CNS Drug Reviews
Vol. 9, No. 4, pp. 343–358

BAY 38-7271: A Novel Highly Selective and Highly Potent Cannabinoid Receptor Agonist for the Treatment of Traumatic Brain Injury

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Keywords: BAY 38-7271 — Cannabinoid agonists — Cannabinoid receptors — Neuroprotection — Traumatic brain injury.

ABSTRACT

Traumatic brain injury (TBI) is the most common cause of mortality and morbidity in adults under 40 years of age in industrialized countries. Worldwide the incidence is increasing, about 9.5 million people are hospitalized per year due to TBI, and the death rate is estimated to be more than one million people per year. Recently BAY 38-7271 has been characterized as a structurally novel, selective and highly potent cannabinoid CB_1/CB_2 receptor agonist *in vitro* and *in vivo* with pronounced neuroprotective efficacy in a rat traumatic brain injury model, showing a therapeutic window of at least 5 h. Furthermore, neuroprotective efficacy was also found in models of transient and permanent occlusion of the middle cerebral artery and brain edema models as well. In this article we review the *in vitro* and *in vivo* pharmacology of BAY 38-7271, the results from acute and subacute toxicity studies, pharmacokinetics and drug metabolism in animals and healthy male volunteers. In phase I studies BAY 38-7271 was safe and well tolerated when administered by i.v. infusion for either 1 or 24 h.

As the doses of BAY 38-7271 in animals needed for maximal neuroprotective efficacy were significantly lower than those inducing typical cannabinoid-like side effects, it is to be expected that the compound will offer a novel therapeutic approach with a favorable therapeutic window for the treatment of TBI or cerebral ischemia.

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INTRODUCTION

Traumatic Brain Injury

Traumatic brain injury (TBI) is the most common cause of mortality and morbidity in adults under 40 years of age in industrialized countries. Worldwide the incidence is increasing, about 9.5 million people are hospitalized per year due to TBI and the death rate is estimated to be more than one million people per year (26,62). In the US approximately 1 million head-injured people are treated in hospitals. From these patients, about 270,000 people experience a moderate or severe TBI, about 70,000 of them die from head injury, and around 80,000 of the survivors live with significant disabilities as a result of the injury. US society spends more than $48 billion a year for direct and indirect costs, thus, TBI has an enormous socioeconomic impact. Although strong efforts have been undertaken, no pharmacological tools for effective treatment of TBI are currently available.

General Considerations for Traumatic Brain Injury

One of the key events caused by brain ischemia after TBI is the immediate excessive release of the excitatory neurotransmitter glutamate, which triggers a cascade of intracellular processes leading finally to neuronal death (34). Based on the crucial role of glutamate mediated events in neuronal death, a variety of therapeutic research strategies have focused on postsynaptic inhibition of glutamate transmission, by means of glutamate receptors antagonists (10). Glutamate antagonists directly acting at the postsynaptic glutamate receptors show considerable side effects, including hypotension and psychotomimetic effects in humans, and can cause neurotoxic injury in animals (29,47,66). So far, most glutamate receptor antagonists investigated in clinical trials revealed no therapeutic efficacy due to unfavorable risk-benefit ratio or lack of efficacy (12,30). Consequently, alternative strategies have been developed. These approaches include inhibition of second messenger cascades involved in glutamatergic signaling and blockade of ion channels, which may counteract excessive ischemia-induced neuronal depolarization. The latter mentioned approach led to the discovery that some 5-HT_{1A} receptor agonists, such as BAY × 3702 (repinotan), have a strong neuroprotective potential (13,38).

The above mentioned primary pathophysiological mechanisms themselves trigger more complex and long lasting processes (17). Inflammation following injury, for example, could enhance pathogenesis through release of nitric oxide (NO) and/or cytokines, such as IL-1, IL-6, and TNF, which are elevated for several hours after traumatic brain injury and act as perpetrators (28,54,57). Although neither the relative importance of various mechanisms, nor the time after injury when these mechanisms are operative, is known, large efforts are undertaken to attenuate these processes (for review see ref. 35).

Cannabinoid Receptors and Neuroprotection

Scientific studies on the pharmacology of cannabis were advanced considerably by the identification of the cannabinoid Δ^9-THC as the major active constituent of cannabis (18), the cloning of the predominantly centrally located CB_1 receptor (37) and the predomi-

nantly peripherally located CB_2 receptor (44), the identification of selective CB_1 and CB_2 receptor antagonists (6), as well as the discovery of endogenous cannabinoid receptor ligands, such as arachidonylethanolamide (anandamide) or 2-arachidonoyl glycerol (2-AG).

Both receptors are negatively coupled to adenylate cyclase through heterotrimeric $G_{i/o}$ proteins (50). Furthermore the CB_1 receptor is negatively coupled to N- and P/Q-type voltage sensitive Ca^{2+}- and D-type K^+-channels, and positively coupled to A-type and inward-rectifying K^+-channels (43,50). Thus, activation of CB_1 receptors leads to cell hyperpolarization and inhibition of neurotransmitter release (19,27). In further investigations it was found that cannabinoids attenuate directly or indirectly a multiplicity of cytokines (for review see ref. 21). It has been also reported, that cannabinoids attenuate microglia which, once activated, become ameboidal, phagocytize tissue debris (33), and produce cytokines (22,33,53,65). Additional evidence that CB_1 receptor activation results in attenuation of multifarious pathways is the observation that TNF_α induced NO production on rat brain microglia (60) and mouse brain activated astrocytes (42) could be blocked by naturally occurring and synthetic CB_1 receptor agonists. Recent findings also suggest a role for CB_2 receptors in cannabinoid mediated neuroprotection. Supportive of this assumption are recent reports, demonstrating the expression of CB_2 receptors in neonatal rat brain cortex microglia upon IFN-γ stimulation (11) and regulation of microglial cell migration by CB_2 receptors (61). Taken together, these findings suggest that activation of cannabinoid receptors may offer a unique therapeutic opportunity for TBI not only by beneficial attenuation of immediate but also by attenuation of long lasting or later occurring pathophysiological processes.

Indeed, the assumed neuroprotective efficacy of cannabinoid receptor agonists has been demonstrated in several models. *In vitro*, the non-classical CB_1/CB_2 receptor agonist WIN 55,212-2 was reported to prevent excitotoxicity in hippocampal neurons (55). In addition, these authors demonstrated that WIN 55,212-2 induced neuroprotection could be blocked by the selective CB_1 receptor antagonist SR 141716A, suggesting a CB_1 receptor-mediated mechanism. Although the classical cannabinoid Δ^9-THC has been suggested to act in similar models via receptor-independent mechanisms (24,25), recent studies indicate that the neuroprotective effects of this compound were mediated by activation of CB_1 receptors (1). Also in rat models of transient global cerebral ischemia and focal cerebral ischemia, cannabinoid receptor agonists such as WIN 55,212-2 were reported to have neuroprotective properties (46). Supportive of the above mentioned studies were findings, that the endogenous cannabinoids, anandamide and 2-AG, were also effective *in vitro* and *in vivo*, in models of neurodegeneration and neuroprotection after experimental TBI (23,48,59). Even though HU-211 (dexanabinol), a non-psychotropic cannabinoid-type compound, has neuroprotective efficacy *in vivo* in different models (8,32), its neuroprotective efficacy is in all probability not mediated via the CB_1 receptor (9,56). However, the enantiomer of HU-211, the potent cannabinoid receptor agonist HU-210 was shown to reduce ischemic damage in a model of permanent middle cerebral occlusion, probably in part through induction of hypothermia (31, for review see ref. 21).

Recently, we have characterized BAY 38-7271 (Fig. 1) as a structurally novel, selective and highly potent cannabinoid CB_1/CB_2 receptor agonist *in vitro* and *in vivo* (15,39,40) with strong neuroprotective properties.

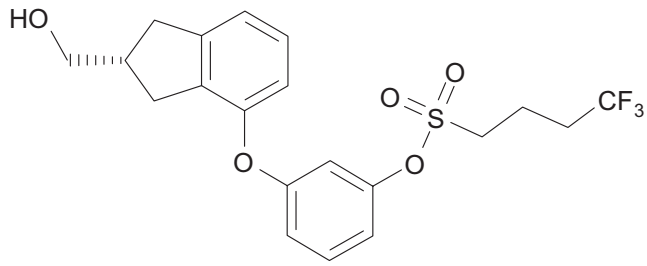

Fig. 1. Structure of BAY 38-7271.

PHARMACOLOGY

In Vitro

BAY 38-7271 was characterized *in vitro* as a highly selective and highly potent CB_1 receptor agonist with partial agonistic properties at the CB_2 receptor. The results of saturation and competition experiments are summarized in Table 1. [^{3}H]BAY 38-7271 binding was saturable at CB_1 and CB_2 receptors and Scatchard analysis fit best with the one site model (40). Depending on tissue and species, both B_{max} and K_d values differed only by a factor of approximately 3 (values from human cortex membranes excluded). Preliminary experiments revealed that in human cortical membranes BAY 38-7272 had slightly lower B_{max} values than in rat brain membranes; whereas no significant difference in K_d values has been detected. At CB_2 receptors, BAY 38-7271 showed comparable B_{max} and K_d values, and there was no evidence for selectivity towards either receptor subtype. However, competition experiments revealed a slightly lower affinity at the human recombinant CB_2 receptor. Results of further investigations revealed only minor interactions at the micromolar range with other binding sites such as adenosine A_3 receptor (IC_{50} = 7.5 μM), peripheral $GABA_A$ benzodiazepine receptor (IC_{50} = 971 nM), melatonin ML_1 receptor (IC_{50} = 3.3 μM), and at the monoamine transporter (IC_{50} = 1.7 μM). Signal transduction studies on brain cortex membranes using the [^{35}S]GTPγS technique revealed high signal transduction efficacy for BAY 38-7271 at human (63.4 ± 2.3% over base level) and at rat

TABLE 1. B_{max}, K_d, and K_i values of BAY 38-7271 at rat brain membranes, human cortex membranes, human recombinant CB_1, and human recombinant CB_2 receptors (radioligand [^{3}H]BAY 38-7271)

	B_{max} (pM/mg)	K_d (nM)	K_i (nM)
Rat brain membranes	3.01 ± 0.46	1.84 ± 1.44	0.46 ± 0.19
Human cortex membranes*	0.23*	2.10*	1.09 ± 0.05
Recombinant human CB_1 receptor	0.87 ± 0.13	0.87 ± 0.13	1.85 ± 0.71
Recombinant human CB_2 receptor	1.88 ± 0.21	4.24 ± 0.18	5.96 ± 0.83

* Values from one preliminary experiment, all other values are means ±standard deviations for at least three independent experiments, each performed in triplicate. The data were reported in ref. 40.

brain membranes (52.6 ± 5.6%). At the CB_1 receptor BAY 38-7271 has been characterized as a full agonist compared with reference compounds such as CP 55,940 (40).

In Vivo

Behavioral studies

The *in vitro* characterization of BAY 38-7271 as a highly potent cannabinoid CB_1 receptor agonist was confirmed *in vivo* in the hypothermia and the drug discrimination assay, two behavioral models highly sensitive to cannabinoid CB_1 receptor activation (4,36,51,63). In the rat hypothermia assay, BAY 38-7271 induced a potent and dose-dependent reduction in core body temperature (ED_{50} = 0.02 mg/kg, i.v., and 0.5 mg/kg i.p.). Moreover, it was found that the hypothermic effects of BAY 38-7271, either i.v. or i.p., were comparable in magnitude to those induced by the cannabinoid reference compounds: HU-210, CP 55,940, or WIN 55,212-2, and somewhat more pronounced than those induced by Δ^9-THC (40). The order of potency seen with these compounds is consistent with data obtained in similar hypothermia assays (36,52) and is in agreement with the *in vitro* binding data. Further evidence for an involvement of cannabinoid CB_1 receptors in hypothermia was suggested by the finding that pretreatment with the selective cannabinoid CB_1 receptor antagonist SR 141716A (52) blocks the hypothermic effects of various cannabinoid CB_1 receptor agonists, including BAY 38-7271 (40,52). Thus, it was found that at 1 mg/kg i.p. SR 141716A completely abolished hypothermia induced by BAY 38-7271, 0.02 mg/kg i.v.

The cannabinoid CB_1 receptor agonist profile of BAY 38-7271 was further confirmed in two drug discrimination assays, in which rats were trained to discriminate from vehicle, a relatively low dose (0.03 mg/kg i.p.) of either CP 55,940 (40), or of BAY 38-7271 (15). It has been demonstrated previously that the discriminative stimulus induced by a cannabinoid CB_1 receptor agonist is highly sensitive and specific (4,5, 63,64). In either CP 55,940 or BAY 38-7271 drug discrimination assays, each of the cannabinoid CB_1 receptor agonists induced complete generalization. Their order of potency closely resembled that obtained in the hypothermia or *in vitro* binding assays. Thus, it was found that BAY 38-7271 induced complete and dose-dependent generalization by either i.v. and i.p. administration in both drug discrimination assays (CP 55,940 drug discrimination: ED_{50} = 0.0004 mg/kg i.v. and 0.015 mg/kg i.p.; BAY 38-7271 drug discrimination: ED_{50} = 0.001 mg/kg i.v. and 0.018 mg/kg i.p.). This finding supports the suggestion that the discriminative effects of these compounds are mediated by activation of cannabinoid CB_1 receptors. This suggestion is also supported by the finding that, in both drug discrimination assays, the discriminative effects of BAY 38-7271 and CP 55,940 could be blocked in a dose-dependent and complete manner by SR 141716A (3 mg/kg, i.p.). These results are in accordance with other studies, which reported that the discriminative effects of Δ^9-THC, CP 55,940 or WIN 55,212-2 could be blocked by the cannabinoid CB_1 receptor antagonist (16,49,63,64).

The finding that relatively high doses of SR 141716A are needed to block the discriminative effects of BAY 38-7271 or CP 55,940 as compared to the relatively low doses which are sufficient to block the hypothermic effects of these compounds, is compatible with the concept that the discriminative and hypothermic effects induced by cannabinoid

CB_1 receptor agonists are mediated by receptor populations with different degrees of spare receptors (14,15,20).

The liability of BAY 38-7271 to induce physical dependence was assessed in rats, according to the method described by Aceto et al. (2,3) and Tsou et al. (58). In this study, rats were treated during 7 days with BAY 38-7271 (0.5 mg/kg, i.p., b.i.d.) or vehicle. At 1 h after the last treatment, half of the rats of each group were injected with either SR 141716A (1 mg/kg, i.p.) or vehicle. Behavioral effects (i.e., withdrawal symptoms) induced after abrupt withdrawal and after precipitated withdrawal were scored during a 60-min period. Since withdrawal symptoms, such as ptosis, and occasional biting, grooming, and locomotor stimulation were mild and only visible after precipitated withdrawal, it was concluded that physical dependence liability of BAY 38-7271 is low and is not essentially different from that of other cannabinoid CB_1 receptor agonists (2,3,58).

Neuroprotection

The neuroprotective efficacy of BAY 38-7271 has been investigated in rat models of TBI (acute subdural hematoma, SDH), reperfusion injury (transient middle artery occlusion, tMCA-O), permanent focal cerebral ischemia (permanent middle artery occlusion, pMCA-O) and brain edema. During surgery and infusion of the drug or vehicle, the body temperature was monitored and maintained within the physiological range ($37.0 \pm 0.5°C$) using a warming pad. After recovery from anesthesia the animals were returned to their home cages.

Acute SDH was induced by a unilateral injection of non-heparinized, autologous blood into the subdural space over a part of the somatosensory cortex of Wistar rats (41); post-surgical survival time was 7 days. Infarct volumes were determined by serial cresyl fast violet stained coronal sections in combination with a computer assisted image analysis system. For comparison of individual experiments, infarct volumes of treatment groups were expressed as percent from the respective controls, which were set to 100%. A strong reduction of the cortical damage over a broad dose range was achieved when BAY 38-7271 was infused for 4 h starting immediately after induction of the SDH. Mean infarct volume reductions of 49, 61, 70, and 62% were found at the doses of 1, 10, 100, and 1000 ng/kg/h, respectively. A graded decrease of efficacy was observed at lower (0.1 ng/kg/h; 37% infarct volume reduction) and higher (10,000 ng/kg/h; 38% infarct volume reduction) doses (Fig. 2A), which suggested a wide u-shape dose-response curve. When BAY 38-7271 was administered with a delay of 3 h after induction of SDH, mean infarct volume reductions of 36, 59, and 48% could be observed at doses of 100, 300, and 1000 ng/kg/h, respectively. Even when BAY 38-7271 administration was started at 5 h after induction of SDH, a significant infarct volume reduction of 42 and 49% at 300 and 1000 ng/kg/h could be observed (Fig. 2B). Strong neuroprotective efficacy has been seen when BAY 38-7271 was administered immediately after SDH either by infusion for 1 h (49, 65, and 53% infarct volume reduction at 0.01, 0.1, and 1.0 μg/kg/h, respectively) or for only 15 min (53% infarct volume reduction at 1 μg/kg). Significant neuroprotection (37, 64, and 47% at 1, 3, and 10 μg/kg of BAY 38-7271, respectively) has been also seen when the drug was infused for 15 min starting at 5 h after injury (Fig. 2C) (39,40).

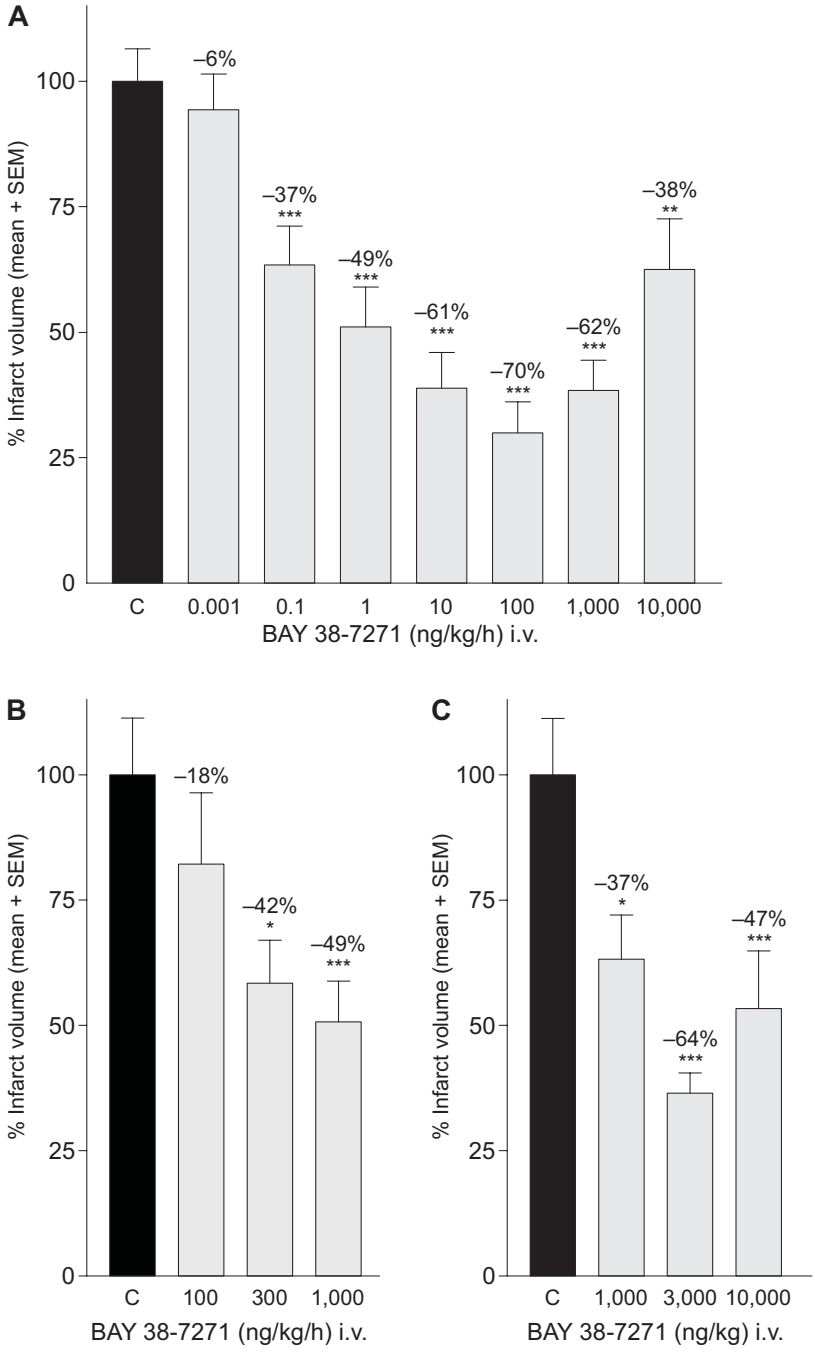

Fig. 2. Neuroprotective efficacy of BAY 38-7271 in the rat SDH model when administered (**A**) immediately after induction of SDH, or (**B**) with a 5 h delay as a 4 h i.v. infusion, and (**C**) neuroprotective efficacy when BAY 38-7271 was administered as 15min short-time infusion at 5 h after SDH. Infarct volumes were determined at 7 days after SDH and calculated as% of infarct volume of the respective control group; which was set to 100%. Values above bars indicate the% infarct volume reduction compared to controls. C, control; $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$; $n = 8–12$.

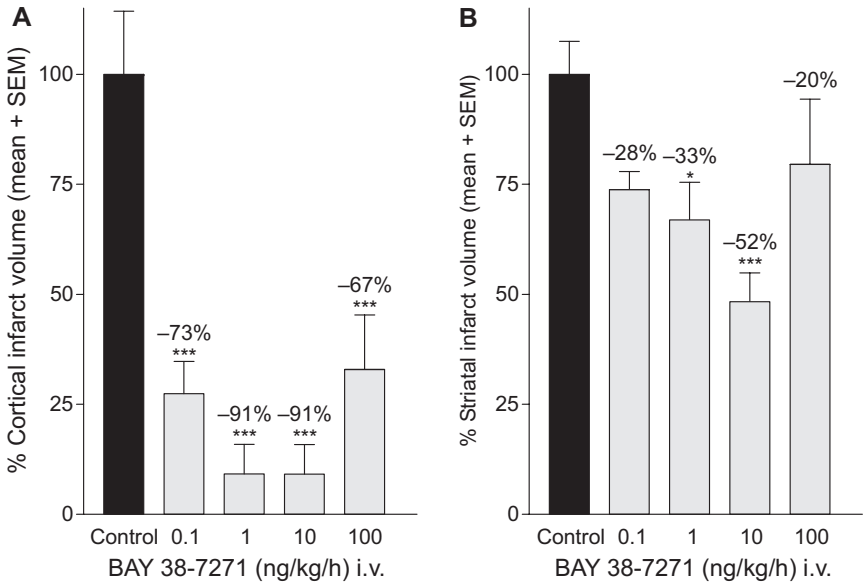

Fig. 3. Neuroprotective efficacy of BAY 38-7271 in the rat tMCA-O model on (**A**) cortical and (**B**) striatal infarct volumes. BAY 38-7271 was administered as continuous i.v. infusion for 4 h after a 1 h occlusion period immediately after reopening of the vessel. Infarct volumes were determined 2 days after SDH and calculated as % of infarct volumes of the respective control group; which was set to 100%. Values above bars indicate the % infarct volume reduction compared to controls. $*P < 0.05$; $***P < 0.001$; $n = 9–10$. Reproduced with permission from ref. 39.

tMCA-O (45) was induced by the modified intraluminal filament occlusion technique in Wistar rats (67), and infarct volumes were determined at 2 days after occlusion. The reperfusion injury induced by 1-h occlusion of the artery was strongly reduced in the cerebral cortex by 73, 91, 91, and 67% at 0.1, 1, 10, and 100 ng/kg/h, of BAY 38-7271, respectively (Fig. 3A). Striatal infarct volumes were smaller than cortical but BAY 38-7271 at 1 and 10 ng/kg/h reduced them by 33 and 52%, respectively (Fig. 3B) (39).

pMCA-O was performed in Long Evans rats according to a standard surgical procedure (7) involving unilateral permanent electrocoagulation of the middle cerebral artery and its branches. During surgery and drug administration body temperature was kept in the physiological range; infarct volumes were determined at 7 days after surgery. BAY 38-7271 was neuroprotective in this model. At 1000 ng/kg/h it reduced infarct volume by 27%, when infused for 4 h, starting immediately after occlusion. At lower and higher doses less neuroprotective efficacy was obtained; indicating that the dose-response curve was relatively flat (40).

Brain edema studies were performed according to a published method (39) using Wistar rats. Intracranial pressure (ICP) and brain water content were determined at 24 h after SDH. When BAY 38-7271 was infused for 4 h starting immediately after SDH, it reduced brain water content. At 250 ng/kg/h the drug reduced brain water content by 20% as compared to vehicle-treated controls. The SDH-induced increase in brain water content was reflected by an increase in the ICP. At 250 ng/kg/h BAY 38-7271 reduced ICP by 28% (39).

TABLE 2. Pharmacokinetic parameters (geometric mean) of BAY 38-7271 after i.v. administration to different animal species and humans

	Rats (male)	Dogs (female)	Humans (male)
Number of experiments	3	3	32 (4–6 per dose)
Dose (μg/kg)	2	0.6	0.07–1.7[b]
Route	i.v. infusion (T = 2 h)		i.v. infusion (T = 1 h)
$C_{max,norm}$ (kg/L)	0.138	0.200	1.02–1.31
AUC_{norm} (kg · h/L)	0.432	0.473	1.78–2.17
CL (L/kg/h)	2.31	2.11	0.46–0.53[c]
V_{ss} (L/kg)	4.31	2.93	1.72 — 2.79[c]
$t_{1/2}$ (h)	1.83[d]	1.23[d]	7.66–12.4[c]
Interval[a]	4–8	2.08–8	~8–t_n
Fu (%)	0.28	0.10	0.06

[a] Used for regression to determine $t_{1/2}$.
[b] Related to body weight of 70 kg.
[c] Data at doses between 40–120 μg. Terminal $t_{1/2}$ not assessable at lower doses.
[d] Values do not represent terminal $t_{1/2}$ due to limitations in sensitivity of analytical method.
t_n, Time of last sample with a concentration above limit of quantification.

PHARMACOKINETICS AND METABOLISM

The pharmacokinetics of BAY 38-7271 (parent compound) and [^{3}H]BAY 38-7271-radioactivity (unchanged compound and radioactive metabolites) were investigated in Wistar rats and Beagle dogs after intravenous infusion. Important pharmacokinetic parameters of BAY 38-7271 are summarized and compared with human data in Table 2.

BAY 38-7271 was infused over two hours at 2 and 10 μg/kg to male rats and at 0.6 and 3 μg/kg to female dogs. In either species its pharmacokinetics was dose proportional. Dose proportionality was also observed when BAY 38-7271 was administered as 4-week continuous infusion at infusion rates of 3, 10, and 30 μg/kg/h to rats and 0.3, 1, and 3 μg/kg/h to dogs. No accumulation or auto-induction was found with BAY 38-7271 by continuous intravenous infusion for 4 weeks.

BAY 38-7271 was rapidly eliminated from plasma in different animal species. The plasma clearance was moderate to high in rats and dogs, 2.3 and 2.1 L/kg/h, respectively. The volume of distribution was high (V_{ss} = 4.31 L/kg in rats and V_{ss} = 2.93 L/kg in dogs). The dominant elimination half-life was 1.8 h in rats (interval: 4–8 h after start of a 2 h infusion at 2 μg/kg) and 1.2 h in dogs (interval: 2.08–8 h after start of a 2 h infusion at 0.6 μg/kg). At higher doses of BAY 38-7271 its terminal elimination half-life in plasma was longer in both species. It was 36 h in rats (interval: 24–72 h after start of a 2 h infusion at 10 μg/kg) and 7.6 h in dogs (interval: 8–48 h after start of a 2 h infusion at 3 μg/kg). During the slow terminal elimination phase the plasma drug concentration was less than 1% of the maximal plasma concentration.

BAY 38-7271 was bound to human plasma proteins to a considerable extent. *In vitro*, free drug fractions in plasma differed substantially among species; they were 0.28% in

rats, 0.1% in dogs, and 0.06% in humans. In human plasma BAY 38-7271 was bound primarily to albumin and acidic α-1-glycoprotein.

Whole-body autoradiography studies with [^{3}H]BAY 38-7271 in rats displayed a rather homogeneous distribution pattern. Low to moderate radioactivity concentrations were observed in the majority of the organs and tissues studied. The radioactivity (parent compound, radioactive metabolites and tritiated water) penetrated the blood-brain-barrier. The radioactivity concentrations in brain were similar to blood. At 24 h after administration of [^{3}H]BAY 38-7271, the residual radioactivity was moderate, with highest concentrations of the drug in organs with excretory function. Only 7.5% of the dose was found in the freeze-dried sample of the residual animal excluding gastrointestinal tract. There was a further drop of the residual radioactivity to 1.8% of dose at day 7 after administration of the drug. There was no indication of irreversible binding or retention of [^{3}H]BAY 38-7271 radioactivity in organs and tissues of rats.

[^{3}H]BAY 38-7271-radioactivity was eliminated mainly via the biliary/fecal route in rats and dogs. In rats, 78% of the radioactivity was found in feces and only 7% was excreted in urine during 7 days after infusion of the drug. In dogs the corresponding values were 83% in feces and 5% in urine.

Incubation of BAY 38-7271 with microsomes from different species revealed that the cyclopentyl moiety of BAY 38-7271 was the main target of metabolic degradation, However, when the drug was incubated with human hepatocytes in sandwich culture the glucuronide conjugate of BAY 38-7271, M-4, was formed as the major metabolite.

In vivo, in rats BAY 38-7271 was subjected to intensive metabolism. In rat plasma, M-3, a 2-carboxy-cyclopentyl derivative of the drug, was the main circulating metabolite. Only 5–6% of the dose administered has been recovered in the 0–48 h rat urine fractions. The metabolic pattern in rat urine fractions was complex, with only traces of unchanged drug present. In the rat bile fractions, besides several minor biotransformation products, two major metabolites, glucuronide conjugate of BAY 38-7271, metabolite M-4, and metabolite M-3 were detected.

The metabolites, M-3 and M-4, were also important metabolites of BAY 38-7271 in the dog plasma, but the metabolic profiles of the drug in dog plasma and urine were more complex than in the rat.

TOXICOLOGY

The acute intravenous toxicity of BAY 38-7271, at single bolus doses, was studied in mice and rats. Due to limited solubility the drug could not have been administered at single doses higher than 1.2 mg/kg. At the doses used no animals died during 14 days after treatment, but typical cannabinoid CNS side effects have been observed in both species during the initial observation period (0–5 h). BAY 38-7271 has a large margin of safety, since its pharmacologically effective doses in rats ranged from 0.0001 to 10 μg/kg and there was no mortality with the 1200 μg/kg dose of the drug.

In subacute toxicity studies BAY 38-7271 was administered by continuous i.v. infusion for 28 days to Wistar rats (0, 3, 10, or 30 μg/kg/h) and beagle dogs (0, 0.3, 1, or 3 μg/kg/h). These studies did not reveal any indication for specific organ toxicity in hematology, clinical chemistry or in histopathological investigations. The clinical findings

observed in the rat at high doses (decreased activity, increased sensitivity to noise) are considered to be the result of an exaggerated pharmacodynamic effect ("cannabinoid action"). Decreased food intake and body weight gain were considered to be secondary to the clinical symptoms associated with the continuous infusion. In dogs no clinical findings were evident up to the highest dose tested. However, from two incidentally overdosed dogs it was shown that comparable exaggerated pharmacodynamic effects (decreased activity, loss of balance, reduction of reflexes, tremor, lateral recumbency, rolling eyes) can also occur in dogs. A no observed effect level (NOEL) of 3 μg/kg/h has been established for rats and dogs after subacute continuous i.v. infusion of BAY 38-7271.

The genotoxic potential of BAY 38-7271 was investigated in three different test systems: *Salmonella*/microsome test, chromosome aberration test *in vitro* with CHO cells, and micronucleus test in male mice. There were no indications for a point mutagenic potential in the *Salmonella*/microsome test with and without metabolic activation. In the chromosome aberration *in vitro* test a clastogenic effect was evident with BAY 38-7271 only in a cytotoxic dose range. However, the *in vivo* micronucleus test performed at clearly toxic dose levels revealed no indication of chromosomal aberrations up to i.v. — doses of twice 1.2 mg/kg. Thus, BAY 38-7271 is not considered to pose a mutagenic risk for humans.

A pilot developmental toxicity study showed no indications for teratogenicity. Some equivocal embryotoxic effects have been observed, but only in a maternally toxic dose range.

CLINICAL STUDY

To investigate safety, tolerability, pharmacodynamic effects and pharmacokinetics in man, the first phase I study was designed as a randomized, double-blind, short-term infusion study. BAY 38-7221 was administered to volunteers at six dose steps (5, 10, 20, 40, 80, and 120 μg i.v. over 1 h). Ethical approval was obtained from the Ethics Committee of the North-Rhine Medical Council. The study was conducted in accordance with the Declaration of Helsinki (1964) in the revised version of 1996 (Somerset West), the ICH GCP Guideline (Note for Guidance on Good Clinical Practice) and the German drug law (Arzneimittelgesetz, AMG).

Thirty-eight healthy male Caucasian subjects (median age 31.5 years; range 23–45 years); body weight: 80.0 ± 10.5 kg (range: 60.0–96.0 kg); height: 180.7 ± 6.6 cm (range 170.0–197.0 cm) were originally enrolled in the study. Two of these subjects received placebo treatment only. In total, 36 treated subjects completed the trial. All administered dosages were safe and well tolerated. Four adverse events were reported after BAY 38-7271 and three after placebo administration. The intensity of all adverse events was mild. Two of the four adverse events were related to BAY 38-7271. About 11 hs after start of the 5-μg infusion, one subject reported headache, which disappeared 9 h later; another subject complained of dryness of mouth 45 minutes after start of the 120 μg infusion. Symptoms resolved 2.5 h later. No clinically relevant changes in vital signs (heart rate, blood pressure), ECG and clinical chemistry, hematology and urinalysis were observed. Body temperature was determined sublingually before and up to 48 h after start of infusion. After infusion of 120 μg BAY 38-7271 body temperature was slightly but not

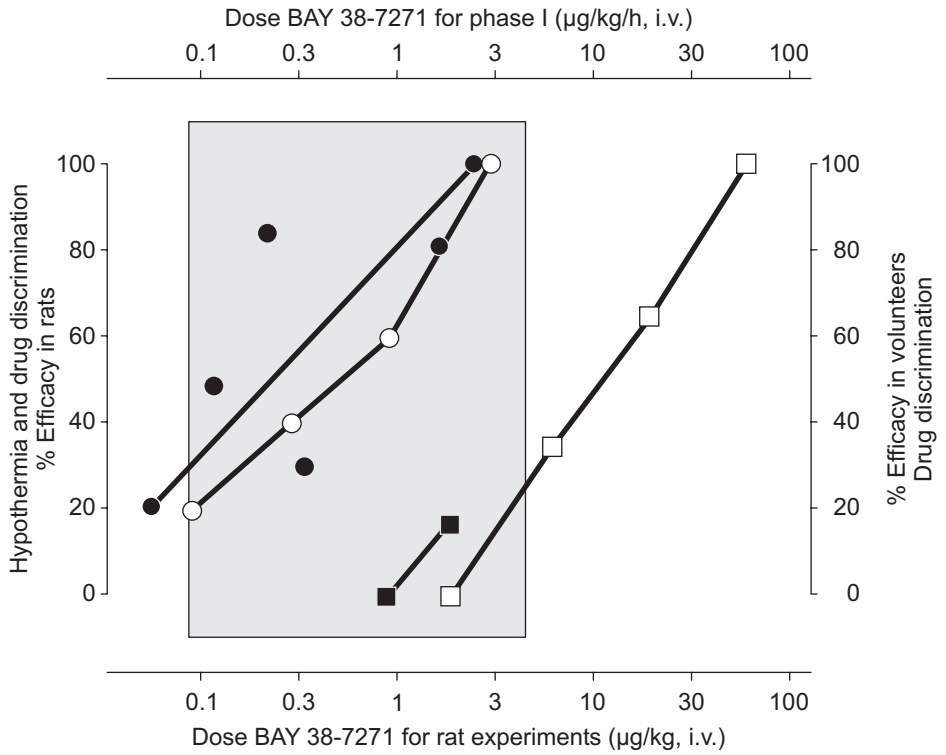

Fig. 4. Preclinical data from hypothermia (□) and drug discrimination (○) experiments were compared with the corresponding results obtained from Phase I studies (hypothermia ■, drug discrimination ●). The neuroprotective dose range in rats is similar to the dose range inducing significant signals in the rat and human drug discrimination test. Expected therapeutic range in humans is highlighted by the gray box (0.1–4 µg/kg/h, 70 kg body weight).

statistically significantly reduced (approx. –0.3°C) in comparison to placebo treatment. At the end of each dose step (second study period) subjects had to answer in which of the two study periods they believe they received the test drug. The results of this "end of study questionnaires" were listed (Böttcher et al., 2003; in preparation). However, when the results of the preclinical drug discrimination and hypothermia experiments were compared with the corresponding results obtained from phase I studies, the expected neuroprotective dose range in humans will likely range from 0.1–4 µg/kg (Fig. 4).

BAY 38-7271 concentrations in plasma and urine were determined with a fully validated gas-chromatographic method with mass spectrometric detection (negative chemical ionization mode) with a limit of quantification of 5 ng/L (plasma) and 25 ng/L (urine), respectively. $^{2}H_{5}$ BAY 38-7271 was used as an internal standard. BAY 38-7271 plasma concentrations increased in a dose-proportional fashion until the end of infusion with low to moderate inter-subject variability. The statistical analysis of $C_{max,\,norm}$ data supports the assumption of linear pharmacokinetics. Maximum plasma concentrations ranged from 75.7 ng/L (5 µg) to 1870 ng/L (120 µg; geometric mean values). The decline in concentration following the end of infusion could be described by a three-compartment model

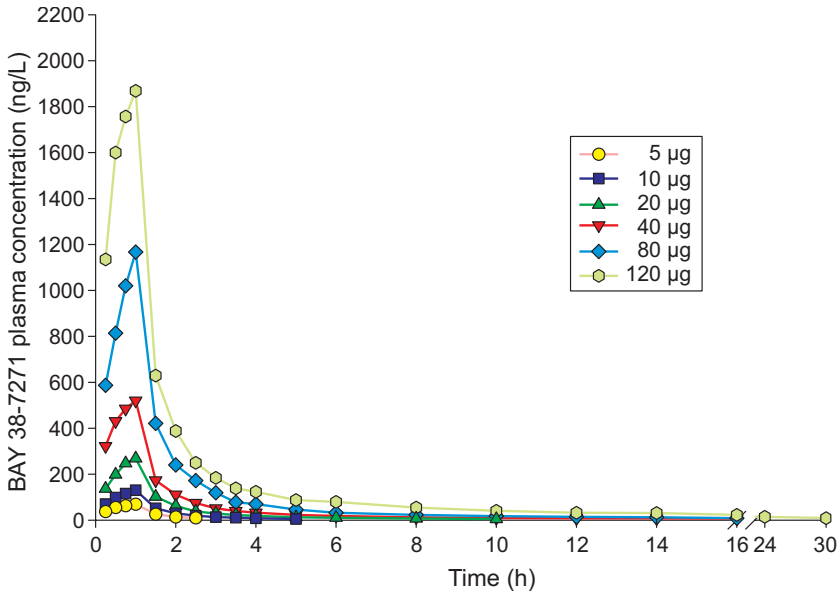

Fig. 5. BAY 38-7271 plasma concentration vs. time curves following i.v. infusion over 1 h to healthy male volunteers (geometric mean values, $N = 4–6$ per dose).

with distribution $t_{1/2}$ values of 0.21 and 1.81 h (dominant half-life). A terminal half-life of 8–12 h was determined in the highest dose steps (40–120 µg). BAY 38-7271 had a large volume of distribution (V_{ss}) of 1.72–2.79 L/kg consistent with animal data (see Table 2). Unchanged BAY 38-7271 was not detectable in urine. The expected BAY 38-7271 plasma concentrations ($C_{P,ss}$) upon continuation of the infusion to steady-state ranged between 150 ng/L (5 µg/h) and 3600 ng/L (120 µg/h) (calculated as quotient of infusion rate and a clearance of 33.3 L/h).

CONCLUSIONS

BAY 38-7271 was characterized as a highly potent and selective CB_1/CB_2 receptor agonist with pronounced neuroprotective efficacy in various models. BAY 38-7271 was also neuroprotective when administered at 5 h after injury by infusion for either 4 h or 15 min. The doses of BAY 38-7271 needed for maximal neuroprotective efficacy were significantly lower than those capable of inducing typical cannabinoid-like side-effects. It is, therefore, expected that the compound will offer a novel therapeutic approach with a favorable therapeutic window for the treatment of TBI and cerebral ischemia. Initial studies in healthy male subjects indicated that BAY 38-7271 is safe and well tolerated.

ADDENDUM: Chemical names of drugs mentioned by code number

CP 55,940: (–)-cis-3-[2-hydroxy-4(1,1-dimethyl-heptyl)phenyl]-trans-4-(3-hydroxypropyl) cyclohexanol.

HU-210: (–)-11-OH-8-tetrahydrocannabinol-dimethylheptyl.

SR 141716A: N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamidehydrochloride.

WIN 55,212-2: (*R*)-4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphtalenylcarbonyl)-6H-pyrrolo[3,2,1-ij]quinolin-6-one.

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