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Prostacyclin Analogue TTC-909 Reduces Memory Impairment in Rats With Cerebral Embolism

YOSHIMI UCHIYAMA-TSUYUKI,[1] KAZUAKI KAWASHIMA, HIROAKI ARAKI AND SUSUMU OTOMO

Department of Pharmacology, Research Center, Taisho Pharmaceutical Co., Ltd., 1-403, Yoshino-cho, Ohmiya, Saitama 330, Japan

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UCHIYAMA-TSUYUKI, Y., K. KAWASHIMA, H. ARAKI AND S. OTOMO. *Prostacyclin analogue TTC-909 reduces memory impairment in rats with cerebral embolism.* PHARMACOL BIOCHEM BEHAV **52**(3) 555-559, 1995.—The effects of the stable prostacyclin analogue TTC-909 on memory impairment in the water maze task and on neuronal damage were studied in rats with cerebral embolism induced by injecting polyvinyl acetate (PVA) into the right internal carotid artery and the ensuing embolism extending out into the right middle cerebral artery. Areas supplied by the lenticulostriate artery were most markedly damaged. In the water maze test, the PVA-embolized rats took longer to reach the platform than did the nontreated control rats. To some extent, repeated administrations of TTC-909 (200 ng/kg, IV) overcame this impairment in water maze learning in the rats. We assume that the vasodilating effects of TTC-909 maintain this blood supply to the ischemic area and that TTC-909 prevents the development of thrombosis around the PVA particles in the arterial capillaries, as a result of antiplatelet aggregative effects. These two mechanisms are likely to be involved in memory improvement. TTC-909 may prove effective for treating subjects with stroke and other cerebrovascular disorders.

Cerebral embolization Polyvinyl acetate Neuronal degeneration Memory impairment Water maze Prostacyclin analogue TTC-909 Internal capsule

CEREBRAL embolism resulting in ischemia and infarction is a major clinical problem. Various forms of experimental microembolization of the brain have been used as models for the production of cerebral ischemia (intra-arterial injection of microspheres, arachidonate, adenosine diphosphate, homologous blood clots, or air) (5-8,14,15,27). Embolization resulted in microinfarctions, a decrease in cerebral blood flow, and secondary brain edema with changes in the oxidative metabolic pathway (2,3,9,20,21). It has been reported that rats with cerebral embolism, as induced by microspheres injections, had an impaired passive and active avoidance response, and water maze learning was diminished (12,13,31). Cerebral embolism induced by polyvinyl acetate (PVA) injection into the internal carotid artery led to a low mortality rate (24,25). The embolization induced brain edema and changes in monoamine metabolism (24,25). In this model, the area supplied by the lenticulostriate artery was markedly damaged. Patients with an infarction in the internal capsule have an acute syndrome, thereby suggesting frontal lobe dysfunction (18,32). Memory deficits and behavioral changes following internal capsule lesions were also noted in rats (26,33). All of these observations led to the notion that the cerebral PVA-embolized rat might be a useful model to examine vascular-type disturbances in learning and memory. Prostacyclin (PGI2), a prostaglandin synthesized by endothelial cells lining the cardiovascular system, is a potent vasodilator and inhibitor of platelet aggregation (16,22,28). PGI2 is unstable and has a short half-life; derivatives were prepared (11,29). TTC-909 [methyl-5-([1S,5S,6R,7R]-7-hydroxy-6-[(E)-(S)-3-hydroxy-1-octenyl]bicyclo[3.3.0]oct-2-en-3-yl)pentanoate] is a chemically stable PGI2 analogue that incorporates into lipid microspheres. Shima et al. reported that TTC-909 improves postischemic low cerebral blood flow and glucose metabolism and reduces brain edema produced by occluding the distal middle

[1] To whom requests for reprints should be addressed.

cerebral artery (MCA) in stroke-prone spontaneously hypertensive rats (SHRSP) (30). Accordingly, our study was done to examine the effect of repeated TTC-909 administrations on behavioral impairment and neuronal damage in PVA-embolized rats.

METHODS

Animals

Male Wistar rats, weighing 320–350 g, were used at the beginning of the experiments. The animals were housed in groups of three in wire-mesh cages under conditions of controlled temperature (25 ± 1°C) and light (0700–1900). Food and water were freely available in the home cage.

Embolization

We performed cerebral embolization according to the method described by Nishi et al. (24), with minor modifications. The rats were anesthetized with Nembutal (40 mg/kg, IP) and placed in the supine position. After median incision of the neck skin, the right carotid artery bifurcation was exposed. The right external carotid and pterygopalatine arteries were ligated. The right common carotid artery was occluded with a vascular clamp. An incision was made in the external artery, and a polyethylene tube was inserted through the incision into the common carotid artery. The clamp occluding the common artery was removed. For embolization, 8 μl of 6.3% wt./vol. PVA in a solution of ethanol (49% by volume) was injected into the internal carotid artery through the tubing for 13 s using a infusion pump (Harvard Apparatus, South Natick, MA). Immediately after we removed the polyethylene tube, we ligated the external carotid artery below the punctured site and closed the wound. Rats were divided into three groups: a) nontreated control (n = 11), b) vehicle (intralipid)-treated PVA-embolized (n = 12), and c) TTC-909–treated PVA-embolized (n = 11). Intralipid, an emulsion of soya bean oil, egg lecithin, and glycerol is a standard component of parenteral nutrition prescribed clinically. TTC-909, 200 ng/kg daily for 15 days, was given IV immediately after PVA embolization. Sham-operated rats served as nontreated controls, These rats had no neurologic symptoms, histopathologic damage, or memory impairment, as confirmed in other experiments. All animals were subjected to each of the following three behavioral tests.

Open-Field Behavior

We observed the general behavior of the rats in the open-field test, as described by Hall (10). Two parameters were measured for 3 min: ambulation, expressed as the total number of sectors crossed by the rat; and rearing, expressed as the frequency with which it stood on its hindlimbs. The open-field test was run 24 days after cerebral embolization.

Passive Avoidance Task

We tested rats in a step-through-type passive avoidance task, using the method of Araki et al. (1). The acquisition trial was started 15 days after PVA embolization. The rat was placed in the illuminated compartment and allowed to enter the dark one; as soon as it did so, the door was closed and an unescapable scrambled foot-shock (6 mA) was delivered through the grid floor for 3 s. After 24 h, the rat was again placed in the illuminated compartment and the latency to enter the dark compartment was measured (retention test), if it took longer than 300 s, a ceiling score of 300 s was assigned.

Water Maze Task

We conducted the water maze task according to the method of Morris (23), with minor modifications. Briefly, rats were trained and tested in a pool composed of a circular black plastic water tank, 100 cm in diameter and 50 cm high, filled to a depth of 30 cm with water (26 ± 1°C) and made opaque by the addition of india ink. The experiment was done 10, 11, 12, and 21 days after PVA embolization. Rats were subjected to two sessions per day at 4-h intervals. One session consisted of four trials at 1-min intervals. In each trial of the day, four start positions were used in a nonrecurring, pseudorandom sequence. For each trial, the rat was placed in the water, facing the wall at the designated start position. Each rat was allowed to search for the platform for a maximum of 120 s. The latency time to find the escape platform, and the path swum by the rat, were recorded on a video tracker (G-2120; Muromachi-Kikai Co., Ltd., Tokyo, Japan). If the rat failed to escape within the time limit, it was guided to or placed on the platform, remained there for 30 s, and then was removed and given the next trial. Immediately after the eighth trial, each rat was given TTC-909 or vehicle, IV, and returned to the home cage. Then, 9 days after the last acquisition test, the same rats were used for the retention test.

Histologic Analysis

The animals were anesthetized and perfused with 10% formaldehyde in 0.1 M phosphate-buffered saline 25 days after embolization. Their brains were then removed, embedded in paraffin, cut into 5-μm-thick sections, and stained with hematoxylin-eosin for verification of infarcted areas.

Data Analysis

The following statistical analyses were made to assess the differences in values between groups. In the results of the open-field and passive avoidance tests, the differences were determined with Scheffé's test following one-way analysis of variance (ANOVA). Differences in the water maze test were determined with Scheffé's test following Kruskal–Wallis test at each trial, and with Friedman's test following nonparametric, two-way ANOVA at each session. For the incidence of infarction in each brain region, we used χ^2 test.

RESULTS

Neurologic Symptoms

In the presence of cerebral embolization, the contralateral paw was paralyzed, and circling or rolling behavior was occasionally present. These neurologic deficits gradually disappeared within 1–3 days. Sham-operated rats had no neurologic symptoms. A total of 13.0% of the rats died within 24 h. There were no significant differences in the mortality rate between TTC-909- and vehicle-treated groups.

Open-Field Test

In the open-field test, there were no significant differences among groups (numbers of ambulation: nontreament, 49.9 ± 5.8; PVA–vehicle, 39.0 ± 13.0; PVA–TTC-909, 50.2 ± 3.4; and number of rearings: nontreatment, 7.4 ± 1.1; PVA–vehicle, 5.3 ± 1.9; PVA–TTC-909, 8.4 ± 0.9) (Fig. 1).

Passive Avoidance Test

In the retention test, normal rats exhibited long response latencies to enter the dark compartment. The latency time of

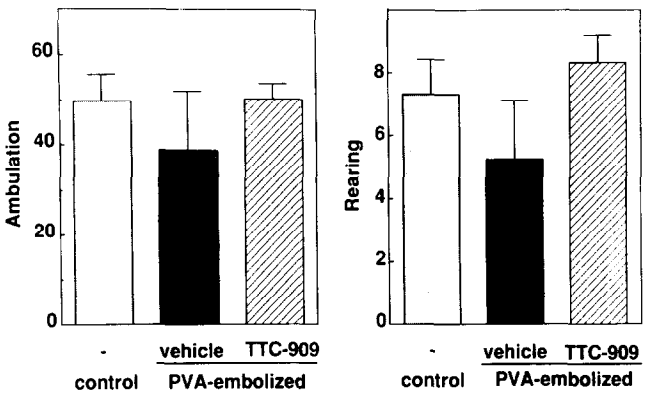

FIG. 1. Ambulation and rearing behavior in the open-field test in rats with cerebral embolization produced by an injection of PVA. Values are means ± SEM.

embolized rats decreased slightly but did not differ significantly from that of nontreated rats. TTC-909 also had no effect on the latency in PVA-embolized rats [latency time (s): nontreatment, 254.3 ± 31.2; PVA-vehicle, 200.1 ± 38.1; PVA-TTC-909, 154.3 ± 55.1] (Fig. 2).

Water Maze Test

Figure 3 shows the effects of TTC-909 on impairment of the acquisition test in the water maze task. The PVA-embolized rats swam as well as the nontreated control rats; however, they took longer to reach the platform than did the control rats. Differences at each trial were as follows: Trial 1, $H(2) = 8.394, p < 0.01$; 2, $H(2) = 8.753, p < 0.05$; 4, $H(2) = 8.552, p < 0.01$; 6, $H(2) = 6.333, p < 0.05$; 8, $H(2) = 10.326, p < 0.01$; 9, $H(2) = 10.554, p < 0.05$; 10, $H(2) = 12.793, p < 0.01$; 12, $H(2) = 7.827, p < 0.05$; 13, $H(2) = 10.166, p < 0.01$; 15, $H(2) = 11.954, p < 0.01$; 16, $H(2) = 5.547, p < 0.05$; 17, $H(2) = 10.482, p < 0.01$; 18, $H(2) = 10.564, p < 0.01$; 19, $H(2) = 4.939, p < 0.05$; 20, $H(2) = 11.359, p < 0.01$; 21, $H(2) = 13.402, p < 0.01$; 22, $H(2) = 15.396, p < 0.01$; 23, $H(2) = 11.519, p < 0.01$; 24, $H(2) = 7.607, p < 0.05$. Differences at each session were as follows: Session 1, $H(1) = 4, p < 0.05$; 2, $H(1) = 4, p < 0.05$; 3, $H(1) = 4, p < 0.05$; 4, $H(1) = 4, p < 0.05$; 5, $H(1) = 4, p < 0.05$; 6, $H(1) = 4, p < 0.05$. Treatment with TTC-909 improved the acquisition significantly in the following trials: Trial 2, $H(2) = 8.753, p < 0.05$; 7, $H(2) = 5.188, p < 0.05$; 9, $H(2) = 10.554, p < 0.01$; 10, $H(2) = 12.793, p < 0.01$; 11, $H(2) = 8.441, p < 0.01$; 13, $H(2) = 10.166, p < 0.05$; 14, $H(2) = 4.688, p < 0.05$; 20, $H(2) = 11.359, p < 0.05$; 21, $H(2) = 13.402, p < 0.01$; 23, $H(2) = 11.519, p < 0.05$. Differences at each session were as follows: Session 1, $H(1) = 4, p < 0.05$; 2, $H(1) = 4, p < 0.05$; 3, $H(1) = 4, p < 0.05$; 4, $H(1) = 4, p < 0.05$; 5, $H(1) = 4, p < 0.05$; 6, $H(1) = 4, p < 0.05$. In the retentive test, the PVA-embolized rats showed evidence of memory impairment: Trial 26, $H(2) = 7.934, p < 0.05$; 27, $H(2) = 6.339, p < 0.05$; 28, $H(2) = 11.132, p < 0.01$; Session 7, $H(1) = 4, p < 0.05$. TTC-909 improved retentive ability: Trial 28, $H(2) = 11.132, p < 0.05$; Session 7, $H(1) = 4, p < 0.05$.

Histopathology

The embolization induced by PVA caused a midline shift and neuronal damage that spread variably in the vicinity of the right-middle cerebral artery in the majority of rats. Lesions were also scattered in the anterior choroidal artery and anterior and posterior cerebral arteries. No neuronal damage was present in the cerebellum and brain stem, or in the contralateral hemisphere. In the vehicle-treated PVA embolized rats ($n = 11$; deleting one which died), there was no infarction in one animal. Of these 11 animals, neuronal damage occurred in the internal capsule (six), striatum (three), thalamus (eight), cerebral cortex (two), hippocampus (four), and hypothalamus (one). In the TTC-909-treated PVA embolized rats ($n = 9$; deleting two which died), one animal had no infarction. Of these nine, neuronal degeneration occurred in the internal capsule (two), striatum (four), thalamus (six), and hippocampus (four). No infarction occurred in the cerebral cortex and hypothalamus. The incidence of infarction in each region did not differ between TTC-909-treated and vehicle-treated PVA rats.

DISCUSSION

When a specific model is used to test the effects of drugs, it is important to know the extent of the ischemic lesions. However, in the type of model we used, inherent variability in location and size of each lesion produced could not be avoided. Histopathology showed that areas supplied by the lenticulostriate artery (including the internal capsule, striatum, and anterior part of the thalamus) were markedly damaged. The CA1 subfield of the dorsal hippocampus and frontal cortex were also affected. Because collateral arterial circuits are rich in the cortical regions, the areas supplied by the lenticulostriate artery were affected more severely than were the cortical regions. These results are similar to those

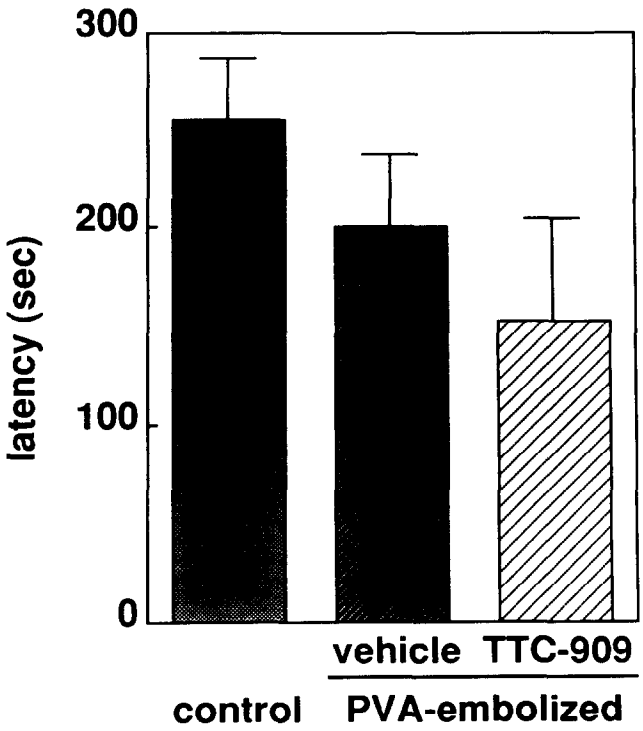

FIG. 2. Effects of cerebral embolization on passive avoidance response in rats. The acquisition trial was started 15 days after the operation, and the retention test was carried out 24 h later. Values are means ± SEM.

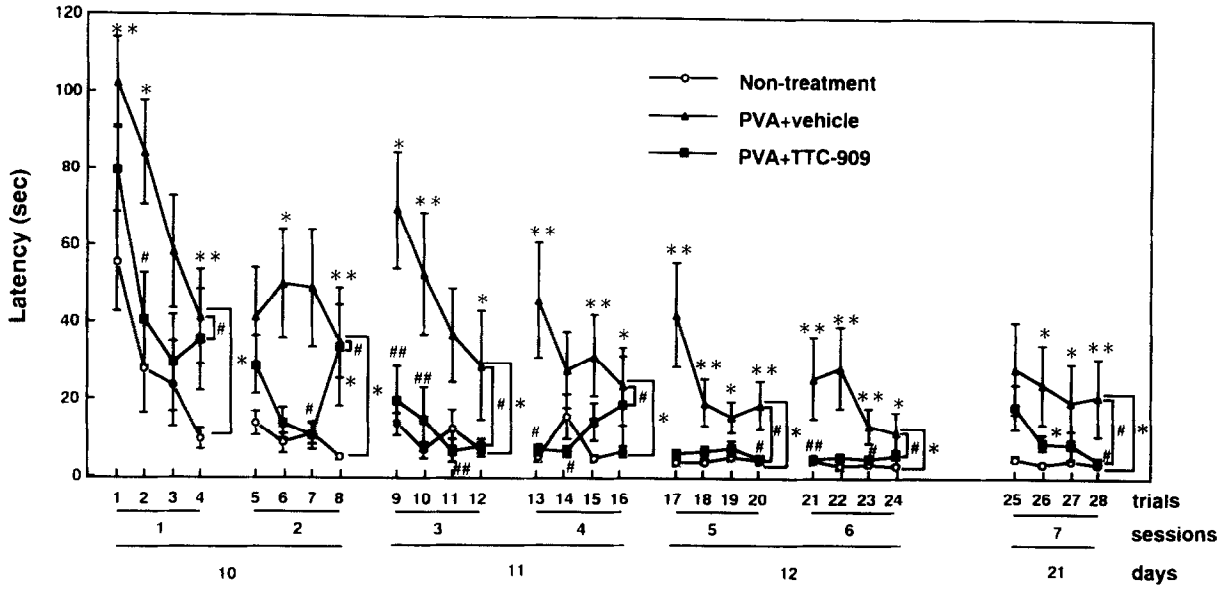

FIG. 3. Effects of TTC-909 on impairment in the water maze task in embolized rats. TTC-909 (200 ng/kg, IV) was administrated daily for 15 days after PVA embolization. $*p < 0.05$, $**p < 0.01$ vs. nontreatment; $\#p < 0.05$ vs. PVA-embolized vehicle treatment.

observed by Nishi et al. (24). In the present study, two different types of memory tests were given to PVA-embolized rats: passive avoidance and water maze. No memory deficits were evident in case of the passive avoidance test, whereas in microsphere-embolized rats, we noted impairment in the acquisition of passive avoidance response (12,13,31). Differences between each result may be related to the extent of neuronal damage. On the other hand, in the water maze task the embolized rats showed disturbances, yet they swam as well as the nontreated control rats. Therefore, impairment in water maze performance is not due to deficits in swimming ability; memory impairment may be associated with neuronal degeneration. The internal capsule forms the largest pathway of the forebrain and provides a conduit for afferent and efferent connections of the neocortex with the thalamus, and it contains large numbers of efferent projections to the midbrain, pons, medulla, and spinal cord (17). In patients with infarction in the inferior genu of the internal capsule, the acute syndrome is accompanied by fluctuating alertness, inattention, memory loss, apathy, abulia, and psychomotor retardation, suggesting frontal lobe dysfunction (18,32). Memory deficits and behavioral changes following internal capsule lesions were also noted in rats (26,33). Memory impairment in PVA-embolized rats may be associated with neuronal degeneration in the internal capsule. We suggest that the cerebral PVA-embolized rat may be a useful model for the vascular type of disturbance related to learning and memory. Treatment with TTC-909 had ameliorating effects in the acquisition and retention part of the water maze test. It has been reported that TTC-909 (100 and 200 ng/kg, IV) improved postischemic, low cerebral blood flow induced by the occlusion of MCA in SHRSP (30). We refered to these data and determined the dose and regimen of TTC-909 used in the present study. Cerebral embolization induces brain edema and changes in energy metabolism (2,3,9,20,21). TTC-909 improved glucose metabolism and reduced brain edema produced by occlusion of the MCA in SHRSP rats (30). TTC-909 seems to maintain the blood supply to the ischemic area for a vasodilating effect, and it prevents the development of thrombosis around the PVA particles in the arterial capillaries for an antiplatelet aggregating effect. Ischemia-induced impairment is most sensitive to hypothermia (4,19). However, we confirmed that TTC-909 in a dose of 100 ng/kg did not effect body temperature. We believe that the mechanism of action of TTC-909 is not related to hypothermia. If ingestion of TTC-909 leads to revascularization of ischemic brain tissue by dislodging trapped PVA particles and/or enhancing circulation through its vasodilating action, memory improvement would be achieved. Our evidence that TTC-909, a stable PGI2 analogue incorporated into lipid microspheres, improved the memory of PVA-embolized rats, suggests that TTC-909 warrants further study for the possible treatment of patients with cerebrovascular disorders, including stroke.

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