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SM-20220, a Potent Na^+/H^+ Exchange Inhibitor, Improves Consciousness Recovery and Neurological Outcome Following Transient Cerebral Ischaemia in Gerbils

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Abstract

We studied the cerebroprotective effect of SM-20220 (*N*-(aminoiminomethyl)-1-methyl-1*H*-indole-2-carboxamide methanesulphonate), a newly synthesized Na^+/H^+ exchanger (NHE) inhibitor, in Mongolian gerbil global ischaemia.

Transient cerebral ischaemia was induced by clipping both common carotid arteries for 30 min followed by 24 h reperfusion. Intravenous administration of SM-20220 (0·3 or 1·0 mg kg^{-1}) immediately after reperfusion significantly shortened the consciousness recovery time ($P < 0{\cdot}01$). SM-20220 also improved the neurological outcome (McGraw's scale) after reperfusion. At the dose of 1·0 mg kg^{-1}, the mortality rate was significantly reduced at 24 h after reperfusion ($P < 0{\cdot}01$).

This study shows that NHE is involved in the aggravation of cerebral function, represented by consciousness recovery, and neurological outcome following transient forebrain ischaemia, and that its inhibitor may exert protective effects on post-ischaemic brain damage.

Cerebral ischaemia causes energy disturbances and loss of neuronal function. Multiple mechanisms have been implicated in the development of brain damage following cerebral ischaemia (Pulsinelli 1992). Loss of ion homoeostasis, especially calcium and sodium overload during and after reperfusion, is also an important factor in ischaemic brain damage (Pulsinelli 1992; Siesjö 1992; Chen et al 1998). The Na^+/H^+ exchanger (NHE) is expressed in neurons and glial cells, and in some other cell types. NHE plays an important role in the regulation of cell volume and intracellular pH (pHi) (Pizzonia et al 1996; Orlowski & Grinstein 1997). Excessive activation of NHE by a decrease in pHi during ischaemia/reperfusion causes significant elevation of intracellular Na^+. The increase in intracellular Na^+ leads to Ca^{2+} overload via Na^+/Ca^{2+} exchangers in neurons and glial cells (Siesjö 1992; Matsuda et al 1997). It is well established that Ca^{2+} overload is a major mechanism of ischaemic cell damage (Pulsinelli 1992; Siesjö 1992). Therefore, it seems that inhibitors of NHE should interrupt the vicious circle that causes Ca^{2+} and Na^+ excess. Although protective effects of NHE inhibitors, such as amiloride and its analogues, have been investigated in simulated ischaemic conditions using cultured glial and neuronal cell damage in-vitro (Kempski et al 1988; Vornov et al 1996), the cerebroprotective effects of NHE inhibition in-vivo are unstudied. SM-20220 (*N*-(aminoiminomethyl)-1-methyl-1*H*-indole-2-carboxamide methanesulphonate) is a potent inhibitor of NHE. With regard to its NHE inhibitory effect, this compound was 50 times more potent than ethylisopropylamiloride (EIPA), a well characterized inhibitor of NHE (Itoh et al 1998). Most recently, we demonstrated that SM-20220 was a highly selective inhibitor of NHE and significantly ameliorated cerebral oedema and infarction in a rat middle cerebral artery occlusion model, thereby suggesting that a selective inhibitor of NHE has beneficial effects against ischaemic brain damage (Kuribayashi et al 1999).

In this study, we investigated the effects of SM-20220 on consciousness recovery and neurological outcome after transient cerebral ischaemia using Mongolian gerbils (*Meriones unguiculatus*).

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Materials and Methods

Induction of cerebral ischaemia

We used a total of 49 adult male gerbils, 60·9–76·5 g (Japan SLC, Hamamatsu, Japan). The gerbils were housed under regulated conditions of temperature (23 ± 2°C), relative humidity (55 ± 10%) and illumination (12-h light–dark cycle).

The gerbils were anaesthetized with halothane (induction at 4% and maintenance at 1·5% in 70% N_2O/30% O_2) and placed in the supine position. A midline cervical incision was made, and both common carotid arteries were carefully isolated and exposed. Transient forebrain ischaemia was induced by clipping both common carotid arteries for 30 min, which was subsequently followed by reperfusion. Complete reperfusion was visually verified after removal of the clips. Sham-operated gerbils were anaesthetized and the common carotid arteries were surgically exposed, without induction of ischaemia. Body temperature was maintained at 37°C by use of a heating pad (BWT-100, Bioresearch Center, Nagoya, Japan) during the surgical procedure and ischaemia. Anaesthesia was discontinued immediately after drug or vehicle administration.

Evaluation of consciousness recovery and neurological outcome

The time required for recovering the righting reflex was measured as an index of consciousness recovery (Kuribayashi et al 1994). Thereafter, the gerbils were transferred to individual cages. The neurological outcome of each gerbil was observed carefully and scored at 2, 4, 6 and 24 h after reperfusion using the modified stroke index of McGraw (McGraw 1977), which is as follows: 1, hair roughed up, tremor and paucity of movements; 2, head cocked; 3, circling behaviour; 4, splayed-out hind limb; 8, seizures; 17, comatose; 20, death. The higher the score, the more severe was the neurological deficit. The cumulative neurological score was calculated as the sum of the scores for each assessment. The number of deaths was ascertained during ischaemia, 0, 2, 4, 6 and 24 h after reperfusion. This data was recorded by a person without any previous information. The experiment was performed in a soundproof room to avoid noise interference.

Drug administration

SM-20220 was synthesized in our research centre. It was dissolved in 8% polyethylene glycol 400. SM-20220 (0·3 or 1·0 mg kg^{-1}) or vehicle was administered via the penile vein immediately after reperfusion. The volume of the solution administered was 3·0 mL kg^{-1}.

Statistical analysis

Consciousness recovery and neurological score were expressed as the mean ± s.e.m. Bartlett's test, followed by Williams's multiple-comparison test, was performed for analysis of consciousness recovery. Shirley-Williams's multiple-comparison test was performed for analysis of neurological score. The chi-squared test was performed for analysis of survival rate. A value of $P < 0·05$ was considered significant.

Results and Discussion

During carotid occlusion, one death was observed. The number of gerbils per group was 12. SM-20220 significantly shortened consciousness recovery time in a dose-dependent manner ($P < 0·01$). The time for recovery of the righting reflex was 1373 ± 62, 1129 ± 67 and 885 ± 50 in the group receiving vehicle, 0·3 mg kg^{-1} SM-20220 and 1·0 mg kg^{-1} SM-20220, respectively (Table 1). All sham-operated gerbils recovered the righting reflex within 66 s (43 ± 5 s) after discontinuation of anaesthesia.

The cumulative neurological scores, as determined after reperfusion, are illustrated in Figure 1. SM-20220 improved the neurological score dose-dependently. At 1·0 mg kg^{-1}, the neurological score started to significantly differ from the vehicle group at 2 h after reperfusion ($P < 0·01$). A dose of 0·3 mg kg^{-1} reduced the neurological score, but significance was not observed until 6 h after reperfusion ($P < 0·05$). The neurological score of all sham-operated gerbils was 0 at all observation points.

Table 1. Effect of SM-20220 on consciousness recovery following 30 min of bilateral common carotid artery occlusion in gerbils. The time for recovery of the righting reflex is shown.

Drug	Time (s)
Vehicle	1373 ± 62
SM-20220 (0·3 mg kg^{-1})	1129 ± 67**
SM-20220 (1·0 mg kg^{-1})	885 ± 50**

Drugs were administered intravenously immediately after reperfusion. Results are mean ± s.e.m. of 12 experiments. **$P < 0·01$, compared with the vehicle group (Bartlett's test, followed by Shirley-Williams's multiple-comparison test).

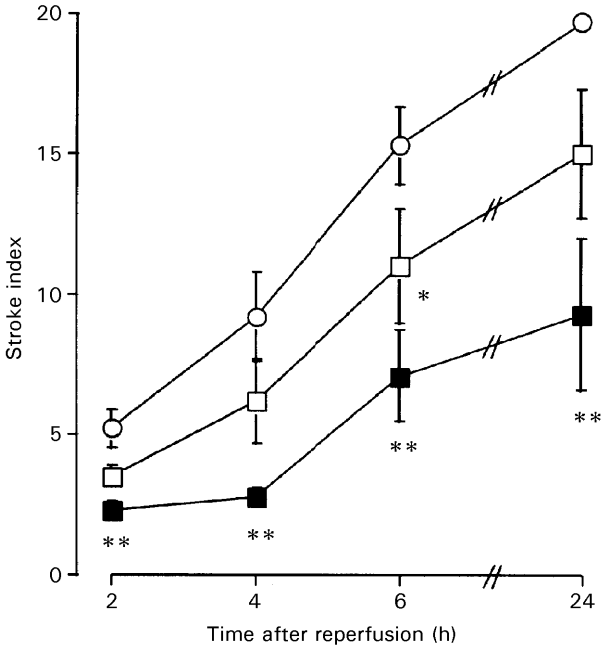

Figure 1. Effect of SM-20220 (□, 0·3 mg kg^{-1}; ■, 1·0 mg kg^{-1}) and of vehicle (○) on stroke index following 30 min of bilateral common carotid artery occlusion in gerbils. Drugs were administered intravenously immediately after reperfusion. Results are mean ± s.e.m. of 12 experiments. *$P < 0.05$, **$P < 0.01$, compared with the vehicle group (Shirley-Williams's multiple-comparison test).

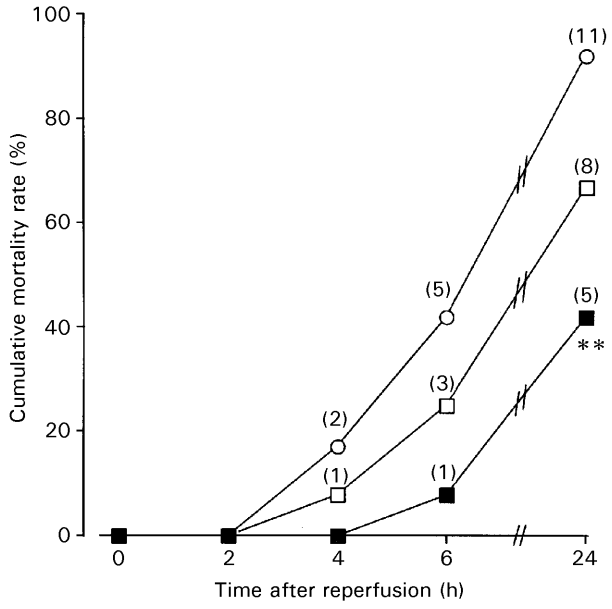

Figure 2. Effect of SM-20220 (□, 0·3 mg kg^{-1}; ■, 1·0 mg kg^{-1}) and of vehicle (○) on cumulative mortality rate following 30 min of bilateral common carotid artery occlusion in gerbils. Drugs were administered intravenously immediately after reperfusion. Numbers in parentheses indicate the number of dead gerbils in 12 experiments. **$P < 0.01$, compared with the vehicle group (chi-squared test).

The cumulative mortality rate at 24 h after reperfusion was 92%, 67% and 42% in the group receiving vehicle, 0·3 mg kg^{-1} SM-20220 and 1·0 mg kg^{-1} SM-20220, respectively (Figure 2). A significant difference was observed in the 1·0 mg kg^{-1} group ($P < 0.01$).

Bilateral common carotid artery occlusion in the gerbil produces a fairly uniform ischaemia in the telencephalon without ambiguity, resulting in neurological deficits and death (Fujisawa et al 1986). Improvement in neurological outcome and mortality may be important for cerebroprotection against ischaemic stroke. We therefore used the gerbil global ischaemia model in this study. SM-20220 did not affect blood pressure, heart rate, spontaneous motility, behaviour, maximal electroshock and pentetrazole-induced seizures, thiopental-induced sleeping time or body temperature as confirmed by a general pharmacological study. Hence, the cerebroprotective effect of SM-20220 in this study is not attributable to drug-induced alterations in physiological variables and secondary CNS behavioural effects. These results suggest that NHE is involved in cerebral function represented by the consciousness recovery and neurological outcome following transient forebrain ischaemia. NHE inhibition causes a reduction in platelet-activating factor formation in endothelial cells (Ghigo et al 1988) and prevention of neutrophil chemotaxis (Simchowitz & Cragoe 1986). Platelet-activating factor and accumulated neutrophils appear to be factors aggravating ischaemic brain injury (Bielenberg et al 1992; Jiang et al 1998). Recently, Phillis et al (1998) reported that EIPA attenuated ischaemia-induced amino-acid release in rats. It has been widely assumed that glutamate toxicity is one of the factors leading to cell death after cerebral ischaemia (Meldrum & Garthwaite 1990). Thus, NHE inhibitors may play various beneficial roles in ischaemic brain damage. In conclusion, this study demonstrated that SM-20220 represents a new class of drugs for the treatment of ischaemic stroke.

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