor allem am α_{2B} – Adrenorezeptor, welcher für die periphere vasokonstriktive Reaktion auf hypotensive Effekte verantwortlich ist.² Indem der Sympathikustonus und die autonomen Reflexe erhalten bleiben und Etomidat und ABP-700 keine analgetische Wirkung zeigen, werden die Reize zum Beispiel einer Laryngoskopie oder endotrachealen Intubation nicht gedämpft. Hierzu müsste zusätzlich ein Opiat in adäquater Dosierung

verabreicht werden.³ In den Studien in **Kapitel 2** und **Kapitel 3** wurden Tachykardien und ein systolischer Blutdruckanstieg bereits bei Abwesenheit der obengenannten Reize beobachtet. Es ist daher anzunehmen, dass eine Laryngoskopie oder endotracheale Intubation die Inzidenz und die Ausprägung der Tachykardien und des Hypertonus weiter verstärken. Höhere Dosierungen von ABP-700 waren zusätzlich mit exzitatorischer Symptomatik wie zum Beispiel unwillkürlichen Muskelzuckungen assoziiert. Es ist möglich, dass diese kardiovaskuläre Hyperdynamik von einem generellen Erregungsstatus mitverursacht wird.

ABP-700 zeigt ähnlich wie Etomidat eine bemerkenswerte respiratorische Stabilität. Es erzeugte, im Gegensatz zu einigen anderen intravenösen Anästhetika, keine zentrale Apnoe und bei keinem der Studienteilnehmer wurden signifikante Sättigungsabfälle beobachtet. Bei einigen Probanden, die ABP-700 verabreicht bekommen hatten, wurden lediglich kurze, klinisch nicht relevante Apnoe-Episoden beobachtet. Die Atemfrequenz der Probanden, die ABP-700 verabreicht bekamen, war höher im Vergleich zu den Kontrollen. Dies führte jedoch nicht zu einem signifikanten Unterschied der PaCO_2 -Werte.

ABP-700 erfüllt also größtenteils auch die 3. und 4. Voraussetzung eines idealen Anästhetikums, nämlich die eines minimalen Effekts auf Hämodynamik und Spontanatmung. Die Zunahme der Herzfrequenz und des systolischen Blutdrucks sind zwar nicht ideal, dennoch sind sie im klinischen Alltag einer kardiovaskulären Depression vorzuziehen. Darüber hinaus würde man im klinischen Einsatz ABP-700 beispielsweise zusammen mit einem Opiat verabreichen, welches die Effekte von ABP-700 auf den Sympathikus dämpfen und damit die Zunahme von Herzfrequenz und Blutdruck verringern sollte.

Im Gegensatz zu Etomidat verursachten sowohl ein Bolus als auch eine kontinuierliche Infusion von ABP-700 keine Nebenniereninsuffizienz. Der Plasma-Cortisol Spiegel nach einem ACTH-Stimulationstest bei ABP-700-Probanden war nicht signifikant unterschiedlich verglichen mit dem der Kontrollpersonen. Diese Beobachtung stimmt mit den Resultaten der präklinischen Studien überein. Eine Nebenwirkung, die bei Probanden nach Verabreichung von ABP-700 relativ häufig beobachtet wurde, war das Auftreten von unwillkürlicher Muskelaktivität (Involuntary Muscle Movement, IMM). In der Bolusstudie, die in **Kapitel 2** beschrieben wird, zeigten 36 von 50 Probanden (72%), die ABP-700 bekamen, IMM. In der Infusionsstudie aus **Kapitel 3** waren dies 20 von 25 (80%) der Probanden. Die unwillkürliche Muskelaktivität variierte zwischen einem dezenten Zucken eines einzelnen Fingers und Myoklonien am ganzen Körper. Die Inzidenz und die Schwere der IMM nahm mit steigender Dosierung zu.

Etomidat wird mit Postoperativer Übelkeit und Erbrechen (Postoperative Nausea and Vomiting, PONV)^{4,5} in Verbindung gebracht, wobei von Inzidenzen bis 40% gesprochen wird. Jedoch fanden Studien, die die Lipidemulsionen von Etomidat mit der von Propofol verglichen, keine signifikanten Unterschiede in der Inzidenz von PONV. Dies legt nahe, dass

die Emetogenität von Etomidat ihren Ursprung in der Formulierung des Präparates hat, und nicht im Anästhetikum selbst.⁶ ABP-700 zeigt ebenfalls emetogene Eigenschaften, allerdings ist die Inzidenz relativ moderat. In der Bolusstudie litten 2 von 50 Probanden (4%) unter PONV, bei der kontinuierlichen Infusion waren es 6 von 50 Probanden (24%). Die Tatsache, dass ABP-700 nicht in einer Lipidemulsion gelöst ist, erklärt möglicherweise die niedrigere Inzidenz von PONV im Vergleich zu Etomidat. Eine bei ABP-700 relativ selten beobachtete Nebenwirkung war Injektionsschmerz, ein Phänomen, das ebenfalls bei mehreren anderen intravenösen Anästhetika wie Propofol und Etomidat beschrieben wird.

Alle relevanten Nebenwirkungen von ABP-700 sind auch bei Etomidat zu beobachten. Eine Nebenniereninsuffizienz findet sich jedoch nicht, so dass ABP-700 gegenüber Etomidat eine grundlegende Verbesserung in Hinsicht auf das Nebenwirkungsprofil darstellt. Die klinisch relevanteste Nebenwirkung von Etomidat ist die unwillkürliche Muskelaktivität. Die Ursache hiervon konnten in den Phase I Studien in den Kapiteln 2 und 3 nicht eruiert werden.

In den **Kapiteln 2 und 3** wurde darüber hinaus der Effekt einer Prämedikation mit 1,0 mg/kg KG Fentanyl untersucht. Fentanyl wurde zusätzlich zu den ABP-700 Dosierungen gegeben, die das günstigste Verhältnis zwischen klinischem Effekt und Nebenwirkungen zeigten. Für die Bolus Studie waren das 0,25 mg/kg KG und 0,35 mg/kg KG, für die Infusionsstudie 50 µg/kg KG/min. Die Gabe von Fentanyl änderte die Zeit bis zum Einsetzen der hypnotischen Wirkung, die Wirkungsdauer und die Zeit bis zur Erholung nicht signifikant. Allerdings war ein ausgeprägter Abfall der BIS-Werte zu beobachten. Zudem konnte Fentanyl die Inzidenz und das Heftigkeit von IMM reduzieren.

ABP-700 hat also vorteilhafte Eigenschaften für ein intravenöses Anästhetikum: es sorgt für ein schnelles Einsetzen und Abklingen des klinischen Effekts, es verursacht keine kardiovaskuläre oder respiratorische Depression und hat ähnliche Nebenwirkungen wie Etomidat ohne eine Nebenniereninsuffizienz zu verursachen. Das kardiovaskuläre Profil von ABP-700 könnte es zu einem geeigneten Induktionsanästhetikum für hämodynamisch instabile Patienten machen. Allerdings ist das Auftreten von unwillkürlicher Muskelaktivität eine beunruhigende Nebenwirkung. Neben dem unbekannten Ursprung dieser Aktivität – worauf im weiteren Verlauf noch näher eingegangen werden wird – könnte die potentielle Auswirkung auf den Einsatz im klinischen Alltag erheblich sein, wenn sie nicht zum Beispiel durch gleichzeitige Verabreichung von Opiaten oder Benzodiazepinen adäquat kontrolliert werden kann. Wie schon besprochen, führt die gleichzeitige Verabreichung von Fentanyl und auch von Benzodiazepinen zu einer Reduktion von unwillkürlicher Muskelaktivität. Diese Medikamente beeinflussen allerdings auch den Atemantrieb und die Wirkungsdauer des Hypnotikums. Man könnte natürlich auch Muskelrelaxantien verwenden um die unwillkürliche Muskelaktivität komplett zu eliminieren; dies würde allerdings dazu führen, dass der Patient intubiert werden müsste, und der vorteilhafte Effekt der Aufrechterhaltung der selbstständigen Atmung bei ABP-700 wäre zunichte gemacht. Darüber hinaus kann der

„Schalter-Effekt“ von ABP-700 im Klinikalltag durchaus eine Herausforderung darstellen. Ein zu schneller Wirkungseintritt und vor allem ein zu schnelles Ende des hypnotischen Effekts wären bei der Anwendung als Induktionsmittel unerwünscht. Der Anästhesist sollte zum Beispiel genug Zeit haben, um die Narkose auf ein volatiles Unterhaltsanästhetikum umzustellen und nicht befürchten müssen, dass der Patient noch während der Intubation aufwacht. Ob ABP-700 gerade schnell genug (aus)wirkt oder doch zu schnell ist noch unklar. Um diese Frage beantworten zu können, müsste in weiteren Studien untersucht werden, ob beispielsweise eine Laryngoskopie nach einem ABP-700 Bolus toleriert wird. Außerdem wäre es ratsam, in derartigen Studien eine klinisch wirksame Dosis eines Opiates zusätzlich zu ABP-700 zu verabreichen, um den Klinikalltag so genau wie möglich zu simulieren.

Zusammenfassend ist ABP-700 ein Hypnotikum, das durchaus vorteilhafte Eigenschaften hat. Sein Mehrwert im Klinikalltag ist im Vergleich zu den etablierten Anästhetika noch unklar.

Wie zuvor bereits erwähnt, ist die auffälligste Nebenwirkung von ABP-700 das Auftreten von unwillkürlicher Muskelaktivität, oft in Kombination mit anderer exzitatorischer Symptomatik wie Tachykardien, Hypertonus und Tachypnoe. In **Kapitel 4** und **Kapitel 5** wird die Ätiologie dieser Muskelaktivität und der potentielle Entstehungsmechanismus mittels verschiedener pharmakologischer Herangehensweisen weiter untersucht: Pharmakologische Methoden in vitro und in vivo in **Kapitel 4** und pharmakokinetisch-pharmakodynamisches Modeling an Probandengruppen in **Kapitel 5**.

In **Kapitel 4** wird erforscht ob die IMM von konvulsiver Natur sind. Hierzu wurden im Tierversuch vierzehn Beagles über zwei Stunden suprathérapeutische Dosen ABP-700 verabreicht. Bei allen vierzehn Hunden traten IMM auf. Fünf von vierzehn Hunden erlitten einen epileptischen Anfall. Allerdings traten diese Phänomene allesamt zu verschiedenen Zeitpunkten auf und auch das Elektroenzephalogramm zeigte Unterschiede. Die IMM fanden meist während der ABP-700 Infusion statt, die epileptischen Anfälle hingegen in den Minuten und Stunden nach dem Ende der Infusion. Diese Beobachtung hat zu unserer Hypothese geführt, dass die Ätiologie der IMM und der Krampfanfälle unterschiedlich sind, und, dass die Krampfanfälle nicht von ABP-700 hervorgerufen wurden, sondern von hohen Plasmakonzentrationen des Metaboliten CPM-acid. Um diese Hypothese zu testen wurden elektrophysiologische Experimente durchgeführt. Auf der Oberfläche von *Xenopus laevis* Oozyten wurden der $\alpha_1\beta_3\gamma_{2L}$ GABA_A Rezeptor und der $\alpha_1\beta$ Glycin Rezeptor, zwei Rezeptoren, die in der Anästhesiologie von grundlegender Bedeutung sind, exprimiert. An diesen wurden die konzentrationsabhängigen, modulierenden Effekte von ABP-700 und CPM-acid analysiert. Die Experimente zeigten, dass hohe Konzentrationen von sowohl ABP-700 als auch CPM-acid den GABA_A Rezeptor und den Glycin-Rezeptor hemmen, ein bekannter Mechanismus der Epilepsie. Konzentrationen von CPM-acid, die hoch genug sind um zur Hemmung des GABA_A Rezeptors zu führen, wurden tatsächlich bei den Hunden festgestellt,

jedoch waren diese CPM-acid Konzentrationen ungefähr einhundert Mal höher als die höchste Konzentration, die in den klinischen Studien am Menschen gemessen wurde.

Weil bei Probanden, bei denen IMM aufgetreten waren die BIS und MOAA/S Werte regelmäßig nicht korrespondierten, wurde das Verhältnis zwischen IMM und den Messungen des klinischen Effektes in **Kapitel 5** weiter analysiert. Hierzu wurde ein rezirkulatorisches pharmakokinetisch-pharmakodynamisches Modell entwickelt, welches die Daten von 266 Probanden aus fünf Phase I Studien beinhaltet. Die Pharmakokinetik von ABP-700 war vergleichbar mit der des vorläufigen Dreikompartimentmodelles aus **Kapitel 2**. Sie wird gekennzeichnet durch kleine Kompartimentvolumina und eine schnelle Ausscheidung. Für das pharmakodynamische BIS-Modell musste eine sekundäre Effektsite in das Modell aufgenommen werden um ein gut funktionierendes Modell zu schaffen: neben der suppressiven Effektsite wurde eine „exzitatorische“ Effektsite hinzugefügt. Diese sekundäre Effektsite steht mit dem Auftreten der unwillkürlichen Muskelaktivität in Verbindung und die Schwelle hierfür nimmt mit zunehmendem Alter ab. Darüber hinaus konnte eine Prämedikation mit einem Opiat oder einem Benzodiazepin den Erregungseffekt, oder auch Enthemmungseffekt, reduzieren.

Für Etomidat wurden bereits mehrere Methoden erprobt um das Auftreten solcher Bewegungen zu reduzieren oder gänzlich zu verhindern. Medikamenten mit einer unterdrückenden Wirkung auf das zentrale Nervensystem wie Opiate,⁷⁻¹⁰ Benzodiazepine,^{11,12} Dexmedetomidin,^{13,14} Thiopental,¹³ Lidocain¹⁵ und Magnesium¹⁶ scheint dies zu gelingen. Eine andere Möglichkeit ist die fraktionierte Gabe von Etomidat.^{17,18}

Der tatsächliche Mechanismus, der verantwortlich für das Entstehen dieser Muskelaktivität ist, ist noch immer unbekannt. Es ist allerdings sehr unwahrscheinlich, dass sie eine epileptogene Ursache hat.¹⁷ Verschiedene Studien haben während Etomidatgaben ein Elektroenzephalogramm aufgezeichnet und konnten feststellen, dass unwillkürliche Muskelaktivität nicht mit epileptiformen Paroxysmen einhergeht.^{17,19-21} In den klinischen ABP-700 Studien wurde bislang kein vollständiges EEG mitaufgezeichnet. Allerdings ist es basierend auf den Resultaten aus **Kapitel 4** sehr unwahrscheinlich, dass die Probanden in den klinischen Studien tatsächlich Krampfanfälle erlitten.

Doenicke et al. und Kugler et al. präsentierten die Hypothese, dass die Ätiologie der unwillkürlichen Muskelaktivität auf ein Ungleichgewicht in der Wirkung des Anästhetikums an verschiedenen Stellen innerhalb des zentralen Nervensystems zurückzuführen sei.¹⁷ Diese Hypothese postuliert, dass niedrige Konzentrationen des Anästhetikums die hemmenden neuronalen Netzwerke früher unterdrücken als die erregenden neuronalen Netzwerke. Mögliche Erklärungen für dieses Ungleichgewicht sind Unterschiede in der lokalen zerebralen Durchblutung, oder Affinitätsunterschiede.²⁰ Dies wird unterstützt durch Beobach-

tungen verschiedener anderer Studien, dass ZNS-hemmende Prämedikation die Inzidenz der unwillkürlichen Muskelaktivität reduziert (siehe oben) und, dass höhere Dosierungen von ABP-700 oder Etomidat, mehr unwillkürliche Muskelaktivität verursachen.

Im PK-PD Modell von ABP-700, welches in **Kapitel 5** beschrieben wurde, wurde die sekundäre Effektsite mit einem Risiko für unwillkürliche Muskelaktivität assoziiert. Niedrigere EC_{50} -Werte für diese enthemmende Effektsite wurden bei jenen Probanden beobachtet, die auch eine ausgeprägtere unwillkürliche Muskelaktivität hatten. Der EC_{50} an dieser Effektsite war höher bei Probanden, die mit Opiaten oder Benzodiazepinen prämediziert wurden. Diese Beobachtung unterstützt die Hypothese von Doenicke und Kugler, dass der Beginn der Wirkung an verschiedenen Stellen im zentralen Nervensystem nicht synchron verläuft.

Die große Frage ist letztendlich was die Ursache für dieses Wirkungsungleichgewicht sein könnte. Es ist wahrscheinlich, dass auf molekularer Ebene die unwillkürliche Muskelaktivität zumindest teilweise vom $GABA_A$ Rezeptor moduliert wird. McGrath et al. konnten zeigen, dass, wenn die Struktur von Etomidat derartig verändert wird, dass sie den $GABA_A$ Rezeptor nicht mehr moduliert, keine unwillkürliche Muskelaktivität bei Ratten beobachtet werden konnte.²² Auffällig ist ebenfalls, dass im PK-PD Modell in **Kapitel 5** eine interindividuelle Variabilität bezüglich der Anfälligkeit für die unwillkürliche Muskelaktivität besteht. Es gibt verschiedene Erklärungen für diese Observation, die gleichzeitig auch das Wirkungsungleichgewicht erklären könnten. Ein Deutungsversuch ist die unterschiedliche Verteilung von verschiedenen Subtypen des $GABA_A$ Rezeptors.²³ Weil Etomidat, und damit auch ABP-700, eine sehr spezifische Bindungsstelle am $GABA_A$ Rezeptor besetzt, könnte ein Unterschied in der Verteilung der Subtypen innerhalb des ZNS eine höhere Anfälligkeit für unwillkürliche Muskelaktivität verursachen. Propofol zum Beispiel bindet weniger spezifisch am $GABA_A$ Rezeptor, was erklären könnte, warum die Inzidenz und das Ausmaß von unwillkürlicher Muskelaktivität bei Propofol niedriger ist als bei Etomidat und ABP-700. Eine andere Erklärung wäre, dass aufgrund der schnellen Wirkung von Etomidat und ABP-700 eine interindividuelle Variabilität in der Verteilung oder dem Metabolismus besteht.

Schlussfolgerungen und Zukunftsperspektiven:

Im ersten Teil dieser Dissertation wurde das neue Anästhetikum ABP-700 erforscht und beurteilt, in wie fern es einem „idealen“ Anästhetikum nahekommt. ABP-700 hat vorteilhafte Eigenschaften, vor allem in Bezug auf hämodynamische und respiratorische Stabilität. Allerdings hat es auch eine signifikante Nebenwirkung, welche sich in der Entwicklung mehrerer GABA_A Rezeptoragonisten als problematisch herausgestellt hat: die der unwillkürlichen Muskelaktivität. Obwohl diese Aktivität nicht konvulsiv zu sein scheint, ist sie dennoch unerwünscht im Klinikalltag und der Mehrwert von ABP-700 als potentielles etabliertes Anästhetikum muss weiter erforscht werden.

Nachdem die fünf in dieser Dissertation besprochenen Phase I Studien abgeschlossen waren, wurde eine Phase II Studie gestartet um ABP-700 als Sedativum zu untersuchen. Leider wurde diese Studie abgebrochen, weil der derzeitige Sponsor, The Medicines Company, sich entschieden hatte, nicht weiter in das Molekül zu investieren. Verschiedene Überlegungen können dazu führen, dass ein Pharmaunternehmen beschließt, ein Entwicklungsprogramm zu beenden. Oft spielen finanzielle Gründe eine erhebliche Rolle, vor allem, wenn ein Molekül auf den ersten Blick nicht vielversprechend erscheint. In manchen Fällen wird das geistige Eigentum des Moleküls an ein anderes Unternehmen oder an eine Holding verkauft, die sich möglicherweise dazu entschließt, die Entwicklung weiterzuführen. In der Anästhesie gibt es verschiedene Moleküle, deren Entwicklung vom ursprünglichen Sponsor gestoppt wurde, um später von anderen Betrieben weitergeführt zu werden und letztendlich erfolgreich den Markt zu erreichen. Beispiele hierfür sind Sevofluran²⁴ und Remimazolam.^{25,26} Auch wenn die Entwicklung von ABP-700 im Moment stillsteht, bedeutet das nicht unbedingt, dass sie nicht wieder aufgenommen wird.

Der zweite Teil dieser Dissertation richtet den Fokus auf die Suche nach dem Ursprung der von ABP-700 ausgelösten unwillkürlichen Muskelaktivität. Dieses Phänomen ist ebenfalls ein Problem bei verschiedenen anderen GABA_A Rezeptor Agonisten wie Propofol, aber auch experimentellen Anästhetika wie AZD3043.²⁷ In dieser Dissertation konnte konkludiert werden, dass die Muskelaktivität höchstwahrscheinlich keinen epileptischen Ursprung hat sondern eher Folge eines Ungleichgewichtes im ZNS bei Narkosebeginn ist. Diese Annahme wird gestützt durch das in **Kapitel 5** beschriebene PK-PD Modell. In diesem Modell war es notwendig eine sekundäre Effektsite zu implementieren um den erregenden Effekt auf den BIS zu berücksichtigen. Da die unwillkürliche Muskelaktivität zunehmend ein Problem für neue GABA_A Rezeptor Agonisten wird, ist es wichtig, den Mechanismus hinter diesem Phänomen weiter zu untersuchen um die Entwicklung von GABA_A Rezeptor Agonisten auch in Zukunft zu voran zu treiben.

Während des Entwurfs der Phase I Studien von ABP-700 wurde das Ausmaß der unwillkürlichen Muskelaktivität nicht vorhergesehen. Wäre das der Fall gewesen, hätten wir das Studienprotokoll mit vollständigen EEG Untersuchungen ergänzt. Für zukünftige Studien zu ABP-700 ist es essenziell, diese EEG Messungen zu inkludieren. Erstens, um zu bestätigen, dass auch beim Menschen die unwillkürliche Muskelaktivität nicht epileptogener Natur ist, und zweitens, wozu auch fMRI Studien beitragen könnten, um zu analysieren, ob in manchen Teilen des Gehirns die Wirkung des Anästhetikums schneller eintritt. Diese Studien würden die Hypothese, dass unwillkürliche Muskelaktivität von einem Wirkungsungleichgewicht verursacht wird und hemmende Hirnregionen früher betroffen sind als erregende, untermauern.

Diese Dissertation möge Einsichten für zukünftige Entwicklungsprogramme von neuen Anästhetika im Allgemeinen und speziell für GABA_A-Rezeptor Agonisten bieten. Hoffentlich bietet sie Forschern auf dem langen und kurvigen Weg auf der Suche nach einem neuen Anästhetikum einige Wegweiser in Richtung Ziel: die Zulassung durch die zuständigen Behörden und schließlich eine Implementierung im Klinikalltag.

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APPENDICES

List of abbreviations

List of publications

Curriculum Vitae

Acknowledgements / Dankwoord

List of abbreviations

ACTH	Adrenocorticotrophic hormone
AE	Adverse Event
AUC	Area under the Curve
BIS	Bispectral Index
BMI	Body Mass Index
Ce/EC	Effect-site concentration
Ce50/EC ₅₀	Concentration resulting in 50% of maximum effect
CL	Clearance
CNS	Central Nervous System
Cp	Plasmaconcentration
CPM-acid	Primary metabolite of CPMM/ABP-700
CPMM/ABP-700	Cyclopropyl-methoxycarbonyl metomidate
CV	Coefficient of variation
DEFF	Parameter for drug effect
ECG	Electrocardiograph
EEG	Electroencephalograph
E _{max}	Maximum effect
EMG	Electromyograph
Fen	Fentanyl
GABA	γ-Aminobutyric Acid
IMM	Involuntary Muscle Movements
INV	Invasive
k _{e0}	Equilibration rate constant for the effect-site compartment
MAP	Mean Arterial Pressure
MOAA/S	Modified Observer's Assessment of Alertness/Sedation
MTD	Maximum Tolerated Dose
NIBP	Non-invasive Blood Pressure
PaCO ₂	Partial pressure of carbon-dioxide
PaO ₂	Partial pressure of oxygen
PD	Pharmacodynamics
PK	Pharmacokinetics
Q	Flow
SpO ₂	Oxygen saturation
t _{1/2}	Half-life
V	Volume of compartment
γ	Steepness of slope

List of publications

- **Valk BI**, Struys MMRF, Etomidate and its analogues: A Review of Pharmacokinetics and Pharmacodynamics. *Clinical Pharmacokinetics* 2021. Online ahead of print.
- **Valk BI**, Eleveld DJ, Meyer P, Meier S, den Daas I, van Amsterdam K, Campagna JA, Sweeney SP, Absalom AR, Struys MMRF. Modeling the Effect of Excitation on Depth of Anesthesia Monitoring in γ -Aminobutyric Acid Type A Receptor Agonist ABP-700. *Anesthesiology*. 2021 Jan 1;134(1):35-51.
- **Valk BI**, McGrath M, Lehoux D, Zerler B, Marota JJA, Raines DE. Toxicologic and Inhibitory Receptor Actions of the Etomidate Analog ABP-700 and Its Metabolite CPM-Acid. *Anesthesiology* 2019 Aug; 131(2):287-304
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Curriculum Vitae

Beatrijs Irene Valk was born on the 24th of September 1994 in Amsterdam, as the eldest child of Jens Valk and Saskia Hagel. Together with her younger brother Floris, she grew up in the Drentsche Aa region, near Groningen. After graduating from the Willem Lodewijk Gymnasium in Groningen in 2012, she enrolled in medical school at the University of Groningen.

She was accepted into the Honours College of the University of Groningen during her Bachelor studies. As a part of this, Beatrijs participated in several small research projects, first at the department of Critical Care and subsequently at the department of Anesthesiology of the University Medical Center Groningen. There, under supervision of Prof. Dr. Michel Struys, her interest in performing research was sparked. She proceeded to write her master thesis at the department of Anesthesiology, which then evolved into an MD/PhD program. In this program, the last two years of the Master of Medicine are combined with two years of full-time research. During her PhD, she spent six months at the Massachusetts General Hospital in Boston, USA under supervision of Douglas Raines, where she worked on the receptor pharmacology of the GABA_A receptor. She graduated from medical school in 2020, completing her clinical rotations in Oldenburg, Germany.

During her medical studies, Beatrijs played the flute and piccolo flute at the student orchestra Mira, where she served on the board of 2015-2016 as treasurer. In her free time, she enjoys reading, baking, yoga and hiking.

Beatrijs is married to Björn Schwarze. They live together in Oldenburg, Germany. Beatrijs has started her residency training in Anesthesiology at the Universitätsklinik für Anästhesie, Intensivmedizin, Notfallmedizin und Schmerztherapie at the Klinikum Oldenburg in 2021, staying on as a post-doc researcher at the department of Anesthesiology at the UMCG.

Dankwoord / Acknowledgements

Toen ik in 2012 begon met mijn studie geneeskunde was ik ervan overtuigd dat onderzoek doen niets voor mij zou zijn. In een lab experimenten met muizen of ratten doen zonder een patiënt te zien – want toen dacht ik nog dat dat onderzoek doen is - was mijn absolute horrorscenario. Min of meer per ongeluk belandde ik toch in het onderzoekswereldje omdat ik een onderzoeksproject ‘moest’ doen in het kader van het Honours college. Daar had ik voor gesolliciteerd omdat ik naast geneeskunde ook wat anders wilde doen, zoals kunstgeschiedenis of rhetorica. Eenmaal bezig met een paar projectjes begon het enthousiasme voor research toch te kriebelen, vooral omdat het vrij weinig te maken had met mijn eerdere schrikbeeld. Deze observatie in combinatie met een inspirerende week in the middle of nowhere/Drenthe en een opmerking van mijn voormalig wiskundeleraar en mentor die in mijn achterhoofd bleef zitten, deden mij uiteindelijk overwegen een MD/PhD traject te starten. Toen zich op de afdeling anesthesiologie het voor mij perfecte PhD-project aandiende stond mijn keuze vast. Nu, zes jaar later, ben ik ontzettend trots op het resultaat. Een mooi proefschrift, ontelbare fantastische ervaringen en vele inspirerende mensen die ik op deze ‘long and winding road’ heb mogen ontmoeten. Met sommige mensen kruiste mijn weg zich slechts kort, en samen met andere mensen heb ik een groot deel van de weg af mogen leggen. Deze mensen wil ik graag ontzettend bedanken.

Allereerst mijn eerste promotor, **professor dr. M.M.R.F. Struys**. Beste Michel, toen ik als tweedejaars studentje uw kantoor binnenliep om over een klein onderzoeksproject voor het Honours college te praten had ik nog nauwelijks een idee over wat onderzoek doen inhield, laat staan dat het iets was waar ik in geïnteresseerd zou kunnen zijn. Na al die jaren kunnen we denk ik wel concluderen dat dit het geval is. In de afgelopen zeven (!) jaar ben ik grotendeels dankzij u gegroeid als wetenschapper, maar ook zeker als mens en als arts. Bedankt voor uw vertrouwen, de talloze goede gesprekken, uw goede raad en vooral de fantastische ondersteuning die ik iedere PhD-er toewens.

Mijn tweede promotor, **professor dr. A.R. Absalom**. Beste Tony, jij bent voor mij het schoolvoorbeeld van een goede hoogleraar. Zowel op klinisch als op wetenschappelijk gebied werk je op een heel hoog niveau en deel je graag je kennis met iedereen die het wil horen. Bedankt voor je substantiële bijdrages, inhoudelijk en taalkundig, aan de manuscripten, het vele sparren en je aanstekelijke enthousiasme voor het vak anesthesie.

Mijn co-promotor, **dr. Douglas Eleveld**. Beste Douglas, af en toe is het me nog steeds een raadsel hoe je in vredesnaam al die getallen en onbegrijpelijke formules om kan zetten in een prachtig, begrijpelijk PK/PD model. Maar wat ben ik blij dat dat zo is, want zonder jou was ik niet ver gekomen met mijn proefschrift. Heel erg bedankt voor je toegankelijkheid als

ik er toch weer een keer niet uitkwam, de goede gesprekken en de cake die elke maandag weer nèt iets anders was.

My supervisor at the Massachusetts General Hospital, **dr. Douglas E. Raines**. Dear Doug, this PhD-thesis literally would not have existed without you. Your team's development of ABP-700 is after all central to it. The six months you invited me to spend at MGH was one of the most inspirational, motivational, and simply most fun times of my life. Thank you very much, again, for that wonderful opportunity and for making this PhD-thesis possible.

Dear **members of the reading committee**, thank you very much for carving out the time of your busy agendas to assess this PhD-thesis. It means a lot!

Our collaborators on the other side of the pond, **Steven Sweeney** and **Dr. Jason Campagna**. Thank you for allowing me to play a substantial role in the trials of this fascinating drug, your inexhaustible enthusiasm and your valuable input into the manuscripts. Steve, thank you for providing me with a place to stay in the Boston area.

Beste **Kai**, bedankt voor het elke keer weer in ontvangst nemen van mijn USB-stickje, voor de stapels papier waar ik de artefacten uit spaghetti-plots kon omcirkelen, en je enorme hulp bij de data-analyses. Ik denk dat we nu wel kunnen zeggen dat we het Annovation-mapje voorlopig dicht kunnen laten.

Dearest **Megan**, thank you so much for showing me how to conduct our experiments, for lengthy discussions ranging from politics to baking shows, for your quirkiness, and for the regular Earl Grey Tea (with milk!). **Anita, Konstantina, Kat, Dan, Ryan, Joe, Steve, Rooma, Alissa and everyone else from Beecher Lab**, thank you for the lunch times, the dinners, the drinks, the many (many) trips to J.P. Licks and all the adventures we shared in- and outside of lab. You all have made my time in Boston one of my dearest memories that I'll cherish forever.

Beste **Peter** en **Sascha**, bedankt voor de leerzame gesprekken, de gezellige onderzoeksdagen en jullie visie op de manuscripten. Peter, bedankt dat je de tijd hebt genomen om mijn wetenschappelijke Duits nog wat bij te schaven. Sascha, bedankt voor de gezelligheid in Miami!

Het team van **QPS Groningen: Sjaak, Sandra, Gonda, Mariska en Khalid**, bedankt voor het wegwijs maken in de wereld van Adverse Events, de MedDRA, en de interessante en productieve meetings tijdens de planning van trials, al dan niet in een strandrestaurant in Miami. Lieve **Yvette, Hasan en studynurses van QPS Groningen**, bedankt voor de gezellige onderzoeksdagen. Elke keer als ik langs een Multivlaai filiaal kom, denk ik aan jullie!

Thank you to all **co-authors** not already named, who have contributed to the articles presented in this PhD-thesis. Your input is highly valued!

De **meer dan 350 vrijwilligers** die deelnamen aan de klinische studies, ontzettend bedankt voor jullie onmisbare bijdrage aan dit proefschrift. Dankzij jullie weten we weer een heel klein beetje meer over de werkingsmechanismen hypnotica.

Aan al mijn **medeonderzoekers** met wie ik in de loop van de jaren het 'hok' heb mogen delen, bedankt voor de gezellige, soms ook wat minder productieve, werkdagen, de peptalks en de inspirerende onderzoeksomgeving die we hebben mogen delen. Ik heb van jullie allemaal ontzettend veel geleerd.

De leden van het **onderzoeksbureau**. Bedankt voor jullie ondersteuning in de complexe logistiek en organisatie van de klinische studies en de vele lunch- en koffiepauzes. **Rob**, bedankt voor je scherpe blik, niet alleen op het gebied van onderzoek, en het feit dat ik altijd bij je aan mocht kloppen. Dankzij jou is mijn zelfvertrouwen een stuk beter ontwikkeld.

Het secretariaat en stafondersteuning van de afdeling Anesthesiologie, vaak op de achtergrond maar o zo belangrijk! Lieve **Joke** en **Audrey**, bedankt voor het puzzelen in drukke hoogleraaragenda's en jullie hulp en ondersteuning. Lieve **Minke**, de afgelopen jaren was jij voor mij de stabiele factor op de afdeling. Vanaf dat ik begon als 'klein studentje' tot en met nu was je er altijd als ik eens een keertje stoom moest afblazen, of ergens aan twijfelde, of gewoon als ik de weg even niet wist, een beetje als een grote zus. Daarnaast wist je altijd wel een gaatje te vinden in de agenda, en dacht je enorm mee. Heel erg bedankt daarvoor! Ik ben vereerd dat jij mijn paranimf bent.

De **Junior Scientific Masterclass**, bedankt voor het financieel mogelijk maken van dit onderzoekstraject. Bedankt voor jullie vertrouwen! **Jans** en **Maria**, bedankt voor de organisatie van de COMPASS-week, waar mijn besluit een MD/PhD-traject na te streven vast kwam te staan, en dat ik altijd even langs mocht komen voor een babbeltje.

Beste **meneer Van Kammen**, bedankt voor het planten van dit zaadje. U was de eerste die mij op het idee bracht dat ik misschien wel eens zou kunnen promoveren. Nu is het eindelijk zover. Ook heel erg bedankt voor uw ondersteuning in turbulente tijden, waar ik nog steeds veel aan heb.

Liebe **Kolleginnen und Kollegen der Abteilung AINS am Klinikum Oldenburg**, vielen Dank für die Einführung in die klinische Anästhesie. Bei euch habe ich entdeckt, dass Anästhesie auch praktisch wirklich mein Fach ist, und nicht nur theoretisch.

Lieve **Julia**, samen een jaar lang een studentenorkest runnen schept een band. Sinds ons bestuursjaar van Mira weet ik dat er maar weinig mensen zijn die ik zo goed kan vertrouwen als jij. Bedankt voor je steun, je relativerende kracht en je onuitputtelijke enthousiasme.

Lieve **Lisa**, ich bin froh, dass du meine Nachbarin warst im Personalwohnheim des Klinikums, denn seitdem bist du eine meiner besten Freundinnen geworden und seit Januar 2021 ja auch Kollegin! Vielen Dank für die Kaffee- und Teemomente, Feierabendbiere, und dein immer offenes Ohr und gute Ratschläge. Die werden sicherlich nützlich sein, wenn du als meine Paranimf auftrittst.

Lieve **opa's en oma's**, bedankt voor jullie support van kleins af aan! Ik heb aan jullie mijn nieuwsgierigheid en mijn liefde voor kunst en literatuur te danken, zodat ik ook nog wat te doen heb naast geneeskunde. Lieve opa Paul, bedankt voor het al op vroege leeftijd stimuleren van een kritische (wetenschappelijke) houding. Soms gaat mijn kritische blik misschien in een andere richting dan de jouwe, maar dat heeft me dan ook al een paar chocoladeletters opgeleverd.

Lieve **Floris**, het leven met mij als betweterige grote zus kan niet altijd makkelijk zijn geweest maar wat ben ik blij dat jij mijn grote kleine broer bent! Jouw enthousiasme voor je passies zijn voor mij een groot voorbeeld. Bedankt voor de vele bro-sis avonden in de bioscoop, de vele ritjes in verschillende skiliften en je goed getimede bezoek in Boston.

Lieve **papa en mama**, ik bof maar met ouders zoals jullie! Ontzettend bedankt voor het creëren van een omgeving waarin ik mezelf kon ontwikkelen tot de persoon die ik nu ben, en verder. Bedankt dat ik altijd bij jullie terecht kan voor advies, voor jullie ondersteuning en voor het regelmatig voorhouden van een spiegel. Lieve mama, bedankt voor je relativerende vermogen en het meeleven met alles wat ik doe. Zonder een aantal “Kop d'r veur”s van jou, had dit proefschrift er nu niet gelegen. Bedankt daarvoor! Lieve papa, dat ik, net als jij, voor het vak anesthesie heb gekozen, is natuurlijk geen toeval, al heeft dat denk ik meer te maken met het erven van jouw genen dan de motivatie “omdat mijn vader het ook doet”. Sinds mijn keuze voor de anesthesie ben jij voor mij een unieke combinatie van mentor in het leven en mentor in het vak. Bedankt voor het vervullen van deze speciale rol!

Liefste **Björn**, wat ben ik trots en bevoorrecht dat ik jou aan mijn zijde mag hebben. Bedankt voor je liefde, je heerlijke eten, je onvoorwaardelijke steun en je humor. Als ik weer eens een keer nerveus of onzeker ben weet jij me altijd om mezelf te laten lachen. Naast mijn steun en toeverlaat, ben je ook een groot voorbeeld in je doorzettingsvermogen, zelfdiscipline en optimisme. Het is de afgelopen jaren niet altijd eenvoudig geweest, niet in de laatste plaats omdat we op een gegeven moment op een halve wereld afstand van elkaar woonden na jaren heen en weer trainen, maar toch ook weer wel, omdat we allebei wisten waar we het uiteindelijk voor deden. Gelukkig is nu de gemiddelde afstand die we moeten bereizen om elkaar te zien die van de woonkamer naar de keuken. Ik weet niet waar de 'long and winding road of life' ons nog allemaal heen gaat voeren, maar ik zou me geen betere reisgenoot kunnen wensen dan jij. Van één ding ben ik absoluut overtuigd: dit is de bedoeling!