Sigma-1 Receptor Imaging in the Brain

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Plasma and brain pharmacokinetics of cutamesine in rats

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ABSTRACT

Cutamesine is a sigma-1 subtype-selective receptor agonist (IC_{50} = 17.4 nM), a novel small molecule, with activity in preclinical models of CNS diseases and currently being studied in humans (phase II clinical trials) for depression and stroke indications. There is no literature available that reports the plasma and brain distribution of this centrally acting compound. In this context, we performed a pharmacokinetic study in Wistar rats, to evaluate plasma and brain disposition of cutamesine at the pharmacologically active doses of 0.3 and 1.0 mg/kg. Following intraperitoneal administration, plasma concentrations of cutamesine decreased bi-exponentially and the elimination half-life in plasma and brain were found to be 1.6-3.2 h and 5.1h, respectively. Systemic clearance and central volume of distribution of cutamesine were found to be 5.9 (L/h/kg) and 27.2 L/kg, respectively. The peak levels and $AUC_{(0-\infty)}$ of brain were 2.4 and 5.2 times, respectively, higher compared to the plasma. Cutamesine was shown to have adequate PK profiles that are required for desired CNS effects. In addition, the levels of cutamesine in brain remained above the IC_{50} value of sigma-1 receptor for up to 16 h of post dosing, suggesting that appreciable levels of drug is available to exert its therapeutic actions. This finding also suggests that this drug may be dosed once daily in the clinic.

Keywords: cutamesine, pharmacokinetics, LC-MS, plasma distribution, brain distribution

INTRODUCTION

Cutamesine, (SA4503), is being developed as a potential drug for the treatment of several central nervous system (CNS) disorders including post stroke recovery, major depression, and cognitive deficit *(1, 2, 3, 4)*. Activity for the above mentioned therapeutic indications is postulated due to its high affinity and selectivity for human sigma-1 receptors (IC_{50} = 17.4 nM). It has also been shown to be effective in several pre-clinical animal models *(5)*. However, to our knowledge, other than a mention in a review article *(6)*, there is no literature available that reports the plasma and brain pharmacokinetics of this centrally acting compound. In this context, we performed a pharmacokinetic study in rats to evaluate plasma and brain disposition of cutamesine, at the pharmacologically active doses of 0.3 and 1.0 mg/kg. This observed data was subsequently used to evaluate a population-based pharmacokinetic (PK) model developed for cutamesine from its ^{11}C-labelled tracer (Chapter 9). In addition, this PK data could be used to understand the exposure-response (receptor occupancy- behavioural study outcome) relationship (Chapter 7).

MATERIALS AND METHODS

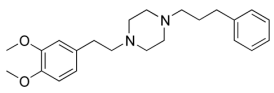
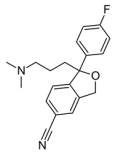
Chemicals and Reagents

Cutamesine (Table 1) was obtained from M's Science Corporation, Kobe, Japan. Citalopram, used as an internal standard, was obtained from Trademex Pharmaceuticals and Chemicals, Shanghai, China. HPLC grade methanol and acetonitrile were obtained from Biosolve Chimie, the Netherlands. Ammonium bicarbonate was obtained from Sigma Aldrich, St.Louis, MO, USA. All other reagents used were of laboratory reagent grade and were used without further purification. All aqueous solutions for UPLC and LC-MS/MS mobile phase were prepared in Milli Q (Millipore, Milford, MA, USA) grade water. Naive rat arterial blood (plasma) and brain were harvested from Wistar Hannover rats purchased from Harlan, Boxmeer, The Netherlands.

Animals

Experiments were performed in male Wistar Hannover rats purchased from Harlan, the Netherlands. The rats were housed in Macrolon cages on a layer of wood shavings in a room with constant temperature (21 ± 2°C) and fixed 12-hour light-dark regime (light phase from 7:00 to 19:00 hours). Food (standard laboratory chow, RMH-B, Hope Farms, The Netherlands) and water were available ad libitum. After arrival the rats were acclimatized for at least seven days. Data for this study was obtained from the animals in Chapter 7. Some of the animals were used in behavioral studies; a wash out period of at least two weeks was allowed before the performance of the pharmacokinetic study described here. Other animals were used in microPET scans for receptor occupancy estimation; samples

Table 1. LC-MS parameters for cutamesine and internal standard

Compound	Molecular Weight	Structure
Cutamesine	368.24	
Citalopram (IS)	324.39	

collected during that scan, between 75 to 180 min post-dosing, were included in the present study. Experiments were performed by licensed investigators in compliance with the Law on Animal Experiments in The Netherlands. The protocol was approved by the Committee on Animal Ethics of the University of Groningen.

Pharmacokinetic study

A femoral artery of the rats was cannulated under isoflurane anesthesia and they were maintained under anesthesia for the duration of the experiment. They were injected intraperitoneally with either 0.3 mg/kg or 1.0 mg/kg of cutamesine dissolved in saline. From various animals in the 1.0 mg/kg group, blood samples were collected at 1, 2, 5, 15, 30, 45, 60, 75, 90, 120, 300 and 1440 min after injection. One animal each was terminated by guillotine at 2, 15 and 120 min, two animals each at 30, 60 and 90 min and additional blood and brain samples were collected at the point of termination. Two uncannulated animals each were terminated at 300 and 1440 min after dosing and blood and brain samples collected without sampling at earlier time points. In the 0.3 mg/kg group, blood sampling was performed as with the 1.0 mg/kg group and one animal each was terminated at 2, 15, 30, 60 and 120 min and further blood and brain samples collected. Additional blood and brain samples collected between 75 to 180 min during the microPET scan study with ^{11}C-cutamesine were included in the analysis (Table 2). The collected blood and brain were immediately placed on crushed ice and plasma obtained by centrifugation. The plasma and brain samples were then stored at -80°C till further analysis.

Analytical procedure

Liquid chromatography- mass spectrometric conditions

Quantitative analysis of cutamesine in plasma and brain was conducted using a Xevo G2 QTof mass spectrometer (Waters Corporation) with ACQUITY UPLC (Waters, Milford, MA, USA). The detectors were UV-detector and time of flight. The analytical column ACQUITY UPLC BEH C18 1.7 µm (2.1 × 50mm, (Waters

Table 2. Number of samples collected at each time point. Parenthesis shows the samples from the microPET scan study

	Time (min)												
	1	**2**	**5**	**15**	**30**	**45**	**60**	**75**	**90**	**120**	**180**	**300**	**1440**
1 mg/kg plasma	1	1	8	8	7	5	5	3 (1)	3 (4)	1 (4)	(3)	2	2
1 mg/kg brain	-	1	-	1	2	-	2	-	2	1*	(4)	2	2
0.3 mg/kg plasma	1	1	4	4	3	2	2	1 (3)	1 (2)	1 (2)	(4)	-	-
0.3 mg/kg brain	-	1	-	1	1	-	1	-	-	1	(1)	-	-

* Data lost during sample preparation

Corporation) was used for the chromatographic separation. Column temperature was set as 40 °C. The flow rate was 0.6 mL/min, starting with 95% of A which goes to 20% in 1.5 min, and goes back to 95% at 2.7 min. The mobile phase A consisted of 10 mM ammonium bicarbonate adjusted to pH 9.4 with 25% ammonia and mobile phase B was acetonitrile. The sample volume injected was 3 µL and the auto sampler was set at 10 °C. The run time was 4 min. LC-MS was operated in positive ionization mode from 50 to 1200 Da. Masslynx V4.1 SCN 803 software was used to operate the LC-MS. Capillary voltage was 0.5 kV. Source temperature and desolvation temperature were set to 150 °C and 600 °C, respectively. Cone gas flow and desolvation gas flow were set to 10 and 1000 l/h, respectively.

Sample preparation

The brain tissue was homogenized with 1 volume of water using Mini bead beater-24, (Biospec products, Bartlesville, OK, USA) for 45 sec at 2.5 (× 1000) oscillations per min.

Stock solutions of cutamesine and citalopram in methanol were prepared at the strength of 1 mg/mL. Working standard solutions of cutamesine of 10-10,000 ng/mL were prepared in methanol from the first stock solution. Citalopram at 100 ng/mL in 80% acetonitrile and 20% 10 mM ammonium bicarbonate was used as internal standard (IS) solution. These stock solutions were stored at approximately 4^0C.

For the calibration curve, various concentrations (for plasma analysis: 0.2, 0.5, 1, 2, 3, 5, 10, 20, 30, 50 ng/mL and for brain analysis: 0.5, 1, 2, 5, 10, 20, 50, 100, 200, 500 ng/mL) of cutamesine were prepared in methanol to a final volume of 50 µL. To this, 50 µL of diluted (1:1 with water) blank plasma or brain homogenate was added.

Similarly, 50 µL of diluted (1:1 with water) plasma or brain homogenate samples were combined with 50 µL of methanol. Protein precipitation was achieved in the samples and calibration curve by the addition of 175 µL of the IS solution, followed by vigorous shaking for 20 min and centrifugation for 15 min (3200 × g for plasma and 7800 × g for brain). The supernatant was directly injected in the UPLC–MS system. A total blank (without analyte and IS) and blank

(without analyte) were injected at the start of the calibration curve and again at the end to test carryover of analytes. Washes were included between standards and samples and between different sample groups.

The methods were tested for signal-response of the calibration standards. The peak area ratios of the cutamesine to the IS versus that of concentration of calibration standards were plotted. The best-fit line was determined by least squares linear regression of the calibration data using a weighting factor of 1/X, where X is the concentration. Concentrations of the analytes were determined using peak area ratios and the regression parameters *(7)*.

Pharmacokinetic data analysis

Pharmacokinetic parameters were calculated by a non-compartmental method (Gibaldi and Perrier 1982) using Microsoft Excel 2007 (Microsoft Corporation, Washington, USA). The area under the plasma concentration versus time curve up to the last quantifiable time point, $AUC_{(0\text{-}t)}$ was obtained by the linear up and log-linear trapezoidal summation. The $AUC_{(0\text{-}t)}$ was extrapolated to infinity (i.e., $AUC_{(0\text{-}\infty)}$) by adding C_{last}/K_{el}, where C_{last} represents the last measurable time concentration and K_{el} represents the apparent terminal rate constant. K_{el} was calculated by the linear regression of the log-transformed concentrations of the drug in the terminal phase. The half-life of the terminal elimination phase was obtained using the relationship $t_{1/2} = 0.693/K_{el}$. The peak plasma concentration (C_{max}) and time to peak (T_{max}) were derived by visual inspection of the data. Systemic clearance (CL) was calculated by the relationship $CL/F = Dose/AUC_{(0\text{-}\infty)}$, where F is bioavailability. The apparent volume of distribution (V_d) was obtained from the equation $V_d/F = Dose/(AUC_{(0\text{-}\infty)}*K_{el})$.

RESULTS

The lower limit of quantification of cutamesine was 0.2 ng/mL. The extraction recovery of cutamesine and citalopram was >95% for both plasma and brain homogenate biological matrices. The chromatographic retention times for citalopram and cutamesine were 1.81 and 1.94 min, respectively. A representative UPLC-MS chromatogram of cutamesine and citalopram is shown in Fig. 1. The mean plasma and brain concentrations versus time profiles of cutamesine following a single intraperitoneal administration to rats are presented in Fig. 2. The mean intra-peritoneal pharmacokinetic parameters for cutamesine are summarized in Table 3. Following intraperitoneal administration, plasma concentrations of cutamesine decreased bi-exponentially and the elimination half-life was found to be 1.6-3.2 h. At 1 mg/kg, where sufficient data points were available, CL and V_d of cutamesine were found to be 5.9 (L/h/kg) and 27.2 L/kg, respectively. The peak levels and $AUC_{(0\text{-}\infty)}$ of brain were 2.4 and 5.2 times, respectively, higher compared to the plasma. The average terminal half-life ($t_{1/2}$) of brain disposition

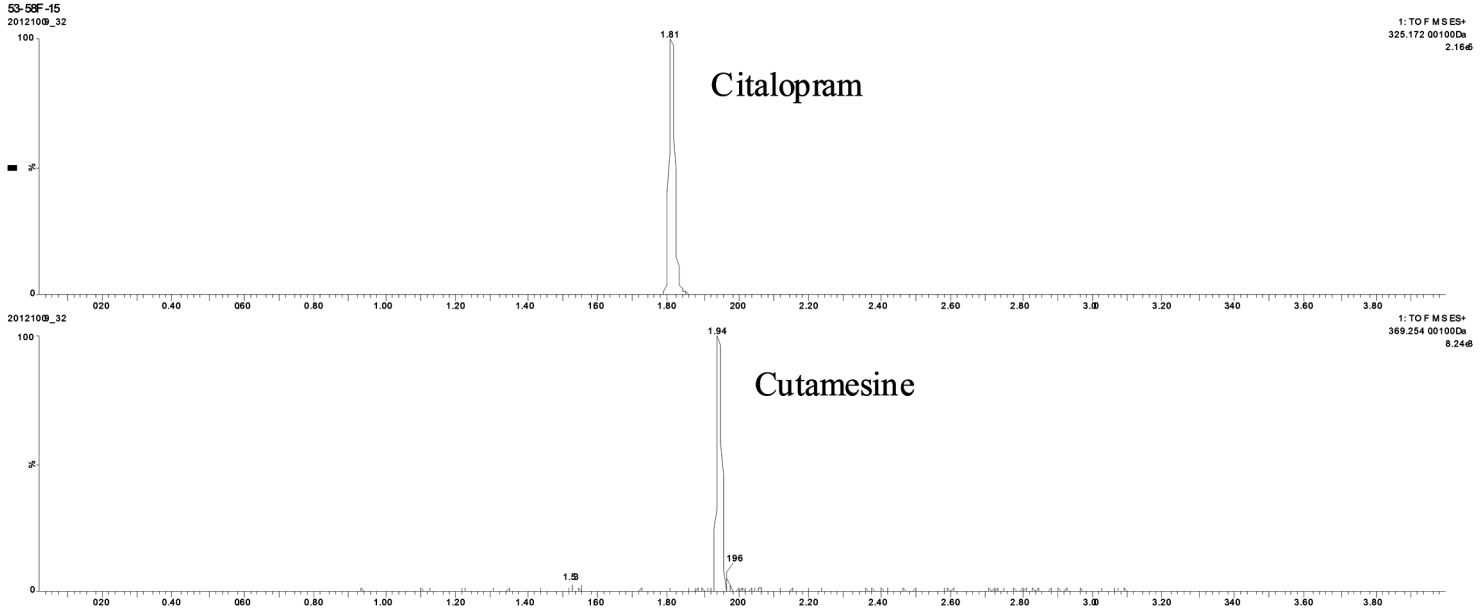


Figure 1. *A representative UPLC-MS chromatogram of cutamesine and internal standard citalopram.*

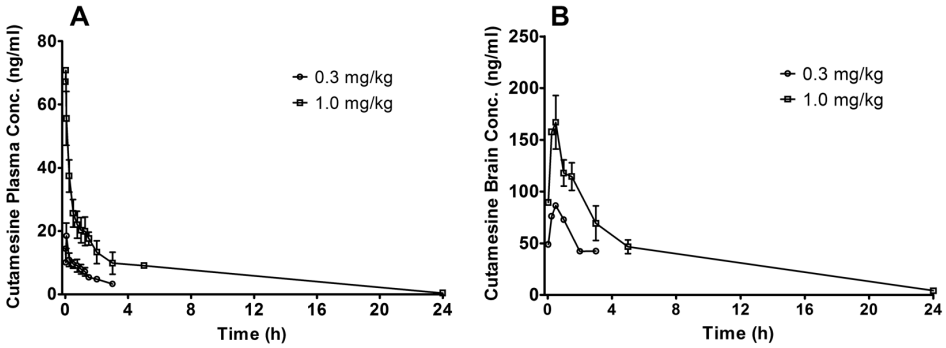


Figure 2. *Concentration-time profiles (mean ± SEM) of cutamesine in plasma (A) and brain (B) after intraperitoneal injection of cutamesine into rats.*

Table 3. Pharmacokinetic (PK) parameters of cutamesine in rats at 0.3 and 1 mg/kg after intraperitoneal administration

	1 mg/kg		0.3 mg/kg	
PK Parameters	**Plasma**	**Brain**	**Plasma**	**Brain**
$AUC_{(0\text{-}3h)}$ (ng×h/mL)	61.5	336.0	20.4	174.3
$AUC_{(0\text{-}\infty)}$ (ng×h/mL)	172.5	896.1	*	*
C_{max} (ng/mL)	70.8	167.0	18.5	86.4
T_{max} (h)	0.03	0.5	0.08	0.5
K_{el} (/h)	0.216	0.135	0.442	0.309
$t_{1/2}$ (h)	3.2	5.1	1.6	2.2
CL (L/h/kg)	5.9	1.2	*	*
V_d (L/kg)	27.2	8.5	*	*

* Sufficient data points were not available to calculate this value accurately

was 5.1 h and longer than plasma. Data from the 0.3 mg/kg dose was sufficient to show that the increase in C_{max} and AUC were dose proportional. Assuming the ^{11}C-cutamesine and cold compound disposition is the same, the $AUC_{(0\text{-}\infty)}$ following an intravenous dose of ^{11}C-cutamesine was used to estimate the bioavailability (Chapter 9). Absolute intraperitoneal bioavailability was found to be 60% for the dose of 1 mg/kg based on radiotracer kinetics.

DISCUSSION

Cutamesine is a novel small molecule, a sigma-1 subtype-selective receptor agonist, with activity in preclinical models of CNS diseases and currently being

studied in humans (phase II) for depression and stroke indications *(1, 5)*. Cutamesine exhibited favorable physicochemical properties that are required for an ideal CNS drug with clogP of 2.52 and aqueous solubility of >31 mg/mL *(2, 3)*. In this article we have quantified the plasma and brain levels of cutamesine using UPLC-MS method. Subsequently, non-compartmental analyses were performed to characterize the pharmacokinetics of cutamesine in rats.

Cutamesine was shown to have adequate PK profiles that are required for desired CNS effects. In addition, the levels of cutamesine in brain remained above the IC_{50} value of sigma-1 receptor for up to 16 h of post dosing, suggesting that appreciable levels of drug is available to exert its therapeutic actions. This finding also suggests that this drug may be dosed once daily in the clinic, if a similar PK profile is observed in humans.

The *in vitro* sigma-1 receptor binding potency (IC_{50} = 17.4 nM), solubility (86 mM), blood brain barrier permeability *(8)* and favorable PK properties (brain) of cutamesine in the preclinical species make it a potential clinical candidate for CNS disease such as depression and stroke.

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