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Neuroprotective effects depend on the model of focal ischemia following middle cerebral artery occlusion

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Abstract

The purpose of the present study was to compare the characteristics of the photochemical-induced thrombotic occlusion model and the thermocoagulated occlusion model of the middle cerebral artery in rats. We evaluated the neuroprotective effects of a NMDA receptor antagonist, (+)-MK-801 (dizocilpine, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cycloheptan-5,10-imine), an α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor antagonist, YM90K (6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione monohydrochloride), a Ca^{2+} channel antagonist, *S*-312-d (*S*-(+)-methyl-4,7-dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)-thieno[2,3-b]pyridine-5-carboxylate), the radical scavengers, MCI-186 (3-methyl-1-phenyl-2-pyrazolin-5-one) and EPC-K_1 (L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyl-tridecyl)-2*H*-1-benzopyran-6yl-hydrogen phosphate] potassium salt), and a calcineurin inhibitor, FK506 (tacrolimus, Prograf). Although all tested agents in the present study attenuated the brain damage in the photochemical-induced thrombotic occlusion model, the radical scavengers did not attenuate the brain damage in the thermocoagulated occlusion model. The time course of brain damage and brain edema formation in the two models was examined. The time course of brain damage was not different in the two models, but the time course of brain edema was quite different. Brain edema formation in the photochemical-induced thrombotic occlusion model was significantly greater ($P < 0.01$) than that in the thermocoagulated occlusion model at all time point studied until 24 h after occlusion of the middle cerebral artery. The present study suggests that the photochemical-induced thrombotic occlusion model has characteristics of both permanent ischemia and ischemia-reperfusion.

Keywords: Cerebral ischemia; Middle cerebral artery occlusion; Neuroprotection

1. Introduction

Protection of neurons from ischemic injury has been observed with various agents including antagonists of glutamate receptors (Gill et al., 1991; Bullock et al., 1994; Yatsugi et al., 1996; Umemura et al., 1997; Park et al., 1998a,b) and Ca^{2+} channels (Sauter and Rudin, 1986; Morikawa et al., 1991; Genba et al., 1993; Takizawa et al., 1995), and free radical scavengers (Abe et al., 1988; Umemura et al., 1994b; Watanabe et al., 1994; Takamatsu et al., 1998). In order to develop a therapeutic agent for stroke treatment, several animal models of focal cerebral ischemia have been developed and used for screening to identify a novel candidate drug. Since the molecular targets for new drug research are diverse, it is important to evaluate a compound in a suitable animal model. Therefore, it is necessary to understand the character of the various models and to select a suitable one.

The photochemical-induced thrombotic occlusion model is based on thrombus formation on injured endothelial cells by molecular oxygen singlet generated with rose bengal and xenon light irradiation (Watson et al., 1985). Based on this technique, the rat middle cerebral artery thrombotic occlusion model was developed and several compounds have been tested in this model (Umemura et al., 1993, 1994a,b, 1995a,b,c,d, 1996, 1997; Matsuno et al., 1993; Kawai et al., 1995; Kaku et al., 1997; Takamatsu et al., 1998). Another well-known model of rat middle cerebral artery occlusion is the thermocoagulated occlusion model, or the 'Tamura model (Tamura et al., 1981)'.

In the present study, we evaluated six neuroprotective agents, a non-competitive NMDA receptor antagonist, (+)-MK-801 (dizocilpine, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[a,d]cycloheptan-5,10-imine), an alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid recep-

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tor antagonist, YM90K (6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione monohydrochloride), a L-type Ca^{2+} channel antagonist, *S*-312-d (*S*-(+)-methyl-4,7-dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)-thieno [2,3-b]pyridine-5-carboxylate), the radical scavengers, MCI-186 (3-methyl-1-phenyl-2-pyrazolin-5-one) and EPC-K_1 (L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyl-tridecyl)-2*H*-1-benzopyran-6yl-hydrogen phosphate] potassium salt), and an immunosuppressant, a calcineurin inhibitor, FK506 (tacrolimus, Prograf), in both the photochemical-induced thrombotic occlusion model and the thermocoagulated occlusion model of middle cerebral artery in rats and investigated differences in the response to these neuroprotective agents.

2. Materials and methods

2.1. Animal preparation

Male Sprague–Dawley rats (Japan SLC, Japan) weighing 280 to 300 g were used. All experiments were performed in accordance with the institutional guidelines of the Hamamatsu University School of Medicine.

Animals were anesthetized with 4% halothane and anesthesia was maintained with 2% halothane in an oxygen 30% and room air 70% mixture. The left middle cerebral artery was occluded by the transorbital approach under an operating microscope. Briefly, a vertical incision was made between the left orbit and the external auditory canal. The temporalis muscle was reflected, and subtemporal craniotomy was performed without removing the zygomatic arch. The main trunk of the middle cerebral artery and olfactory tract were observed through the dura mater. In the photochemical-induced thrombotic occlusion model (Umemura et al., 1993), photoillumination with green light (wave length, 540 nm) was achieved by using a xenon lamp (model L-4887, Hamamatsu Photonics, Japan) over the main trunk of the middle cerebral artery at the olfactory tract through the dura mater. Photoillumination was performed for 10 min after intravenous injection of rose bengal (20 mg/kg). The middle cerebral artery was occluded by thermocoagulation (Tamura et al., 1981) by using a microbipolar electrocoagulator (MICRO-3D, Mizuho, Japan). The middle cerebral artery from proximal to the olfactory tract to the inferior cerebral vein and the lenticulostriate arteries were permanently occluded and transected to avoid recanalization. After occlusion of the middle cerebral artery, the temporalis muscle and skin were closed in layers and anesthesia was discontinued. During the operation, the body temperature of the animals was maintained at 37.5°C with a heating pad (K-module model K-20, American Pharmaseal, USA). The surgical procedure was carried out within 20 min for the photochemical-induced thrombotic occlusion model or within 10 min for the thermocoagulation model. Twenty-four hours after the occlusion, the animals were decapitated under pentobarbital sodium anesthesia. The brain was removed and six preselected coronal sections (from anterior 3.5 mm to anterior 13.5 mm, 2 mm thickness) were made by using a brain matrix (RBS-02, Neuroscience, Japan). Each section was stained with 1% 2,3,5-triphenyltetrazolium chloride in phosphate buffer (pH 7.4). Photographs of the sections were then taken. After correction for swelling (Swanson et al., 1990), the volume of neuronal damage was calculated from the areas of damage at the different coronal sections and their anteroposterior coordinates.

2.2. Drug treatments

(+)-MK-801 hydrogen malerate (RBI, USA) was dissolved in saline and was administrated intravenously (0.1 mg/kg) by single bolus injection (2 ml/kg) immediately after middle cerebral artery occlusion. YM90K was dissolved in saline. The drug (30 mg/kg per hour) was intravenously infused (2 ml/kg per hour) immediately after middle cerebral artery occlusion for 4 h. *S*-312-d was dissolved in polyethylene glycol 400. It (0.3 mg/kg) was administrated intraperitoneally (1 ml/kg) immediately after middle cerebral artery occlusion. MCI-186 was dissolved in saline. The drug (6 mg/kg per hour) was intravenously infused (2 ml/kg per hour) immediately after middle cerebral artery occlusion for 3 h. EPC-K_1 was dissolved in saline and was administered (10 mg/kg) intravenously by single bolus injection (3 ml/kg) 3 h after middle cerebral artery occlusion. FK506 (10 mg/ml for injection) and its vehicle (1% ethanol in 400 mg/ml polyoxyl 60 hydrogenated castor oil) were diluted with saline. FK506 (0.3 mg/kg) or its vehicle was administered intravenously by single bolus injection (2 ml/kg) immediately after middle cerebral artery occlusion.

2.3. Time course of brain damage and edema

The brain damage and brain edema following middle cerebral artery occlusion were evaluated. Three, 6, 12, or 24 h after the middle cerebral artery occlusion, the animals were anesthetized with pentobarbital sodium. For the evaluation of brain damage, the brains were fixed by transcardial perfusion with 10% formalin neutral buffer solution, pH 7.4, following saline perfusion at 100 mm Hg. The brains were then removed and six preselected coronal sections (from anterior 3.5 mm to anterior 13.5 mm, 2 mm thickness) were made using a brain matrix (RBS-02, Neuroscience). Each section was embedded in paraffin wax, and 10-μm thick sections were cut and stained with hematoxylin and eosin. For each animal, the sum of the area of brain damage (Osborne et al., 1987), and the sum of the whole area of cerebrum in six sections were calculated by using a computerized image analysis system. After correction for swelling (Swanson et al., 1990), the brain damage

Table 1
Effects of neuroprotective compounds in the photochemical-induced thrombotic occlusion model of rat middle cerebral artery

Drug treatment		N	Brain damage (mm^3)		
			Total	Cortex	Striatum
(+)MK801	vehicle	9	202.4±8.5	142.8±7.7	59.6±5.3
	0.1 mg/kg	8	126.1±20.3[a]	73.9±15.4[a]	52.1±5.7
YM90K	vehicle	8	195.8±9.8	138.5±8.5	57.3±5.0
	30 mg/kg/h	8	122.6±10.3[a]	70.2±9.2[a]	52.4±5.6
S-312-d	vehicle	9	228.9±9.7	161.9±7.3	67.0±4.0
	0.3 mg/kg	9	164.7±13.3[a]	106.7±10.6[a]	58.1±4.3
MCI-186	vehicle	8	206.2±14.4	142.6±12.1	63.6±6.9
	6 mg/kg/h	8	158.8±27.0	86.2±23.1[b]	72.7±5.7
EPC-K_1	vehicle	8	239.6±10.6	169.0±9.3	70.7±4.0
	10 mg/kg	8	149.3±14.9[a]	87.2±13.6	62.1±4.7
FK506	vehicle	9	225.1±6.5	157.8±5.4	67.3±3.7
	0.3 mg/kg	8	156.6±19.2[a]	100.8±14.5[a]	55.8±5.3

Data represent the means±S.E.M. [a]$P < 0.01$, [b]$P < 0.05$ vs. vehicle group.

in each animal was calculated as the ratio of the area of brain damage to the total area of cerebrum. For evaluation of brain edema, the animals were decapitated and the brains were rapidly removed. The contralateral and ipsilateral hemispheres were immediately weighed separately. Each hemisphere was dried at 100°C for 72 h and then the dry weight of each hemisphere was determined. The brain water content was calculated from the weight difference between the wet and dry tissue. Data represent the difference in the water content of the left and right hemispheres.

2.4. Statistical analysis

Data are presented as the means ± S.E.M. Comparison of two groups was made by using an unpaired Student's *t*-test. $P < 0.05$ was considered significant.

Table 2
Effects of neuroprotective compounds in the thermocoagulated occlusion model of rat middle cerebral artery

Drug treatment		N	Brain damage (mm^3)		
			Total	Cortex	Striatum
(+)MK801	vehicle	8	198.9±6.7	137.8±4.9	61.1±2.7
	0.1 mg/kg	8	136.5±6.2[a]	80.0±8.1[a]	56.5±3.6
YM90K	vehicle	8	198.3±14.1	145.8±13.2	52.5±3.8
	30 mg/kg/h	8	126.4±7.3[a]	80.5±4.6[a]	45.9±5.2
S-312-d	vehicle	10	195.9±11.8	135.9±9.2	60.0±4.3
	0.3 mg/kg	10	135.4±11.6[b]	83.1±10.2[b]	52.3±3.8
MCI-186	vehicle	9	199.0±13.4	129.9±11.4	69.1±4.3
	6 mg/kg/h	9	182.2±16.5	121.8±13.4	60.4±4.2
EPC-K_1	vehicle	8	216.1±6.2	147.0±6.0	69.1±2.8
	10 mg/kg	8	182.9±18.1	118.8±18.0	64.1±2.5
FK506	vehicle	10	180.4±5.2	119.7±6.5	60.7±5.8
	0.3 mg/kg	9	126.7±5.8[a]	75.9±6.7[b]	50.8±6.2

Data represent the means±S.E.M. [a]$P < 0.01$, [b]$P < 0.05$ vs. vehicle group.

3. Results

Physiological variables following the operation were within the normal range. The middle cerebral artery occlusion in both models led to brain damage that affected the cortex and the striatum. The damage to the striatum was not attenuated by any treatment used in the present study.

In the photochemical-induced thrombotic occlusion model, all of the agents tested, (+)-MK-801, YM90K, *S*-312-d, MCI-186, EPC-K_1, and FK506, significantly attenuated the brain damage (Table 1). MCI-186 did not affect the total brain damage, but attenuated the cortical damage.

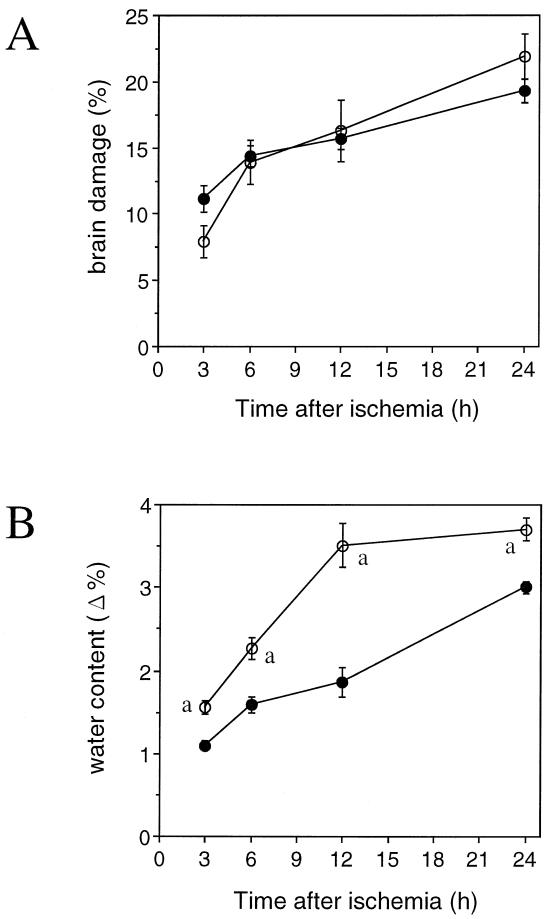

Fig. 1. (A) Time course of brain damage in the photochemical-induced thrombotic occlusion model (open circle) and in the thermocoagulation model (closed circle) of rat middle cerebral artery. The area of brain damage is presented as a percentage of the area of one cerebrum. Each point represents the mean for 7–8 animals, and the bars indicate the S.E.M. (B) Time course of brain edema formation in the photochemical-induced thrombotic occlusion model (open circle) and in the thermocoagulated occlusion model (closed circle) of rat middle cerebral artery. The water content is given as the difference between the left and right hemispheres. Each point represents the mean for 6–7 animals, and the bars indicate the S.E.M. [a]$P < 0.05$ vs. the thermocoagulated occlusion model.

Table 2 shows the extent of brain damage after treatment with each agent in the thermocoagulated occlusion model. (+)MK801, YM90K, *S*-312-d, and FK506 attenuated the brain damage significantly, but MCI-186 and EPC-K_1 did not have an effect in this model.

The time course of brain damage is shown in Fig. 1A. The brain damage seen in both middle cerebral artery occlusion models was not different at any time point studied until 24 h after ischemia. Fig. 1B shows the time course of brain edema. The water content of the contralateral hemisphere in the photochemical-induced thrombotic occlusion model and in the thermocoagulated occlusion model was $78.79 \pm 0.05\%$ and $79.11 \pm 0.03\%$, respectively. The brain edema in the photochemical-induced thrombotic occlusion model was significantly greater ($P < 0.01$) than that in the thermocoagulated occlusion model at all time points studied until 24 h after ischemia (Fig. 1B).

4. Discussion

In the present study, we evaluated six neuroprotective agents in two middle cerebral artery occlusion models in rats. The dosage, route, and duration of treatment of each agent were chosen according to the results of preliminary experiments (data not shown). A reproducible dose or a maximum solvable dose was used in each case.

The ionotropic glutamate receptors antagonists, (+)-MK-801 and YM90K, and a L-type Ca^{2+} channel antagonist, *S*-312-d, ameliorated the brain damage in the two models. Neuroprotective effects of (+)-MK-801 (Gill et al., 1991; Park et al., 1998a,b), YM90K (Bullock et al., 1994; Yatsugi et al., 1996; Umemura et al., 1997), and *S*-312-d (Genba et al., 1993) have been reported. These results, like those for other animal models of focal ischemia, demonstrated that the excessive release of glutamate and the excessive influx of Ca^{2+} through L-type Ca^{2+} channels following ischemia might play a critical role in the development of brain damage in the two models. The immunosuppressant FK506, a calcineurin inhibitor, was also effective in both models. Sharkey and Butcher (1994) first demonstrated that the neuroprotective effects of FK506 are mediated by immunoplilin or calcineurin. Thus calcineurin may mediate the progression of brain damage in both models. These findings suggest that an increase in the intracellular calcium concentration plays a key role in the development of neuronal damage.

There was a difference between the photochemical-induced thrombotic occlusion model and the thermocoagulated occlusion model in the response to the radical scavengers, MCI-186 and EPC-K_1. These compounds were only effective in the photochemical-induced thrombotic occlusion model. MCI-186 and EPC-K_1 can scavenge hydroxyl radicals and are inhibitors of lipid peroxidation (Mori et al., 1989; Watanabe et al., 1994). It has been reported that MCI-186 attenuates ischemic brain damage (Watanabe et al., 1994; Kawai et al., 1997) and brain edema (Abe et al., 1988; Nishi et al., 1989). Neuroprotective effects of EPC-K_1 have also reported (Kuribayashi et al., 1994; Takamatsu et al., 1998). These results suggest that reactive oxidants, especially hydroxyl radicals, may contribute to the development of brain damage more in the photochemical-induced thrombotic occlusion model than in the thermocoagulated occlusion model. There may be sufficient time for free radical generation during the development of brain damage in the photochemical-induced thrombotic occlusion model.

Although the neuroprotective effects of antioxidants in the permanent middle cerebral artery occlusion model in rats have been little reported, α-phenyl-*tert*-butyl-nitrone (Cao and Phillis, 1994), tirilazad (Park and Hall, 1994), and ebselen (Takasago et al., 1997) are reported to be effective in permanent occlusion models. We did not test these compounds. According to Cao and Phillis (1994), α-phenyl-*tert*-butyl-nitrone has a neuroprotective action when administration is started 12 h after ischemia. In the thermocoagulated, permanent, occlusion model used in the present study, the neuronal damage at 12 h after ischemia was almost maximal (Fig. 1A). Thus, it will be difficult to detect such neuroprotective effects in our model. The lack of a neuroprotective effect of tirilazad in the permanent middle cerebral artery occlusion model has also been reported (Hellstrom et al., 1994). The antioxidant ebselen has multiple functions, including an anti-inflammatory effect (Schewe, 1995) and an inhibitory effect on nitric oxide synthase (Zembowicz et al., 1993), which may contribute to the protective effect against ischemic damage. These findings suggest that the neuroprotective effects of antioxidants in the permanent middle cerebral artery occlusion model in rats are detectable under specific ischemic conditions and may be based on multiple functions of the agent.

It is generally considered that reoxygenation during reperfusion provides oxygen as a substrate for numerous enzymatic oxidation reactions that produce reactive oxidants (Chan, 1994, 1996). It is also thought that reperfusion disrupts the blood–brain barrier and exacerbates edema formation (Yang and Betz, 1994). In the present study, the time course of brain damage was not different in the two models, whereas the time course of brain edema formation was quite different. The water content of the brain in the photochemical-induced thrombotic occlusion model was higher than that in thermocoagulated occlusion model at all times up to 24 h after ischemia. These results indicate that a reperfusion-like phenomenon may occur rapidly after middle cerebral artery occlusion in the photochemical-induced thrombotic occlusion model. Although in the photochemical induced thrombotic occlusion model, spontaneous recanalization of the middle cerebral artery occurred about 8 h after occlusion (data not shown), Kawai et al. (1995) demonstrated that during middle cerebral artery occlusion in this model, fibrin-rich microthrombi formation occurred in a time-dependent manner. Accord-

ing to them, the thrombi were only found on the side supplied by the occluded middle cerebral artery, and, just after occlusion, microthrombi appeared on the surface of brain and then extended to the cortex (Kawai et al., 1995). The reperfusion-like phenomenon in the photochemical-induced thrombotic occlusion model may be caused by a change in the balance between consecutive microthrombi formation and their degradation by endogenous thrombolytic substances in microvessels. Their findings support our hypothesis that the reperfusion-like phenomenon might occur early after middle cerebral artery occlusion in this model. The reoxygeneration caused by this reperfusion-like phenomenon might produce reactive oxidants which exacerbate edema formation in this model. For this reason, we could evaluate the neuroprotective effects of radical scavengers in the photochemical-induced thrombotic occlusion model.

In conclusion, in the present study, we investigated the difference in the neuroprotective actions of radical scavengers and the time course of brain edema formation in the photochemical-induced thrombotic occlusion model and the thermocoagulated occlusion model of rat middle cerebral artery. These results suggest that the photochemical-induced thrombotic occlusion model shows characteristics of both permanent ischemia and ischemia-reperfusion. Therefore, the photochemical-induced thrombotic occlusion model may be a suitable model of focal ischemia for the screening of various compounds with different mechanisms of action.

References

Abe, K., Yuki, S., Kogure, K., 1988. Strong attenuation of ischemic and postischemic brain edema in rats by a novel free radical scavenger. Stroke 19, 480–485.

Bullock, R., Graham, D.I., Swanson, S., McCulloch, J., 1994. Neuroprotective effect of the AMPA receptor antagonist LY-293558 in focal cerebral ischemia in the cat. J. Cereb. Blood Flow Metab. 14, 466–471.

Cao, X., Phillis, J.W., 1994. α-Phenyl-*tert*-butyl-nitrone reduces cortical infarct and edema in rats subjected to focal ischemia. Brain Res. 644, 267–272.

Chan, P.H., 1994. Oxygen radicals in focal cerebral ischemia. Brain Pathol. 4, 59–65.

Chan, P.H., 1996. Role of oxidants in ischemic brain damage. Stroke 27, 1124–1129.

Genba, T., Ninomiya, M., Matsunaga, K., Ueda, M., 1993. Effects of a novel calcium antagonist, *S*-(+)-methyl-4,7-dihydro-3-isobutyl-6-methyl-4-(3-nitrophenyl)-thieno[2,3-b]pyridine-5-carboxylate (*S*-312-d), on ischemic amino acid release and neuronal injury in stroke-prone spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 265, 463–467.

Gill, R., Brazell, C., Woodruff, G.N., Kemp, J.A., 1991. The neuroprotective action of dizocilpine (MK801) in the rat middle cerebral artery occlusion model of focal ischemia. Br. J. Pharmacol. 103, 2030–2036.

Hellstrom, H.O., Wanhainen, A., Valtysson, H., Persson, L., Hillered, L., 1994. Effect of tirilazad mesylate given after permanent middle cerebral artery occlusion in rats. Acta Neurochir. 129, 188–192.

Kaku, S., Umemura, K., Mizuno, A., Kawasaki, T., Nakashima, M., 1997. Evaluation of the disintegrin, triflavin, in a rat middle cerebral artery thrombosis model. Eur. J. Pharmacol. 321, 301–305.

Kawai, H., Umemura, K., Nakashima, M., 1995. Effect of argatroban on microthrombi formation and brain damage in the rat middle cerebral artery thrombosis model. Jpn. J. Pharmacol. 69, 143–148.

Kawai, H., Nakai, H., Suga, M., Yuki, S., Watanabe, T., Sato, K., 1997. Effects of a novel free radical scavenger, MCI-186, on ischemic brain damage in the rat distal middle cerebral artery occlusion model. J. Pharmacol. Exp. Ther. 281, 1997.

Kuribayashi, Y., Naritomi, H., Sasaki, M., Sawada, T., 1994. Effects of L-ascorbic acid 2-[3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyl-tridecyl)-2*H*-1-benzopyran-6yl-hydrogen phosphate] potassium salt on cerebral energy state and consciousness recovery following transient forebrain ischemia in gerbils. Arzneim.-Forsch./Drug Res. 44, 995–998.

Matsuno, H., Uematsu, T., Umemura, K., Takiguchi, Y., Asai, Y., Nakashima, M., 1993. A simple and reproducible cerebral thrombosis model in rats induced by a photochemical reaction and the effect of a plasminogen-plasminogen activator chimera in this model. J. Pharmacol. Toxicol. Methods 29, 165–173.

Mori, A., Edamatsu, R., Kohno, M., Ohmori, S., 1989. A new hydroxyl radical scavenger: EPC-K_1. Neurosciences 15, 371–376.

Morikawa, E., Ginsberg, M.D., Dietrich, W.D., Duncan, R.C., Busto, R., 1991. Postischemic (*S*)-emopamil therapy ameliorates focal ischemic brain injury in rats. Stroke 22, 355–360.

Nishi, H., Watanabe, T., Sakurai, H., Yuki, S., Ishibashi, A., 1989. Effect of MCI-186 on brain edema in rats. Stroke 20, 1236–1240.

Osborne, K.A., Shigeno, T., Balarsky, A.-M., Ford, I., McCulloch, J., Teasdale, G.M., Graham, D.I., 1987. Quantitative assessment of early brain damage in a rat model of focal cerebral ischemia. J. Neurosurg. Psychiatry 50, 402–410.

Park, C.K., Hall, E.D., 1994. Dose-dependence analysis of the effect of 21-aminosteroid tirilazad mesylate (U-74006F) upon neurological outcome and ischemic brain damage in permanent focal cerebral ischemia. Brain Res. 645, 157–163.

Park, C.K., Nehls, D.G., Graham, D.I., Teasdale, G.M., McCulloch, J., 1998a. Focal cerebral ischemia in cats: treatment with the glutamate antagonist MK-801 after induction of ischaemia. J. Cereb. Blood Flow Metab. 8, 757–762.

Park, C.K., Nehls, D.G., Graham, D.I., Teasdale, G.M., McCulloch, J., 1998b. The glutamate antagonist MK-801 reduces focal cerebral ischemic brain damage in the rat. Ann. Neurol. 24, 543–554.

Sauter, A., Rudin, M., 1986. Calcium antagonists reduce the extent of infarction in rat middle cerebral artery occlusion model as determined by quantitative magnetic resonance imaging. Stroke 17, 1228–1234.

Schewe, T., 1995. Molecular actions of Ebselen—an antiinflammatory antioxidant. Gen. Pharmacol. 26, 1153–1169.

Sharkey, J., Butcher, S.P., 1994. Immunophilins mediate the neuroprotective effects of FK506 in focal cerebral ischemia. Nature 371, 336–339.

Swanson et al., 1990.

Takamatsu, H., Kondo, K., Ikeda, Y., Umemura, K., 1998. Hydroxyl radical generation after the third hour following ischemia contributes to brain damage. Eur. J. Pharmacol. 352, 165–169.

Takasago, T., Peters, E.E., Graham, D.I., Masayasu, H., Macrae, I.M., 1997. Neuroprotective efficacy of ebselen, an anti-oxidant with anti-inflammatory actions, in a rodent model of permanent middle cerebral artery occlusion. Br. J. Pharmacol. 122, 1251–1256.

Takizawa, S., Matsushima, K., Fujita, H., Nanri, K., Ogawa, S., Shinorara, Y., 1995. A selective N-type calcium channel antagonist reduces extracellular glutamate release and infarct volume in focal ischemia. J. Cereb. Blood Flow Metab. 15, 611–618.

Tamura, A., Graham, D.I., McCulloch, J., Teasdale, G.M., 1981. Focal cerebral ischemia in the rats: 1. Description of technique and early neuropathological consequences following middle cerebral artery occlusion. J. Cereb. Blood Flow Metab. 1, 61–69.

Umemura, K., Wada, K., Uematsu, T., Nakashima, M., 1993. Evaluation

of the combination of a tissue-type plasminogen activator, SUN9216, and a thromboxane A2 receptor antagonist, vapiprost, in a rat middle cerebral artery thrombosis model. Stroke 24, 1077–1081.

Umemura, K., Toshima, Y., Nakashima, M., 1994a. Thrombolytic efficacy of a modified tissue-type plasminogen activator, SUN9216, in the rat middle cerebral artery thrombosis model. Eur. J. Pharmacol. 262, 27–31.

Umemura, K., Wada, K., Uematsu, T., Mizuno, A., Nakashima, M., 1994b. Effect of 21-aminosteroid lipid peroxidation inhibitor, U74006F in the rat middle cerebral artery occlusion model. Eur. J. Pharmacol. 251, 69–74.

Umemura, K., Ishiye, M., Kosuge, K., Nakashima, M., 1995a. Effect of combination of a tissue-type plasminogen activator and an endothelin receptor antagonist, FR139317, in the rat cerebral infarction model. Eur. J. Pharmacol. 275, 17–21.

Umemura, K., Kawai, H., Ishihara, H., Nakashima, M., 1995b. Inhibitory effect of clopidogrel, vapiprost and argatroban in the middle cerebral artery thrombosis in the rats. Jpn. J. Pharmacol. 67, 253–258.

Umemura, K., Ishihara, H., Nakashima, M., 1995c. Anti-platelet effects of clopidogrel in rat middle cerebral artery thrombosis model. Thrombosis Res. 80, 209–216.

Umemura, K., Toshima, Y., Asai, F., Nakashima, M., 1995d. Effect of dietary docosahexaenoic acid enhances thrombolytic effect of tissue-type plasminogen activator in the rat middle cerebral artery thrombosis model. Thrombosis Res. 78, 379–387.

Umemura, K., Gemba, T., Mizuno, A., Nakashima, M., 1996. Inhibitory effect of MS-153 on elevated brain glutamate level induced by the rat middle cerebral artery occlusion. Stroke 27, 1624–1628.

Umemura, K., Shimakura, A., Nakashima, M., 1997. Neuroprotective effect of a novel AMPA antagonist, YM90K, in rat focal cerebral ischemia. Brain Res. 773, 61–65.

Watanabe, T., Yuki, S., Egawa, M., Nishi, H., 1994. Protective effect of MCI-186 on cerebral ischemia; Possible involvement of free radical scavenging and antioxidant actions. J. Pharmacol. Exp. Ther. 268, 1597–1604.

Watson, B.D., Dietrich, W.D., Busto, R., Wachtel, M.S., Ginsberg, M.D., 1985. Induction of reproducible brain infarction by photochemically initiated thrombosis. Ann. Neurol. 17, 497–504.

Yatsugi, S., Takahashi, M., Kawasaki-Yatsugi, S., Koshiya, K., Sakamoto, S., Uematsu, D., Shimizu-Sasamata, M., 1996. Neuroprotective effect of YM90K, a novel AMPA/kainate receptor antagonist, in focal cerebral ischemia in cats. J. Cereb. Blood Flow Metab. 16, 959–966.

Yang, G.-Y., Betz, A.L., 1994. Reperfusion-induced injury to the blood-brain barrier after middle cerebral artery occlusion in rats. Stroke 25, 1658–1665.

Zembowicz, A., Hatchett, R.J., Radziszewski, W., Gryglewski, R.J., 1993. Inhibition of endothelial nitric oxide synthase by ebselen. Prevention by thiols suggests the inactivation by ebselen of a critical thiol essential for the catalytic activity of nitric oxide synthase. J. Pharmacol. Exp. Ther. 267, 1112–1117.