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A Review of the Neuroprotective Properties of the 5-HT$_{1A}$ Receptor Agonist Repinotan HCl (BAY x 3702) in Ischemic Stroke

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ABSTRACT

Repinotan HCl (repinotan, BAY x 3702), a highly selective 5-HT$_{1A}$ receptor agonist with a good record of safety was found to have pronounced neuroprotective effects in experimental models that mimic various aspects of brain injury. Repinotan caused strong, dose-dependent infarct reductions in permanent middle cerebral artery occlusion, transient middle cerebral artery occlusion, and traumatic brain injury paradigms. The specific 5-HT$_{1A}$ receptor antagonist WAY 100635 blocked these effects, indicating that the neuroprotective properties of repinotan are mediated through the 5-HT$_{1A}$ receptor.

The proposed neuroprotective mechanisms of repinotan are thought to be the result of neuronal hyperpolarization via the activation of G protein-coupled inwardly rectifying K$^+$ channels upon binding to both pre- and post-synaptic 5-HT$_{1A}$ receptors. Hyperpolarization results in inhibition of neuron firing and reduction of glutamate release. These mechanisms, leading to protection of neurons against overexcitation, could explain the neuroprotective efficacy of repinotan per se, but not necessarily the efficacy by delayed administration. The therapeutic time window of repinotan appeared to be at least 5 h in in vivo animal models, but may be even longer at higher doses of the drug.

Experimental studies indicate that repinotan affects various mechanisms involved in the pathogenesis of brain injury. In addition to the direct effect of repinotan on neuronal hyperpolarization and suppression of glutamate release this compound affects the death-inhibiting protein Bcl-2, serotonergic glial growth factor S-100 and Nerve Growth Factor. It also suppresses the activity of caspase-3 through MAPK and PKC$\alpha$; this effect may contribute to its neuroprotective efficacy. The dose- and time-dependent neuroprotective efficacy of repinotan indicates that the drug is a promising candidate for prevention.
of secondary brain damage in brain-injured patients suffering from acute ischemic stroke. Unfortunately, however, the first, randomized, double blind, placebo-controlled clinical trial did not demonstrate the efficacy of repinotan in acute ischemic stroke.

INTRODUCTION

Stroke

At the end of the 20th century stroke was the third leading cause of death and was responsible for approximately 5.5 million deaths in the Western World, accounting for approximately 10% of all deaths. From 2002 on, however, it became the second most common cause of death in the world. While 90% of all acute stroke deaths are in people over 65 years of age, traumatic brain injury (TBI) caused by accidents is the most common cause of mortality and morbidity in adults under 40 years of age. Although the survival rates for acute stroke and TBI are improving, they are still low. Acute stroke is not lethal in the majority of patients with only 8–20% of patients dying within the first 30 days. However, permanent neurological deficits remain in 50% of patients and more than 25% require long-term care. Both conditions, stroke and TBI, are associated with an insufficient blood supply in affected brain regions that may lead to motor, cognitive and other behavioral impairments dependent on the location and size of the affected brain area. These outcomes represent a substantial social and economic burden. In 1993, the total cost of stroke for society was estimated to be $18 billion in the USA alone (65,66,122).

Ischemic Cell Death and Different Strategies for Neuroprotection

After the onset of ischemia, loss of energy stores leads to ionic imbalance and the breakdown of the energy-driven membrane potential. This induces neurotransmitter release into the extracellular space and inhibition of their uptake, in particular the uptake of the major mammalian excitatory transmitter glutamate (12,120,132). Subsequently, continuous activation of glutamate receptors [i.e., ionotropic NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid), kainate and metabotropic glutamate receptors] will promote excessive postsynaptic Ca²⁺ influx and indirect mobilization of intracellular calcium stores.

This non-physiological rise in intracellular Ca²⁺, together with the repeated membrane depolarization or even general loss of membrane potential, constitutes a positive feedback loop that triggers an array of downstream intracellular processes that ultimately lead to the degradation of membranes and proteins that are essential for cellular integrity. The two most fundamental downstream mechanisms lead either to a rapid cell necrosis or to a relatively delayed apoptotic-like cell death (83,86). As a result, the neuronal dysfunction and/or death represent new sources of neurotoxic glutamate (133). Thus, at least three fundamental mechanisms can be distinguished in the process of ischemic cell death: excitotoxicity and ionic imbalance, oxidative/nitrosative stress, and apoptotic-like cell death. These mechanisms mediate injuries within neurons, glia, and vascular elements, and at the subcellular level they affect the functional characteristics of mitochondria, nuclei, cell membranes, endoplasmic reticulum, and lysosomes (83,86,94).
Since cell death after ischemia is associated with an overstimulation of the neurons, research has focused on inhibiting neuronal activity immediately after injury and consequently preventing cell death after ischemia. Because of the prominent role of glutamate in excitotoxicity, neuroprotective therapy focused first on glutamate and on glutamate receptors like the NMDA receptor, which contributes prominently to Ca$^{2+}$ influx (27), and also on AMPA receptors (18). Competitive and non-competitive NMDA antagonists have been reported to attenuate histopathology, and in certain instances to alleviate functional outcome (79,108,113,114,130). However, despite their effectiveness in experimental models of traumatic or ischemic brain injury, clinical trials with NMDA receptor antagonists revealed concerns about their safety, efficacy and tolerance. Glutamate antagonists, directly acting at the postsynaptic glutamate receptors, have considerable side effects. They produced hypotension and psychotomimetic effects in humans, and neurotoxic injury in animals (72,109,146). Two possible factors contributing to the unsuccessful clinical outcomes are differences in pathogenic mechanisms and therapeutic windows between clinical and experimental conditions (38). Glutamate antagonists have been shown to lack clinical efficacy and specificity in human trials and their further development has been curtailed (30,40,78).

Consequently, alternative strategies have been explored to alleviate neuronal cell death after cerebral ischemia. These approaches, all of which were designed to counteract the consequences of excessive ischemia-induced neuronal depolarization, include antagonists of voltage-sensitive calcium and sodium channels (14,67,90,110,124,135), glutamate synthesis inhibitors, glutamate release antagonists and inhibitors of second messenger cascades and downstream regulatory pathways involved in glutamatergic signaling (112). It became obvious that the search for new neuroprotective strategies would include stimulation of neuronal processes capable of preventing membrane depolarization by inducing hyperpolarization. Within this framework the serotonin (5-HT) and in particular the 5-HT$_{1A}$ receptor subtypes came into focus because of their hyperpolarizing efficacy, and their widespread distribution in forebrain areas which are prone to ischemic injury (33,115).

**5-HT$_{1A}$ Receptor Agonists in Neuroprotection**

Initially, the role of the serotonergic system in the pathophysiology of cerebral ischemia was not understood, but considerable progress has been made in recent years. The concentration of 5-HT in the cerebral cortex was found to be decreased in the ischemic brain (74). However, neurotoxin that destroys 5-HT system (5,7-dihydroxytryptamine), increases neuronal death after ischemia in gerbil hippocampus (105). In addition, rapid enhancement of serotonergic sprouting was demonstrated in response to excitotoxic stimuli in different brain areas (54,148) suggesting a probable enhanced signaling.

5-HT$_{1A}$ receptor is one of several subtypes through which serotonin exerts its neuronal effects (63). It is widely distributed within the brain, both pre- and postsynaptically. Some 5-HT$_{1A}$ receptors are called autoreceptors, they are located on the cell soma and dendrites of 5-HT neurons (139). Others are highly expressed postsynaptically in areas of the brain that are most sensitive to neuronal damage following ischemic stroke or brain trauma, such as hippocampus (CA1 and CA3 sectors and dentate gyrus), cerebral cortex and basal forebrain nuclei (24,31,106). The 5-HT$_{1A}$ receptors exert an inhibitory effect on the firing activity of neurons in these brain areas.
Stimulation of postsynaptic 5-HT_{1A} receptors is known to cause neuronal hyperpolarization, most likely by activating G protein-coupled inwardly rectifying K^+ channels (16, 33, 68). Consequently, neuronal activity is suppressed due to increased resistance of the neuronal membrane to glutamate-induced depolarization and overstimulation (i.e., excitotoxicity) (7). In addition, 5-HT_{1A} receptor agonists produce voltage-independent blockade of N-, P-/Q-, and T-type voltage-dependent Ca^{2+} channels via G-protein-mediated signaling mechanisms or via a membrane-delimited pathway, respectively (137). Furthermore, 5-HT_{1A} signaling inhibits L-type voltage-dependent Ca^{2+} channel through a G-protein-mediated diffusible cytosolic messenger (19), and by this way abrogate Ca^{2+} entry into nerve cells (121).

It has also been reported that 5-HT_{1A} receptor agonists can reduce glutamate release from nerve terminals (121, 134), which is most likely mediated through activation of presynaptic 5-HT_{1A} receptors located on glutamatergic terminals (95, 116).

Recently 5-HT_{1A} receptor agonism was shown to inhibit apoptotic cell death in cultured neurons from chick embryo (5) as well as in neuronal HN-2-5 cells (1). There is strong evidence to support the role of programmed cell death in cerebral ischemia (82, 91), which points to the importance of increasing the time window for therapeutical intervention (81).

Based on the above considerations, it appears that selective agonists of the 5-HT_{1A} receptor may offer a new approach to neuroprotection in neurodegenerative disorders such as stroke, traumatic brain injury (33) and probably also Alzheimer’s disease (84). Several 5-HT_{1A} agonists, such as buspirone, urapidil, ipsapirone, gepirone, and 8-OH-DPAT have been shown to have neuroprotective properties in different models of cells damage in vitro and in vivo (15, 95, 115, 131). However, all compounds investigated so far had limited 5-HT_{1A} receptor selectivity, affinity and/or intrinsic activity. The limited selectivity may be partly explained by brain region-dependent differences in receptor reserve or in the nature of receptor-effector coupling (31). It was suggested that the therapeutic spectrum of a 5-HT_{1A} receptor ligand could be optimized by increasing the level of intrinsic activity, at different pre- and postsynaptic 5-HT_{1A} receptors populations (31, 32). These findings and considerations stimulated the search for a more selective 5-HT_{1A}-receptor agonist that might exert neuroprotection also by inhibiting neuronal activity.

**REPINOTAN**

**Pharmacokinetics**

Recently, a methylchroman derivative, repinotan HCl (will be referred to as repinotan in this manuscript) also known as BAY x 3702; or R-(−)-2-{4-[(chroman-2-ylmethyl)-amino]-butyl}-1,1-dioxo-benzo[d]isothiazolone hydrochloride (Fig. 1) has been synthesized at Bayer AG Healthcare, Wuppertal, Germany, and characterized in biochemical, electrophysiological and behavioral assays as a novel, highly potent, highly selective and orally active 5-HT_{1A} receptor full agonist (33, 35, 37).

Most importantly, repinotan was found to have pronounced long-lasting neuroprotective effects in animal models of focal cerebral ischemia (permanent middle cerebral artery occlusion, p-MCA-O), of reperfusion injury (transient middle cerebral artery occlusion, t-MCA-O), and of traumatic brain injury (TBI) (33, 61, 62).
The compound is highly soluble in water (2.1 g/100 mL) (34). The half-life of repinotan HCl in plasma is relatively short ($t_{1/2} = 0.6$ h in rat; 0.4 h in rhesus monkeys; 1.2 h in human), and the drug is extensively metabolized. Only 0.5% of the administered drug is found unchanged in plasma or urine of rats (33).

Rapid penetration of the brain tissue is thought to be essential for the neuroprotective efficacy of drugs. However, the distribution of drugs in the ischemic brain is likely to be different than in the normal brain (87). Following cerebral ischemia, biphasic openings of blood-brain barrier (BBB) are common and the severity and duration of the ischemic insult affects the spatial and temporal pattern of drug distribution in the brain (76).

$[^{14}C]$Repinotan passes the intact BBB of healthy rats rapidly and extensively (126, 127). Repinotan passes BBB in unchanged form; its distribution equilibrium of the free drug in blood and brain is reached immediately after the start of infusion. The concentrations of the drug in blood and brain are identical, indicating that the drug crosses BBB freely in both directions with diffusion as a driving force. However, the penetration of repinotan into ischemic brain areas is dependent on time of drug administration in relation to injury. The distribution pattern of $[^{14}C]$repinotan, administered either at 13 min or at 5 h after pMCA-O to rats was identical with that in healthy controls. However, when administered at 18 h after pMCA-O the drug was not able to pass BBB (127).

**Toxicology**

By single i.v. injection, repinotan was well tolerated by dogs and rabbits. It had low to moderate toxicity in rodents (LD$_{50} = 13$ and 27 mg/kg i.v. in rats and mice, respectively). By repeated administration to rats, dogs or primates, repinotan produced predominantly behavioral changes, suggesting that the central nervous system (CNS) is the primary target organ. The threshold plasma concentration for the CNS side effects of repinotan was 50 $\mu$g/L (33,92). Repinotan did not seem to have any effect on male fertility and early embryonic development, when administered to rats before and during the mating period. At doses up to 0.1 mg/kg repinotan had no effects on estrous cycle in rats. Signs of embryotoxicity were observed, but only at maternally toxic doses. Experiments on genotoxicity in seven different test systems revealed neither mutagenic nor clastogenic properties of repinotan. Repinotan was also inactive with respect to DNA repair/damage induction (33,92).

Studies with repinotan in healthy human subjects demonstrated favorable safety and tolerability profiles. The drug had no clinically significant effects on blood pressure, heart rate, ECG, or clinical chemistry (33,107). Some subjects demonstrated a prolonged elimination of repinotan. These subjects had reduced cytochrome P450 II D6 activity (33). Cytochrome P450 II D6 is encoded by a polymorphic gene, and reduced expression of this cytochrome occurs in only 1% of the Asian population, whereas in Caucasians, African
Americans and Hispanics it is 5 to 8% (33). However, prolonged elimination of repinotan was not associated with adverse events in these subjects.

The safety and tolerability of three different doses (0.5, 1.25, or 2.50 mg/day) of repinotan was assessed in patients with severe traumatic brain injury (107). By continuous infusion for 7 days repinotan had no apparent adverse effects on intracranial pressure, hemodynamics or clinical chemistry. As expected, repinotan did not increase the risk of seizures, or the incidence and severity of adverse events. No further safety concerns were raised during the 3 months period after treatment.

**Neuroprotectective Properties**

Neuroprotective properties of repinotan in brain damage after ischemia have been studied *in vitro* and *in vivo*. It has been shown that repinotan activates 5-HT1A receptors effectively in several pathways, which are involved in either neuroprotection or recovery of neuronal function.

**In vitro**

In pre- and post-injury incubation protocols repinotan prevented neuronal damage. Exposure to repinotan protected rat cortical and hippocampal neurons in cultures from apoptosis induced by 25 nM staurosporine (136). Also in mixed rat hippocampal neuronal/glial cultures subjected to 24-h serum deprivation (4) or exposure to glutamate toxicity repinotan was neuroprotective (129). After staurosporine-induced apoptosis, repinotan, at 50 pM to 1 μM, reduced the release of lactate dehydrogenase, DNA fragmentation, and apoptotic body formation in a concentration-dependent manner (136). This indicated a selective and specific mode of action in accordance with its reported high potency and selectivity (33). However, at 5 pM repinotan did not prevent cell death. Also, if staurosporine concentrations were higher than 100 nM repinotan was ineffective The latter may be due to the fact that with increasing concentrations of staurosporine multiple pathways leading to apoptotic cell death are activated, which are not affected by repinotan (136).

**In vivo**

Various accepted models that mimic different aspects of human brain injury were used to examine the neuroprotective properties of repinotan:

1) permanent focal cerebral ischemia model involving occlusion of the middle cerebral artery (pMCA-O) (102),

2) reperfusion injury model by transient middle artery occlusion (tMCA-O) (118), and

3) traumatic brain injury (TBI) model involving induction of acute subdural hematoma (aSDH) (58).

The therapeutic window for repinotan has been also studied. This is extremely important because under clinical conditions there is usually a considerable delay between the onset of stroke, hospitalization, diagnosis, and the first pharmacological intervention (97). Two different administration techniques have been used in the evaluation of neuroprotective properties of repinotan. The compound was administered either by triple bolus injection or by continuous infusion over a 4 h period.
Permanent middle cerebral artery occlusion (pMCA-O)

By triple bolus injection (at 0, 2, and 4 h after pMCA-O), repinotan, at 1 to 10 µg/kg had pronounced neuroprotective effect. The maximal infarct volume reduction was 73% at 3 µg/kg of the drug as assessed in rats at 7 days treatment (62,97). At lower or higher doses repinotan had less neuroprotective efficacy (97).

Repinotan produced a strong and dose-dependent reduction in infarct size when administered by continuous infusion over a 4 h period, starting immediately after occlusion. A reduction was observed at the drug doses of 0.3, 1, 3, 10, and 30 µg/kg/h. Again, a decrease in the efficacy was observed at lower and higher doses of repinotan (U-shape curve) (97). Strongest infarct volume reduction was found within the 1–10 µg/kg/h dose range, with 59–65% infarct reduction (62,97). Other investigators also found similar magnitude of protection after infusion of repinotan at 3 or 10 µg/kg/h for 4 h (57 and 55%, respectively), starting immediately after occlusion in the pMCA-O model (129).

Transient middle cerebral artery occlusion (tMCA-O)

In rats subjected to tMCA-O repinotan (1, 10, or 100 µg/kg/h i.v. for 4 h) was started immediately after reopening of MCA after a 1-h occlusion. The infarct volume was measured two days later. Repinotan reduced cortical brain injury by 81, 97, and 84% at doses of 1, 10, and 100 µg/kg/h, respectively (33,97). In addition, at 1 µg/kg/h the drug reduced injury in the caudate putamen by 67% and completely prevented reperfusion injury in the hippocampus (33). In another study the infarct volume reduction was also measured in the hippocampus at 7 days after i.v. infusion of repinotan (at 1, 3, or 10 µg/kg/h) for a period of 4 h immediately after 10 min of tMCA-O (123). At the lowest dose used (1 µg/kg/h) repinotan caused only a 10% reduction of neuronal damage in the CA1 area of hippocampus; at the higher doses (3 and 10 µg/kg/h) repinotan had significant effect (123). This lack of neuroprotective efficacy at the higher doses was probably due to a strong reduction of mean arterial blood pressure during infusion of the drug (111). DNA fragmentation, as a measure for induced apoptosis in the hippocampus and striatum, was examined using TUNEL-staining and gel-electrophoresis in the latter study. The neuroprotective dose of repinotan (1 µg/kg/h) was administered for 4 h immediately after 10 min tMCA-O; it strongly reduced DNA fragmentation in the hippocampus and striatum, as measured by gel-electrophoresis at 4 days after tMCA-O (123). Furthermore, at 3 days after tMCA-O, the compound reduced the number of TUNEL-stained cells both in the hippocampus (10% reduction) and in the (dorsolateral region of) striatum (123).

Traumatic brain injury (acute subdural hematoma, aSDH)

In rats with traumatic brain injury repinotan was administered either as intravenous (triple) bolus injection immediately, at 2 or at 4 h after injury, or as a 4-h long infusion. By the triple bolus i.v. injection repinotan produced maximal neuroprotection at doses of 10 to 100 µg/kg. There were no differences in the effects produced at the minimal as compared to the maximal doses, with brain damage reduction being 60 ± 15.5 and 64 ± 15% at 10 and 100 µg/kg, respectively (33,62,97).

Repinotan was also administered intravenously at 15 min before injury and as a continuous infusion for 4 h after induction of aSDH. Pretreatment with repinotan (3 or 10 µg/kg/h) produced a 40% reduction in the size of ischemic brain damage. The volumes of damaged brain in the control, high-dose and low-dose groups were 106 ± 33,
61 ± 49, and 59 ± 35 mm³ (mean ± S.D.), respectively (6). Other authors administered repinotan doses ranging from 0.3 to 1000 μg/kg/h by continuous infusion for 4 h, starting immediately after induction of aSDH (post-treatment). Repinotan reduced damaged brain volumes by 76 and 69% at 1 and 10 μg/kg/h, respectively (33,62).

Repinotan has also been tested in a clinically relevant contusion model of traumatic brain injury (TBI) (71). The drug was infused intravenously at 10 μg/kg/h for 4 h, starting at 5 min after injury. Repinotan reduced mean cortical tissue damage volume by ca. 19% (20.18 ± 1.51 vs. 24.89 ± 1.60 mm³ in the vehicle group). The number of morphologically intact hippocampal CA1 and CA3 neurons was also increased by repinotan. The effects of repinotan on vestibulomotor function, spatial learning and memory deficits have been examined in this study. The drug treatment did not enhance vestibulomotor function as assessed in beam balance and beam walk tasks on post-operative days 1–5, but improved Morris water maze learning of traumatized animals (71). Motor function recovery was demonstrated in earlier studies as an improvement in motor behavior deficits after treatment with repinotan. This drug enhanced and accelerated the recovery of abnormal hindlimb grasping reflex and forelimb flexion compared with vehicle-treated controls for up to 42 days after brain injury (46).

In summary, repinotan has been shown to produce beneficial effects in a clinically relevant model of TBI, as concluded from both anatomical and functional parameters. Motor and memory impairments are common following human traumatic brain injury, and a treatment strategy employing repinotan may accelerate functional recovery.

**Dose-response curve and therapeutic time window**

In various in vitro experiments repinotan did not have a clear dose–response curve. In chick neurons there was a dose-response curve for repinotan for neuronal viability, but not for percentage of apoptotic neurons (4). There was also no clear relationship between the drug dose and the firing rate of dorsal root ganglion neurons probably because of the limited range of effective drug concentrations (34). Other experiments indicated, however, that the dose–response curve for repinotan is a wide U-shaped curve (62,75,97,129), which has previously been observed with other compounds (96). This may indicate that repinotan is very potent in a wide low-dose range, but produces adverse effects at higher doses (75,97). In addition, repinotan appeared to be more potent and exhibited a broader dose–response curve and larger dose variability after administration as a 4 h continuous infusion, compared to (triple) bolus injection. This is probably due to the fact that infusion, contrary to bolus administration, maintains brain levels of the compound in the optimal range throughout time of application (97).

To assess the therapeutic time-window of repinotan, the drug was administered by continuous 4-h long infusion with a delay of either 1, 3, or 5 h in the pMCA-O model and with 3 and 5 h in the tMCA-O and aSDH models (97). In the pMCA-O model, delaying infusion by 1 h did not reduce neuroprotective efficacy at either 3 or 10 μg/kg/h (33). After a 3 h delay, neuroprotection was still observed with 48% infarct volume reduction at 3 μg/kg/h (62,97). When administration of repinotan was delayed for 5 h, a dose of 3 μg/kg/h still caused a moderate 26% infarct reduction (33,62). However, when the dose of repinotan was increased to 10 μg/kg/h, the infarct volume was reduced by 43% (97). When in the tMCA-O model the administration of repinotan HCl (10 μg/kg/h) was delayed by 3 or 5 h, the infarct volumes were still decreased by 80% (97). When in the
aSDH model the treatment with repinotan (3 or 10 μg/kg/h) was delayed by 3 h, the drug still produced 74% protection. When the treatment was delayed by 5 h there was still a 54% protection (33,97). There was no difference in the effects of repinotan at the two doses used.

Thus, the therapeutic time window of repinotan appeared to be at least 5 h in in vivo models used to assess the neuroprotective efficacy of the drug. Moreover, because of the suggested broad dose–response curve for repinotan, the therapeutic window might be larger than 5 h when the dose is increased in delayed administration models.

PUTATIVE MECHANISMS UNDERLYING THE NEUROPROTECTIVE EFFECT OF REPINOTAN

Several putative mechanisms of neuroprotective action of repinotan have been examined. These mechanisms included neuronal membrane hyperpolarization, modulation of glutamatergic neurotransmission, antiapoptotic effects, effects on intracellular signaling, and modulation of the effects of neurotrophic factors.

Receptor binding of repinotan

Repinotan has a $K_i$ of 0.19 nM (calf hippocampus), 0.25 nM (rat and human cortex), and 0.59 nM (rat hippocampus). These $K_i$ values reflect very high affinity receptor binding in comparison to many other CNS-active drugs. The 5-HT$_{1A}$ receptor affinity of repinotan is ca. 1.5 to 10 times (depending on the particular brain tissue) and 5 to 15 times lower for other 5-HT$_{1A}$ agonists: 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) and ipsapirone, respectively. Functionally, in both, in vitro and in vivo electrophysiological assays, repinotan was found to suppress neuronal firing more potently than 8-OH-DPAT and ipsapirone. The potency difference was about 1 and 2 orders of magnitude, respectively (34).

Repinotan binds with lower affinity to 5-HT$_7$ ($K_i = 6$ nM), α$_1$- and α$_2$-adrenergic ($K_i = 6$ and 7 nM, respectively), 5-HT$_{1D}$ (36 nM), dopamine D$_3$ and D$_4$ (48 and 91 nM, respectively), σ sites (176 nM) and 5-HT$_{2C}$ (310 nM) receptors. However, the binding profile of repinotan is considered to be relatively selective, as at least one order of magnitude separated its binding to 5-HT$_{1A}$ receptors from its binding to other receptors or binding sites (33,35,36). In addition, the binding is considered to be stereo-selective as the affinity of the (1)-enantiomer, BAY x 3703, was about 10-fold lower than that of repinotan (36). Consequently, it was concluded that the pharmacological effects of repinotan are mediated mainly by 5-HT$_{1A}$ receptors.

Selective Antagonism at the 5-HT$_{1A}$ Receptors by WAY 100635

The conclusion that the effects of repinotan are mediated mainly by 5-HT$_{1A}$ receptors has been supported by multiple studies with a selective 5-HT$_{1A}$ receptor antagonist N-(2-(4-(2-methoxyphenyl)-1-piperazinyl) ethyl)-N-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride (WAY 100635), which has no, or only very low affinity to other putative central receptors (43,69). Pretreatment with WAY 100635 potently and completely blocked the neuroprotective effects of repinotan both in vitro and in vivo (3,4,22,34,36,37,
Moreover, the involvement of α-adrenoceptors and dopamine receptors was excluded since the antiapoptotic effect of repinotan (4) and its other actions at 0.1 mg/kg, i.p. were not comparable with those of either α1- and α2-adrenergic antagonists, prazosin and idazoxan, or of D2-receptor antagonist, raclopride (36).

Chick neurons were found to be insensitive to WAY 100635 (4). It was suggested that WAY 100635 has a lower affinity for the avian than for the rat 5-HT1A receptors. In addition, there were subcellular and regional differences in the inhibitory potency of WAY 100635 (25,77).

Glutamate Release

In order to evaluate whether modulation of glutamatergic neurotransmission may be involved in the neuroprotective properties of repinotan, the effects of the drug on glutamate excitotoxicity were examined in vitro and in vivo.

To study the in vitro effects of repinotan on glutamate excitotoxicity, hippocampal cells were exposed to either 75 mM KCl (96) or to 0.5 mM L-glutamate for 1 h in serum-free medium (129). Basal glutamate release was not affected by repinotan at concentrations up to 5 μM (96). However, repinotan inhibited potassium-stimulated glutamate release in a concentration-dependent manner. Maximal inhibition was achieved at 5–10 μM; at these concentrations the stimulated glutamate release was nearly completely blocked (96). Hippocampal cells, exposed to L-glutamate only, showed a neuronal damage of approximately 70%. If repinotan (0.0001–1 μM) was added immediately after glutamate, it reduced excitotoxic damage in a concentration-dependent manner, with only about 40% neuronal damage at 0.1 and 1 μM. Additionally, the drug preserved the cell morphology and the integrity of neuronal network (129).

In vivo, the effects of repinotan HCl on ischemia-induced glutamate-release were examined in the pMCA-O model using microdialysis technique (96). The drug was administered as a bolus injection directly after pMCA-O. At 1 or 10 μg/kg repinotan reduced release of glutamate by 41.6 ± 10.5 and 54.2 ± 14.1% of control values, respectively. This reduction reached statistical significance only for the lower dose group. This was at variance with other studies, which did not find any difference in the neuroprotective effects of repinotan at these two doses (62,97).

In summary, the 5-HT1A receptor-mediated decrease in ischemia-induced glutamate release may explain the neuroprotective efficacy of repinotan. Repinotan could mediate the decrease in extracellular glutamate through two basic mechanisms: (i) inhibition of glutamate release from synaptic vesicles or from glial cells provoked by reversed glutamate transport and/or (ii) stimulation of glutamate uptake. However, repinotan has not yet been found to interact directly with any of the currently known glutamate transporters, thus making it unlikely that its activity was due to direct modulation of glutamate transport. Inhibition of glutamate release by repinotan is more likely to be mediated by its hyperpolarizing action as demonstrated in vitro as well as in vivo (16,21,33,36,96,116).

Hyperpolarization and Altered 5-HT Neurotransmission

In vitro (33) and in vivo (21) experiments showed that repinotan induced a long lasting, but reversible, inhibition of neuronal firing. Its effective in vitro concentrations were as low as 1 nM. In vivo, biologically active doses of repinotan were 10–100 times lower
In vivo, repinotan-induced hyperpolarization and the subsequent reduction of 5-HT release is thought to be the result of activation of both, pre- and post-synaptic 5-HT$_{1A}$ receptors (17,22,37). By systemic administration repinotan (10–100 µg/kg s.c.) dose-dependently diminished 5-HT output in the dorsal (DR) and median raphe nucleus (MnR) and in two areas of the rat forebrain, the medial prefrontal cortex (mPFC) and the dorsal hippocampus (dHPC) in a region-selective manner (21). Furthermore, the effect of repinotan on 5-HT output was greater than that produced by equivalent doses of other selective 5-HT$_{1A}$ receptor agonists, such as 8-OH-DPAT, alnespirone or ipsapirone under the same experimental conditions (20–22). Repinotan has apparently a comparatively higher potency in vivo (33,36).

At a dose of 100 µg/kg s.c., the maximal effects of repinotan were observed in the MnR and mPFC, and moderate effects in the DR and dHPC with the decrease in release to ~15% and ~45% of the baseline level, respectively. The maximal reduction of the 5-HT output took place at 40–60 min after drug administration. 5-HT values returned to baseline at ca. 26 h after administration, except at the 100 µg/kg dose in mPFC. Application of repinotan in the mPFC and DR (30 µM) diminished the local 5-HT output to 40% and 25% of baseline, respectively (21,22). In addition, local application of the drug inhibited neuron firing activity in the DR to ~20% of baseline, and that in mPFC to ~50% of baseline (21). Thus, since the reduction in forebrain 5-HT output does not appear to depend exclusively on the DR or MnR origin of the 5-HT fibers, post-synaptic together with pre-synaptic 5-HT$_{1A}$ receptors in the raphe also seem to participate in the lessening of serotonergic activity and 5-HT release elicited by repinotan.

The lower firing activity of 5-HT neurons in the dorsal raphe nucleus after chronic administration of repinotan (1.25 mg/kg/day for 2 days) showed subsequent gradual recovery and returned to normal after 7 days of treatment. This recovery of firing activity was much longer after administration of repinotan than other known 5-HT$_{1A}$ agonists. This suggests that the dissociation of repinotan from the 5-HT$_{1A}$ receptors occurs very slowly (37). In contrast, the postsynaptic 5-HT$_{1A}$ receptors (in the hippocampus) showed unaltered responsiveness during long-term administration of repinotan (37). The desensitization of somatodendritic 5-HT$_{1A}$ autoreceptors results in a normalization of the firing activity of 5-HT neurons. Moreover, an enhancement of the tonic activation of the postsynaptic 5-HT$_{1A}$ receptors takes place after a long-term treatment with 5-HT$_{1A}$ receptor agonists (50). The normalized 5-HT release and the enhanced postsynaptic 5-HT$_{1A}$ receptor sensitivity in the target areas tend to strengthen the efficacy of 5-HT in causing membrane hyperpolarization and neuroprotection. Thus, hyperpolarization enhances the efficacy of 5-HT neurotransmission and appears to be one of several mechanisms of the neuroprotective effect of repinotan.

In addition to hyperpolarization and inhibition of glutamate release (16,21,33,96,116) voltage-independent blockade of N-, P-/Q-, and T-type voltage-dependent Ca$^{2+}$ channels via G-protein-mediated signaling mechanisms (137) and direct reduction of glutamate release (121,134) have been suggested as possible mechanisms of the neuroprotective action.
of repinotan. However, no studies have been published in support of the involvement of these mechanisms in the neuroprotective effect of repinotan.

**Repinotan, Hypothermia, and Blood Flow Parameters**

Hypothermia is considered to be one of the classic symptoms of 5-HT₁A receptor activation (34,57,99). Furthermore, hypothermia has been shown to be neuroprotective and to enhance functional outcome following different experimental procedures, including TBI (28,145). Hypothermia has also been reported to improve functional outcome in severely brain-injured patients (71,93). Repinotan reduces body temperature in a dose- and time-dependent manner. The maximal reduction of body temperature after repinotan was 2.5°C, it occurred at 0.5–1 h after drug administration. Hypothermia was no longer observed at 2 or 4 h after i.v., i.p., or p.o. administration (36).

The neuroprotective efficacy of repinotan does not seem to be due to its possible hypothermic effect. In several studies neuroprotective activity of repinotan was found to be independent of its effect on body temperature (33,71). However, since during surgery the body temperature of rats was kept artificially physiological, it is conceivable that after surgery the general stressful condition might mask the hypothermic effect of repinotan. In any case it is still possible that combining repinotan treatment with hypothermia may produce greater beneficial effects than either treatment alone (71).

In addition, the neuroprotective efficacy of repinotan did not seem to depend on its possible effect on the regional cerebral blood flow (rCBF) in animals (33). At two hours after start of continuous i.v. infusion of the drug, the most effective neuroprotective doses of repinotan were 1 or 3 μg/kg/h. At these or higher doses repinotan had only negligible effects on rCBF in the cerebral cortex, nucleus accumbens or thalamic nuclei. Furthermore, repinotan did not affect arterial blood pressure, blood glucose levels, or arterial pH, pCO₂, and pO₂ in different in vivo models (6,75,123,129). Thus, alterations in these parameters did not seem to contribute to the neuroprotective potency of repinotan.

**Involvement of Neurotrophic Factor S-100β**

S-100 is a set of small, highly acidic, calcium-binding dimer proteins of approximately 20 kDa, which are widely distributed in different tissues. The S-100 proteins are produced by a wide variety of normal and neoplastic cells, including brain cells (42,103). Three subtypes of S-100 proteins, formed by dimeric combinations of the α- and the β-chain, are known; S-100αα (αα), S-100αβ (αβ), and S-100ββ (ββ).

In the brain, S-100β is synthesized in and released by astrocytes. Its expression is directly related to the level of serotonin (52) and shown to be triggered by stimulation of 5-HT₁A receptors (8,142). The release of S-100β has been suggested to be involved in the neurotrophic effects of serotonin during early development (8,144), when serotonin stimulates neurite extension and sprouting in serotonergic neurons (138,140). In the adult brain, S-100β plays a role in neuronal plasticity (10,141) and long-term potentiation (47,80).

However, it is controversial whether S-100β is an apoptosis-inducing (42,44) or a neuroprotective protein (13). Because different concentrations of S-100β were used in these studies it is conceivable that S-100β may act as a cell survival factor at nanomolar
and as an apoptosis-inducing agent at micromolar concentrations. In any case, the effect of S-100β seems to depend on the developmental stage of the neurons and their density.

Furthermore, the role of S-100β during ischemia is still unclear. The levels of S-100β are known to be increased in the cerebrospinal fluid of stroke patients (70) and patients with traumatic brain injury (56). The increased S-100β immunoreactivity in the brain was related to the extent of neuronal damage. Therefore, the S-100β level is considered a marker for the activation of astrocytes and the S-100β levels in the blood are used as an indicator of the infarct volume and of prognosis in acute stroke (100, 119). However, it is still unclear whether the increase in S-100β immunoreactivity reflects the astroglial response to injury, whether it causes or enhances neuronal damage or whether it is cellular defense mechanism against oxidative stress.

The role of S-100β during ischemia was examined (3) to elucidate whether S-100β can protect neurons against glutamate- or staurosporine-induced damage and whether S-100β is involved in the protective effect of repinotan against staurosporine-induced apoptosis (136). S-100β (1–10 ng/mL) protected rat hippocampal neurons from both glutamate-induced excitotoxicity and staurosporine-induced apoptosis. In addition, S-100β protected chick embryonic neurons from staurosporine-induced apoptosis. Repinotan increased S-100β levels both in vitro and in vivo. When repinotan was infused at 4 μg/kg i.v. over 4 h to rats, the S-100β levels were elevated at 6 h after termination of the infusion, in striatum but not in the hippocampus (3). This latter finding was in accordance with earlier studies (11). In cultured rat cortical astrocytes, a twofold increase in the release of S-100β was reported after incubation for 6 and 24 h with repinotan, 1 nM (3). Finally, S-100β antibody partially inhibited the anti-apoptotic effect of repinotan against staurosporine-induced apoptosis (3). Thus, S-100β was shown to contribute, at least partly, to the anti-apoptotic effects of repinotan. This result is in accordance with the finding that there is a correlation between increased S-100β availability in the extracellular space and a marked improvement in dendrite recovery after ischemia (117) adding further evidence for a S-100β role as a mediator of 5-HT1A-induced neuroprotection.

Involvement of Nerve Growth Factor (NGF)

The partial 5-HT1A agonist 8-OH-DPAT has been shown to protect chick embryonic neurons against apoptosis induced by serum deprivation. More importantly, the expression of nerve growth factor (NGF) was suggested to be partially involved in its mechanism of action (5).

To examine whether NGF is also involved in the neuroprotective effects of repinotan, the neuronal NGF secretion of chick neurons incubated in medium with or without serum was measured in the presence or absence of the drug (4). Under control conditions (medium with serum), repinotan increased secretion of NGF into the culture medium. Similarly, in vivo, NGF content of rat hippocampus was increased at 6 h after a 4 h-long intravenous infusion of repinotan, 4 μg/kg. The NGF levels returned to baseline levels at 12 h after termination of repinotan infusion. The drug had no effect on NGF content of the striatum. Under conditions of serum withdrawal, the NGF content of the culture medium was increased and not further elevated by repinotan. However, NGF antibodies (10 ng/mL) inhibited the neuroprotective effect of repinotan, 1 μM, in chick neurons suggesting that the drug affected NGF signaling pathway leading to a synergistic effect of NGF and repinotan (4).
Signaling Pathways after 5-HT$_{1A}$ Receptor Stimulation by Repinotan. MAPK-Pathways: Induction of NGF and BCL-2, and Inhibition of Caspase-3

The stimulation of the 5-HT$_{1A}$ receptor can lead to the activation of the mitogen-activated protein kinase (MAPK) cascade (49), and thus influence gene expression. Specifically, the isoforms of extracellular signal-regulated protein kinase (ERK)1/2 of the MAPK family have been linked to the inhibition of apoptosis (143). In the hippocampal neuron-derived cell line HN-2-5 a peak activation of ERK1/2 occurred after 30 min of treatment with repinotan, 100 nM (2). Subsequently, after terminating the drug treatment, the activation decreased dramatically to virtually basal levels in 3 h, which is different from the prolonged activation of ERK observed in 8-OH-DPAT-treated HN-2-5 cells (1) and could be attributed to the lower chemical stability of repinotan in aqueous solution (2). Dose-response analysis revealed that repinotan-triggered ERK1/2 activation was quite significant even at nanomolar concentrations of the drug. Similar to the dose-response curve of 8-OH-DPAT, the ERK1/2 stimulation reached a maximum at 100 nM of repinotan and then remained unchanged up to at least 1 µM.

In fibroblasts, ERK can be activated through the transfected human 5-HT$_{1A}$ receptor (45). In these cells 5-HT$_{1A}$ receptor stimulation eventually leads to activation of ERK (104). In the hippocampal neuron-derived cell line HN-2-5, increased phospholipase C (PLC) activity seemed to be essential for the activation of ERK1/2. This was supported by elimination of the 5-HT$_{1A}$ receptor-mediated activation of ERK1/2 by the PLC$_{b}$ inhibitor U73122 (29). In addition, the general protein kinase C (PKC) inhibitor GFX did not block signaling upstream of ERK1/2 (2), confirming that 5-HT$_{1A}$ receptor-mediated activation of ERK1/2 also in this cell line probably occurs through a Ras → Raf → MEK → ERK1/2 cascade and not through a PKC → Raf pathway. Interestingly, the MAPK pathway is shared by both, NGF and Bcl-2 signaling.

The exact molecular pathway of the effect of repinotan on NGF is still unclear. Other drugs have been shown to induce NGF protein and NGF mRNA by increasing the intracellular cAMP concentrations (101,125,128). As mentioned above, repinotan increased NGF secretion in in vivo (rat) as well as in vitro (chick neurons). In chick neurons, stimulation of 5-HT$_{1A}$ receptors is also coupled to an activation of adenylate cyclase and to an increase in the intracellular c-AMP content (41). In addition, as mentioned earlier, repinotan causes hyperpolarization which induces elevated intracellular potassium concentration, which in turn increases the concentration of NGF-mRNA in cultured hippocampal neurons (89,147).

Similarly, the exact molecular pathway of the effect of repinotan on proteins of the proto-oncogene B-cell lymphoma protein 2 (Bcl-2) family is only partly unraveled (75). The Bcl-2 and its related proteins are known to be major players in cell survival after brain injury. The Bcl-2 family contains both inhibitors (e.g., Bcl-2, Bcl-XL) and promoters (e.g., Bcl-2-associated protein X (Bax), Bcl-2-associated death promoter homologue) of cell death, and the ratio between these two types of proteins contributes to the vulnerability of an individual cell towards damaging stimuli (60). Interfering with Bcl-2 family protein expression by systemically administered drugs was proposed to be an interesting pharmacological approach to stroke therapy, especially during the first 24 h after damage (26,48,64).
Repinotan (1 or 3 μg/kg/h) was infused intravenously immediately after a 45-min ischemic period due to tMCA-O. At 6, 12, 24 h after the start of reperfusion Bcl-2 and Bax protein levels in the cerebral cortex were calculated (75). The treatment resulted in elevated Bcl-2 protein level in the ipsilateral cerebral cortex as early as 6 and 12 h after the start of reperfusion. This effect became more pronounced at 24 h. Repinotan had no effect on Bax content during reperfusion. In both cases, there was no difference between the effects of the drug at the two doses.

Thus, repinotan was found to increase Bcl-2 protein levels, which were shown to be neuroprotective during ischemia and/or reperfusion (33,97,123). The increase in BCL-2 protein content could contribute to the neuroprotection at 6 and 12 h of reperfusion (75).

Bcl-2 promoter activation has recently been shown to be regulated via MAPK (ERK) p42/p44 in vitro (85). Thus, it was hypothesized that stimulation of 5-HT1A receptors with repinotan contributes to the activation of the MAPK/ERK signalling pathway followed by induction of Bcl-2 protein (75), which in turn promotes cellular survival. Although repinotan did not seem to have an effect on Bax content during reperfusion, Bax regulation, similar to Bcl-2, has also been linked to the ERK1/2 cascade in several cell lines (59). However, the knowledge of molecular pathways of Bax protein regulation is still very limited and it is unknown whether Bcl-2 and Bax proteins are regulated via the same, or different, molecular pathways in the brain in vivo, and how this regulation is changed under pathological conditions such as ischemia/reperfusion (75).

Furthermore, through a MAPK cascade, stimulation of the 5-HT1A receptor with repinotan leads to a phosphoinositide-3-kinase (PI-3K)-independent inhibition of the pro-apoptotic, cysteinyl aspartate-specific protease, caspase-3 (2), which actually causes the cytoskeletal breakdown that defines apoptosis (1,49). Since many conditions of neural insult and degeneration culminate in the activation of caspase-3, it is clearly the common downstream protease that has to be somehow inhibited to cause suppression of apoptosis. The 5-HT1A receptor-mediated inhibition of caspase-3 could be used as a general tool to prevent neuronal apoptosis.

Downstream of ERK1/2, the enzyme PKCα has been found to provide a link between the 5-HT1A receptor and the pro-apoptotic enzyme caspase-3 in the hippocampal neuron-derived cell line HN-2-5 (2). Earlier studies already showed that drugs which activate PKC promote cell survival (88), and inhibitors of PKC, such as staurosporine (136), induce apoptosis.

Since phosphorylation is important for proper activation and turnover of the PKCα molecule (51) and one of the different phosphorylation sites of PKCα, the Thr638 residue, is within a MAPK recognition sequence (2), ERK1/2 was thought to regulate PKCα through direct phosphorylation. Likewise, caspase-3 was suggested to be inhibited through phosphorylation by PKCα.

CLINICAL TRIALS

Preclinical studies with repinotan suggested that the drug should be effective in the prevention of secondary brain damage in brain-injured patients suffering from acute ischemic stroke or traumatic brain injury. The safety and tolerability of repinotan at three different doses (0.5, 1.25, or 2.50 mg/day) were assessed in patients with severe traumatic brain injury.
injury (107). On a descriptive basis, the proportion of patients having good outcome or moderate disability based on Glasgow Outcome Scale was somewhat higher in repinotan-treated (60%) than in placebo-treated patients. Further clinical studies were designed to demonstrate the benefit of repinotan treatment in traumatic brain injury and preliminary results appeared to be favorable.

In the completed Phase IIb clinical trial (from 2002 to 2004) in patients with acute ischemic stroke repinotan did not meet the expectations raised by the preclinical studies. This randomized, double blind, placebo-controlled trial has been completed but not yet published. According to the press release from Bayer AG the results were negative (BAYNEWS International, December 23, 2004).

CONCLUSIONS

Repinotan, a drug with a good safety profile (33,92,107), was found to have pronounced neuroprotective effects in various ischemia models (pMCA-O, tMCA-O, and aSDH) that mimic different aspects of brain injury. Under pMCA-O conditions, repinotan HCl caused a strong, dose-dependent infarct reduction, with maximal reduction of infarct volume found in the 1–10 μg/kg dose range (62,97,129). In the tMCA-O model, application of repinotan HCl also resulted in a pronounced reduction in infarct volumes (33,97,123). In an SDH model repinotan was also clearly neuroprotective (6,61,71,97).

In patients with acute ischemic stroke, however, the primary endpoint of the clinical trial was not met and the anticipated clinical benefits of repinotan could not be demonstrated. Preliminary observations in human patients suggested a probable beneficial action of repinotan in traumatic brain injury (107). A detailed comparison of neuroprotective effects, plasma levels, side effects, pharmacokinetic and pharmacodynamic effects of repinotan in human patients and experimental animals is in preparation.

In the experimental models the neuroprotective effects of repinotan were mediated by 5-HT1A receptors, since the specific 5-HT1A receptor antagonist WAY 100635 blocked all effects of repinotan in vivo (21,22,34,36,39,73,96,123). Pretreatment with repinotan was not required to achieve neuroprotection. The drug reduced neuronal degeneration when it was added immediately after induction of injury. Post-treatment seemed to be more effective than pretreatment in the aSDH paradigm (6,33,61,62).

The proposed neuroprotective mechanisms of repinotan are thought to involve neuronal hyperpolarization via activation of G protein-coupled inwardly rectifying K+ channels (33,68). This hyperpolarization is induced through activation of both pre- and postsynaptic 5-HT1A receptors (17,22,37) and reduced glutamate release (16,21,33,36,96,116). In vitro repinotan decreased neuronal damage caused by glutamate in primary hippocampal cultures (96,129) and preserved cell morphology and integrity of the neuronal network (129).

In addition, voltage-independent blockade of N-, P-/Q-, and T, and L-type voltage-dependent Ca2+ channels via G-protein-mediated signaling mechanisms (19,98,137), and direct reduction of glutamate release (121,134) have been suggested as other probable mechanisms by which repinotan may exert its neuroprotective effects. These mechanisms could explain the neuroprotective efficacy of repinotan per se, but not necessarily its efficacy by delayed administration. The therapeutic time window of repinotan appeared to
be at least 5 h in \textit{in vivo} models used to assess its neuroprotective efficacy (33,62,97). Its therapeutic window at higher doses may be even longer than 5 h (97).

It is likely that other processes involved in the pathogenesis of brain injury could be affected by repinotan. The neuroprotective efficacy of repinotan cannot be attributed to induction of hypothermia (33,75,96) or to involve an effect on local cerebral blood flow (33), arterial blood pressure, blood glucose levels, or changes in the arterial pH, pCO$_2$, or pO$_2$ (6,75,123,129). The neuroprotective efficacy of repinotan could be related to its antiapoptotic effect that may be distinct from its hyperpolarizing effect or modulation of
glutamate release. *In vitro*, in mixed neuronal/glial cultures repinotan protected cells from serum deprivation-induced (4), as well as from staurosporine-induced apoptosis (3,136). *In vivo* repinotan protected hippocampal and striatal neurons from apoptosis after tMCA-O (123). All these effects were blocked by the specific 5-HT$\textsubscript{1A}$ receptor antagonist WAY 100635, showing a specific 5-HT$\textsubscript{1A}$ receptor-mediated effect.

Furthermore, repinotan increased the levels of death-inhibiting protein Bcl-2 in rat brains during ischemia/reperfusion (75) and levels of S-100$\beta$, a serotonergic glial growth factor that has been reported to protect cultured neurons against glutamate-induced damage (3). Both of these mechanisms may contribute to the neuroprotective efficacy of repinotan. In addition, repinotan was found to affect the NGF signaling pathway suggesting a synergistic effect with NGF (4). Finally, repinotan has been shown to suppress caspase-3 through MAPK and PKC$\alpha$ (2). This seems to be another pathway through which repinotan could exert its neuroprotective effect.

The ultimate goal of neuroprotective therapy is to restrict the severity of initial damage and to improve neurological outcome and, therefore, the quality of life. In the clinically relevant model of TBI repinotan did not seem to enhance vestibulomotor function as assessed in beam balance and beam walk tasks (71). Earlier studies showed an improved and accelerated recovery of abnormal hindlimb grasping reflex and forelimb flexion as compared with vehicle-treated controls for up to 42 days after brain injury (46). Repinotan also attenuated neocortical lesion-induced spatial learning and memory deficits in a Morris water maze (71). Supportive correlative behavioral, neuroanatomical and neurochemical evidence show that repinotan attenuates NMDA-induced delayed neuronal death in rat MBN when administrated at 2 or 3 days after injury (54,55). Thus, treatment with repinotan seems to be beneficial in a clinical context, since impairments in memory and learning and motor regulation are common following ischemic human brain injury.

In conclusion, the dose- and time-dependent neuroprotective efficacy of repinotan in preclinical studies indicate that this drug is a promising candidate for prevention of secondary brain damage in brain-injured patients suffering from acute ischemic stroke or traumatic brain injury. The anticipated clinical benefits could not be, however, demonstrated in acute ischemic stroke patients in the first comprehensive clinical trial. It is still possible that repinotan could be effective in the treatment of traumatic brain injury in humans.

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