Neuroprotective Effect of TTC-909, an Isocarbacyclin Methyl Ester Incorporated in Lipid Microspheres, on Hippocampal Delayed Neuronal Death of Stroke-Prone Spontaneously Hypertensive Rats

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ABSTRACT—TTC-909 is a newly developed isocarbacyclin methyl ester (TEI-9090) incorporated in lipid microspheres. The neuroprotective effect of TTC-909 was histologically examined in the pyramidal cell layer of the hippocampus CA1 subfield 7 days after transient forebrain ischemia using stroke-prone spontaneously hypertensive rats. TTC-909, given intravenously 10 min after the transient forebrain ischemia, dose-dependently protected against ischemia-related delayed neuronal death. The blood pressure remained unchanged following TTC-909 administration. This finding suggests that TTC-909 has a neuroprotective action on ischemic delayed neuronal death in the hippocampus.

Keywords: Ischemia-related delayed neuronal death, Prostacyclin analogue, Stroke-prone spontaneously hypertensive rat

Prostacyclin (PGI_2) has potent vasodilating and anti-platelet activities (1). However, the clinical use of PGI_2 is limited because of its unstable and short-lasting properties (1). Isocarbacyclin is one of the stable analogues of PGI_2, and TEI-9090, isocarbacyclin methyl ester (methyl 5-{(1*S*,5*S*,6*R*,7*R*)-7-hydroxy-6-[(*E*)-(*S*)-3-hydroxy-1-octenyl]bicyclo[3.3.0]oct-2-en-3-yl}pentanoate, CAS 88931-51-5), is also a chemically stable isocarbacyclin methyl ester (2). TTC-909 is a drug preparation of TEI-9090 incorporated into lipid microspheres. TTC-909 has been shown to possess both vasodilative and anti-platelet activity, similar to PGI_2 (3). In an experiment with a hamster cheek-pouch model, the anti-thrombotic activity of TTC-909 was more potent than that of PGI_2 (4). One advantage of TTC-909 over natural PGI_2 is its accumulation of coating lipid microspheres around the damaged blood vessel walls (5). TTC-909 is thus expected to be of clinical usefulness in the treatment of peripheral vascular disorders. On the other hand, TTC-909 improved the post-ischemic decrease and increase of cerebral blood flow and blood-brain barrier permeability, respectively, and prevented ischemic brain edema produced by occluding the middle cerebral artery in stroke-prone spontaneously hypertensive rats (SHRSP) (6). Thus TTC-909 seems to prevent delayed neuronal death evoked by transient forebrain ischemia. Ozagrel, a highly selective inhibitor of thromboxane A_2 synthase, is used as a therapeutic agent for thromboembolic disorders, cerebral circulatory disorders, ischemic heart diseases and asthma (7). Ozagrel inhibits both the spasms of the basilar artery and the decreases in regional cerebral blood flow, by reducing thromboxane A_2 production and increasing PGI_2 production (7). We report here the effect of TTC-909 and ozagrel on the delayed neuronal death induced by transient forebrain ischemia in SHRSP with the two-vessel occlusion model. SHRSP with two-vessel occlusion is a useful model for examining delayed neuronal death in the hippocampus caused by a transient forebrain ischemia (8, 9). This model is suitable for examining the post-ischemic conditions in human, because the microvascular disorder such as lacunar infarction is considered to have a long history of hypertension.

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Male SHRSP maintained and bred at the Laboratory Animal Center for Biochemical Research, Nagasaki University School of Medicine were used. These animals were fed SP diets containing 0.8% NaCl (Funabashi Farm Co., Chiba) and water ad libitum. Groups of three to four rats were housed in a cage in an air-conditioned room at 21±2℃, humidity of 55±10%, with a 12-hr light-dark schedule (light on 7:00 a.m.). Twelve- to fourteen-week-old SHRSP, each weighing 210−285 g, were supplied for bilateral carotid occlusion. The two-vessel occlusion model of the SHRSP was prepared as described previously (8, 9). In brief, the SHRSP were anesthetized with 1.5% halothane in room air, and body temperature was maintained at 37℃ with a heating pad. The common carotid arteries were surgically exposed bilaterally and then occluded for 10 min with aneurysmal clips. The sham operation was done in the same manner except for clamping the arteries. TTC-909 (Taisho Pharmaceutical

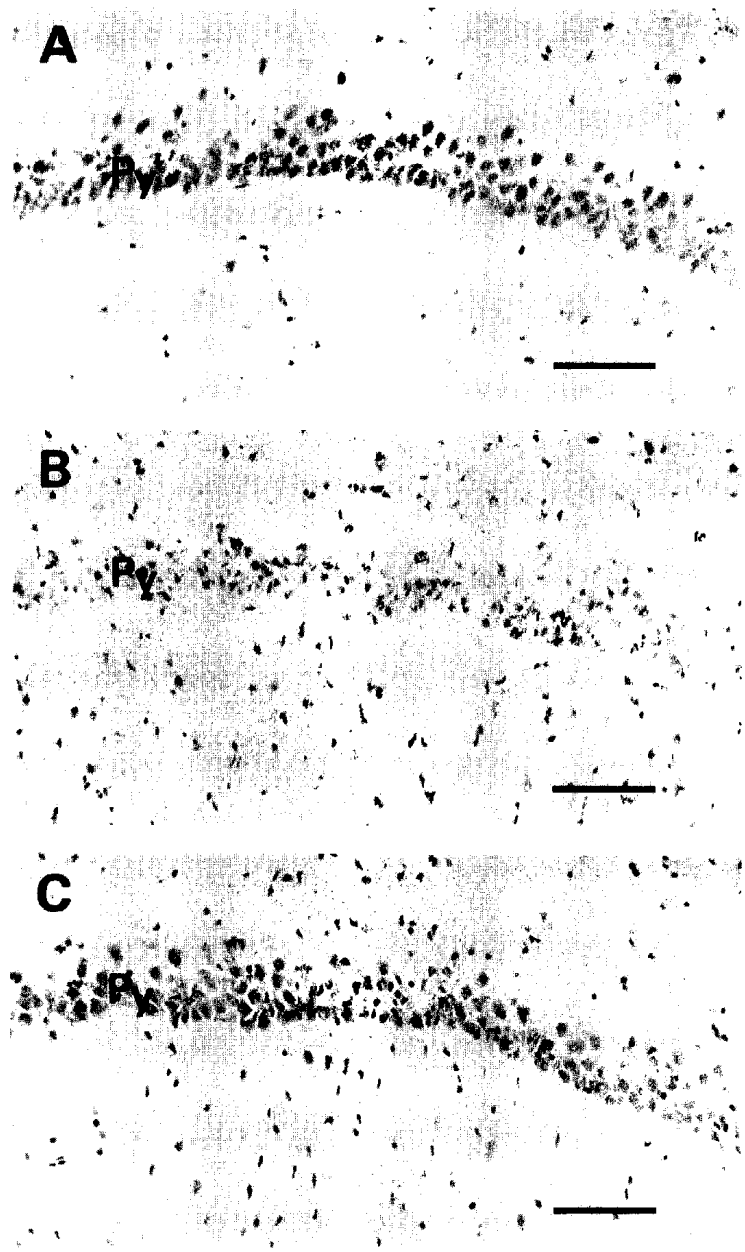

Fig. 1. Representative photomicrograghs showing the effect of TTC-909 on ischemic neuronal damage in the hippocampus CA1 pyramidal cell layer of the SHRSP. Photomicrographs of the hippocampus CA1 subfield were taken 7 days after the sham operation (A) and 10-min occlusion of bilateral common carotid arteries (B and C). Vehicle (B) and TTC-909 at a dose of 100 ng/kg (C) were administered intravenously 10 min after reperfusion. Py=pyramidal cell layer. Bars=100 μm.

Co., Tokyo) dissolved in a vehicle (Intralipid 10%; Otsuka Pharmaceutical Co., Tokyo) was injected intravenously (0.1 ml/100 g body weight) at a dose of 20, 50 or 100 ng/kg 10 min after reperfusion of blood flow. Ozagrel (Kissei Pharmaceutical Co., Matsumoto) was dissolved in saline and was injected intravenously at a dose of 10 mg/kg 10 min after reperfusion. Sham-operated animals were injected with only the vehicle.

After 7 days of survival, the brains were removed under diethylether anesthesia and frozen with isopentane at −30℃. Ten-micron-thick colonal brain sections at the level of plate 28 and 33 of Paxinos and Watson (10) were cut in a cryostat and thaw-mounted onto gelatin-coated glass slides, followed by staining with hematoxylin. The number of pyramidal cells per 1 mm linear length in an area selected at random for each section of the pyramidal cell layer of the hippocampus CA1 subfield was calculated by counting the neurons showing neither necrotic nor shrunken morphology under a microscope with a scale attached to the eye lens (9). The average number of pyramidal cells obtained from two separate sections (plate 28 and 33) of each rat was taken as the data for each rat. The effects of TTC-909 and ozagrel on the blood pressure of SHRSP were examined by measuring systolic blood pressure according to the tail-cuff method using a pneumatic pulse transducer with an electrosphygmomanometer (Narco Biosystem Co., Houston, TX, USA), 1 day before, 1 hr, 1 day and 7 days after drug administration. All animals used for procedures were treated under the Guideline of Animal Care and Use Committee of Nagasaki University. All data are expressed as means±S.E.M. Significance of differences was determined by one-way analysis of variance followed by Dunnett's test for multiple comparisons.

The number of pyramidal cells in the hippocampus CA1 subfield of SHRSP was 176.8±2.6 at 7 days after the sham-operations (Fig. 2). No neuronal damage was observed in the sham-operated SHRSP (Fig. 1A). A 10-min bilateral carotid occlusion of the SHRSP significantly decreased the number of pyramidal cells to 42.9±9.5 ($P<0.01$) at 7 days after the operation (Figs. 1B and 2). When the SHRSP with occlusion were treated with 20 ng/kg of TTC-909 10 min after reperfusion, the injured pyramidal cells were not improved. TTC-909, given at the dose of 50 and 100 ng/kg at 10 min after reperfusion, induced dose-dependent recovery of the occlusion-injured pyramidal cells to the number of 62.4±17.6 and 109.7±22.6, respectively, at 7 post-operative days. TTC-909 at the dose of 100 ng/kg was significantly effective in decreasing the ischemia-induced neuronal damage ($P<0.01$) (Figs. 1C and 2). When ozagrel at 10 mg/kg was administered to the SHRSP with occlusion 10 min

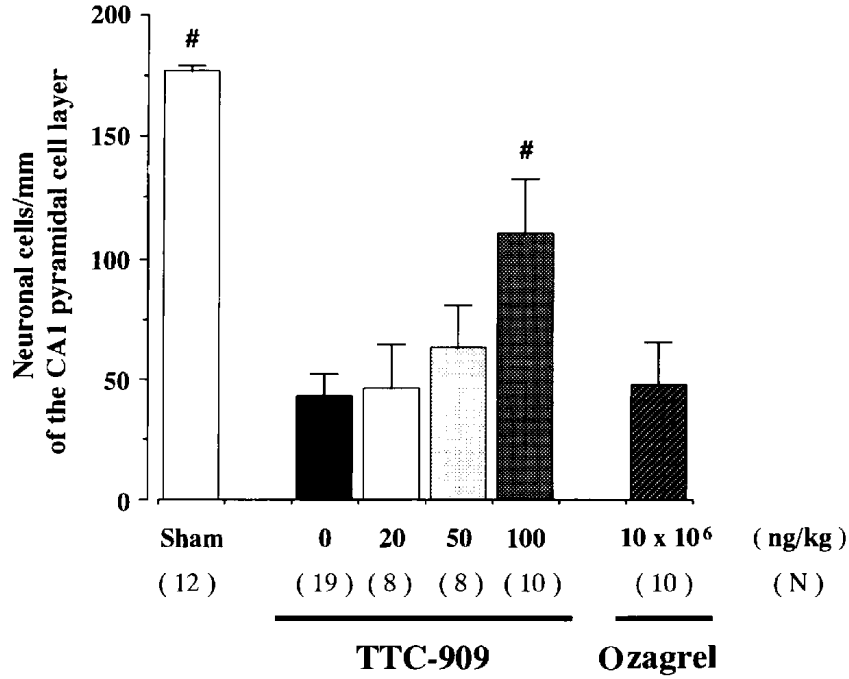

Fig. 2. Effects of TTC-909 and ozagrel on the number of pyramidal cells per 1 mm linear length in the hippocampal CA1 subfield of the SHRSP with occlusion at 7 post-operative days. TTC-909 and ozagrel were administered intravenously 10 min after reperfusion. Each column and vertical bar represent the mean±S.E.M. $^{\#}P<0.01$, vs vehicle (dose=0)-treated group in the occluded SHRSP (Dunnett's multiple comparison test).

Table 1. Effects of TTC-909 and ozagrel on blood pressure of SHRSP with bilateral carotid artery occlusion

Group	Dose (ng/kg)	n	Blood pressure (mmHg) (% of pre)			
			pre	1 hr after	1 day after	7 days after
Sham		12	242±5 (100±2.3)	224±8 (92.6±3.3)	235±7 (97.1±2.8)	256±6 (105.9±2.7)
Vehicle		19	239±5 (100±2.1)	226±6 (94.5±2.5)	249±6 (104.2±2.3)	241±5 (100.8±2.2)
TTC-909	20	8	247±7 (100±2.8)	253±7 (102.3±2.7)	238±9 (96.5±3.6)	248±8 (100.3±3.3)
	50	8	249±7 (100±2.8)	242±5 (97.0±1.9)	257±4 (103.0±1.8)	244±5 (98.0±1.9)
	100	10	235±5 (100±2.1)	227±6 (96.4±2.6)	248±6 (105.5±2.6)	241±7 (102.6±2.9)
Ozagrel	10×10^6	8	249±5 (100±2.2)	254±10 (101.8±4.1)	253±12 (101.5±4.7)	236±4 (94.5±1.8)

Values are mean±S.E.M.

after reperfusion, the injured pyramidal cells were not improved.

Table 1 shows the effects of TTC-909 and ozagrel on the blood pressure of SHRSP with occlusion. Any dose of TTC-909 or ozagrel did not affect the blood pressure of SHRSP at any period examined during the experiment. The 10-min bilateral carotid occlusion and reperfusion did not alter the blood pressure of SHRSP.

The present study demonstrated that a single administration of TTC-909 prevented ischemic neuronal damage in the hippocampus CA1 subfield of SHRSP following transient forebrain ischemia. This effect of TTC-909 appeared in a low dose of 100 ng/kg. Post-ischemic administration of TTC-909 was effective in this study. These characteristics are beneficial for the clinical treatment of ischemic brain disorder, because the systemic side effects can be avoided by using a low dose of drugs. Drugs that excessively decrease blood pressure may worsen the local cerebral perfusion. In this experiment, no dose of TTC-909 affected the systemic blood pressure. In models of global cerebral ischemia, the cerebral blood flow increases transiently after reperfusion, followed by long-lasting decreases (11). The capillary permeability in the blood-brain barrier also increases after transient forebrain ischemia (12). These progressive impairments of microcirculation in the regions vulnerable to ischemia may play a role in the generation of ischemic neural injury. There is evidence that treatments with TTC-909 significantly improved the decreased regional cerebral blood flow in the ischemic penumbra and significantly reduced the increased permeability in the ischemic center and rim, 7 days after middle cerebral artery occlusion in SHRSP (6). The lipid microspheres-coating of TTC-909 has the ability to accumulate around the damaged vascular walls, but not in normal tissue (5). These findings support the idea that TTC-909 improves post-ischemic events in local cerebral blood flow and blood-brain barrier permeability, leading to an attenuation of ischemic neuronal death in the hippocampus CA1 subfield of SHRSP.

It has been reported that ozagrel has the ability to decrease infarct size evoked by middle cerebral artery occlusion in rats (7). In the present study, we did not observe any neuroprotective action of ozagrel even at a high dose of up to 10 mg/kg. The occlusion of middle cerebral artery acutely developed the neuronal damage in the striatum and frontoparietal cortex. In the two-vessel occlusion model of SHRSP used here, the delayed neuronal death occurred in the pyramidal cells of the hippocampus CA1 subfield. These differences between the two models in the damaged brain region and the progression of the injury may reflect the effects of ozagrel. Although the half-lives of TTC-909 and ozagrel in plasma level are about half an hour, the difference between TTC-909 and ozagrel is their permeability to the blood-brain barrier (13, 14). TTC-909 can pass through in the brain and change to its active metabolite (TEI-7165) (13). However, ozagrel has very low blood-brain barrier permability (14). In an in vitro experiment, TEI-9090 and its active metabolite (TEI-7165) protected against BAY K 8644 (an L-type voltage-gated Ca^{2+} channel agonist)-induced striatal dysfunction, in a concentration-dependent manner (15). As excessive Ca^{2+} entry seems to be pertinent as a major etiological factor of neuronal death, a direct effect of TTC-909 on the central nervous system may also be operative for the attenuation of the ischemic neuronal death in the hippocampus CA1 subfield of SHRSP.

In conclusion, the present findings with the two-vessel occlusion model of SHRSP provide evidence that TTC-909 has a neuroprotective action on delayed neuronal death evoked by transient forebrain ischemia.

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