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European Journal of Pharmacology 455 (2002) 127–133

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Effect of a platelet-activating factor antagonist, E5880, on cerebrovasospasm following subarachnoid hemorrhage in a canine double-hemorrhage model

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Received 7 October 2002; accepted 11 October 2002

Abstract

We investigated the effects of a platelet-activating factor (PAF) antagonist, E5880 (1-ethyl-2-[*N*-(2-methoxy)benzoyl-*N*-[(2)-2-methoxy-3-(4-octadecycarbamoylox)piperidinocarbonyloxy-propyloxy]carbonyl]aminomethyl-pyridiniumchloride), on subarachnoid hemorrhage-induced prolongation of cerebral circulation time and decrease in the basilar artery diameter in a canine double-hemorrhage model. Animals were assigned to three groups, control (saline), E5880 1.2 mg/kg and E5880 2.4 mg/kg. For measurement of cerebral circulation time, regions of interest were chosen at the basilar artery and the straight sinus in order to obtain time–density curves. Cerebral circulation time was defined as the difference between the arterial and venous peaks. Cerebral circulation time and basilar artery diameter were assessed by intra-arterial digital subtraction angiography (IA-DSA) on Days 0, 2 and 7. The prolongation of cerebral circulation time following subarachnoid hemorrhage was significantly inhibited by intravenous administration of 2.4 mg/kg of E5880. Basilar artery constriction was also reduced by E5880. Thus, E5880 had preventive effects on the prolongation of cerebral circulation time and the vasoconstriction of basilar artery in this model. These results suggest that E5880 may have a preventive effect on neurological symptoms aggravated by cerebrovascular lesions following subarachnoid hemorrhage.

Keywords: PAF (platelet-activating factor); Cerebral vasospasm; Intra-arterial digital subtraction angiography; Cerebral circulation time (canine model); Subarachnoid hemorrhage

1. Introduction

Delayed cerebral vasospasm, occurring approximately 3–14 days after subarachnoid hemorrhage, is an important cause of mortality and neurological dysfunction, and affects patient prognosis. Although many agents with different mechanisms, such as thromboxane synthetase inhibition (Suzuki et al., 1989), neuroprotective agents (Ohta et al., 1986), anti-platelet agents (Fujita et al., 1988; Kobayashi et al., 1982) and myosin light-chain phosphorylation inhibition (Shibuya et al., 1992), have been used for preventive therapy of vasospasm, the therapeutic potencies of these agents are insufficient.

Platelet-activating factor (PAF), a potent inflammation mediator, has contractile activity on vessel smooth-muscle (Akopov et al., 1995; Kim et al., 1993) and induces activation of platelets, which is associated with delayed ischemic neurological deficits following subarachnoid hemorrhage. PAF also causes direct damage to nerve cells (Nogami et al., 1997; Prehn and Krieglstein, 1993). Further, animal experiments (Hirashima et al., 1993, 1996) and several clinical papers (Hirashima et al., 1994a,b, 1997) have suggested the participation of PAF in cerebral

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PII: S0014-2999(02)02586-4

vasospasm, delayed ischemic neurological deficits and cerebral infarction.

Hirashima et al. (1996) have reported prevention of cerebrovasospasm following subarachnoid hemorrhage in rabbits treated with the PAF receptor antagonist, E5880 (1-ethyl-2-[*N*-(2-methoxy)benzoyl-*N*-[(2)-2-methoxy-3-(4-octadecycarbamoylox)piperidinocarbonyloxy-propyloxy]-carbonyl]aminomethyl-pyridiniumchloride). They reported that neurological deterioration was largely prevented in rabbits by intravenous administration of E5880. In addition, basilar artery constriction was reduced in treated animals. Further, the E5880-treated group exhibited ischemic changes less frequently than those in the control group. Thus, they suggest that PAF may play a role in the pathogenesis of vasospasm following subarachnoid hemorrhage and that administration of E5880 is a promising approach for its prevention.

So far, there have been no reports showing the preventive effect of PAF antagonists in cerebrovasospasm in a canine model, which is generally used for the evaluation cerebrovasospasm following subarachnoid hemorrhage. Cerebral hemodynamics are important for the evaluation of cerebral vasospasm together with the angiographic changes of main trunk artery; thus, we thought it necessary to assess cerebral circulation time.

In the present study, we investigated whether intravenous administration of E5880 prevents subarachnoid hemorrhage-induced prolongation of cerebral circulation time and decreases basilar artery diameter in a double-hemorrhage canine model.

2. Material and methods

The protocol was approved by the Animal Care and Use Committee at Eisai Tsukuba Research Laboratories. All surgical procedures and postoperative care of the animals were carried out according to the Manual on Laboratory Animals at Eisai Tsukuba Research Laboratories.

2.1. Animal preparation

Twenty-one beagle dogs of both gender, each weighing 10.2–15.2 kg and approximately 2 years old, were used for this study. All surgical, cerebral angiographic (CAG) and intra-arterial digital subtraction angiography (IA-DSA) procedures were performed after animals had been anesthetized with an intravenous injection of sodium pentobarbital (300 mg/body).

On Day 0 (before subarachnoid hemorrhage), animals were intubated in the supine position, and ventilated mechanically to keep an end-tidal pCO_2 of 38–42 mm Hg. Spontaneous respiration was permitted after anesthetization. Arterial blood pressure and pulse rates were monitored via femoral artery. Using fluoroscopic guidance, a transfemoral catheter was placed into the vertebral artery for CAG and IA-DSA. An angiographic catheter of 5 french (Medikit, Tokyo, Japan) was advanced to the C5 spinal level under fluoroscopic guidance. After that, animals received 1 mg/kg of suxamethonium chloride (Succcin®, Yamanouchi, Tokyo, Japan) for immobilization. CAG was performed by injecting 5 ml of iomeprol (Iomeron®, Eisai, Tokyo, Japan) for measurement of basilar artery diameter. A magnification standard was included in each radiograph. Then, dogs were positioned laterally and their heads were fixed. IA-DSA was performed by injecting 3 ml of iomeprol for measuring cerebral circulation time.

Subarachnoid hemorrhage was simulated by injecting the cisterna magna with fresh unheparinized arterial blood. In the present study, the "two-hemorrhage" or "double-hemorrhage" canine model (Varsos et al., 1983; Zabramski et al., 1986) was used. After angiograms were obtained, the animals were placed in a prone position. The cisterna magna was punctured percutaneously with a No. 21 spinal needle and 0.3 ml/kg of cerebrospinal fluid (CSF) was removed by spontaneous egress. Thereafter, the injections of fresh autologous nonheparinized arterial blood (0.5 ml/kg) were delivered using a Harvard pump. Animals were kept with the head down both during and 30 min after the procedure. The catheter was then removed and the animals were permitted to awaken and were returned to the housing cages.

Two days after the first blood injection (Day 2), dogs were anesthetized with sodium pentobarbital and IA-DSA was performed. Then, 0.3 ml/kg of CSF was withdrawn, and 0.5 ml/kg of fresh autologous nonheparinized arterial blood was injected into the cisterna magna as described above for Day 0.

2.2. Experimental design

Animals were assigned to three groups. Seven dogs were injected with 0.5 ml/kg of saline (control group). Fourteen animals received E5880. Of the 14 animals, 7 were injected intravenously with E5880 1.2 mg/kg and the other 7 received E5880 2.4 mg/kg. The justification for dose level was determined by preliminary study in a canine double-hemorrhage model. E5880 (0.3 mg/kg) inhibits platelet aggregation induced by PAF (ex vivo) for 8 h in the dog (unpublished data). Saline and E5880 were intravenously administered twice daily for 8 days from Days 0 to 7 after the initial blood injection (Day 7). The injection was done over a period of 30 s.

2.3. Cerebral circulation time

Regions of interest were located at the origin of the basilar artery and the middle of the straight sinus for the measurement of cerebral circulation time (on the lateral view). In order to obtain time–density curves, we recorded the optimal density on each regions of interests using

image Σ-II IS206 (Nippon Avionics, Tokyo, Japan) with reflection by IA-DSA. Cerebral circulation time was defined as the difference between arterial and venous peaks on the time–density curve and was measured on Days 0, 2 and 7.

2.4. Assessment of basilar artery diameter

The diameter of basilar artery was measured at midpoint of the upper, lower and middle thirds of its length, and the values were averaged. The measurement of basilar artery diameter was conducted automatically by Heart ver. 4.00 of the cardiovascular image processor system (CCIP/310/W, Cathex, Tokyo, Japan) on the angiogram. The diameter was measured on Days 0 and 7. The diameter of basilar artery on Day 7 was expressed as vasoconstriction percentage of the baseline (diameter of basilar artery before subarachnoid hemorrhage).

2.5. Statistical analysis

The results are shown as the mean ± S.E.M. Data were analyzed using Kruskal–Wallis test to compare cerebral circulation time on Day 7 among the control, E5880 1.2 and 2.4 mg/kg groups. Furthermore, the change in cerebral circulation time with time was analyzed using Wilcoxon's signed-rank test and compared among Days 0, 2 and 7. Dunnett's multiple comparison test was used to compare the diameter of basilar arteries on Day 7 among the three groups. Probability values of less than 0.05 were accepted as statistically significant.

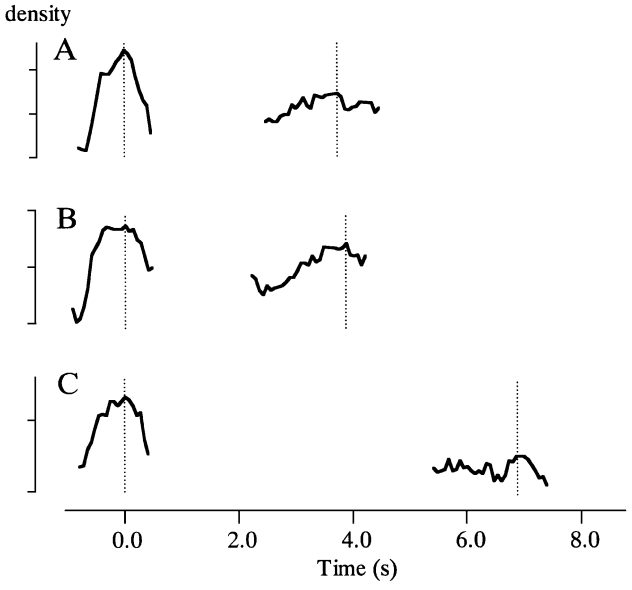

Fig. 1. Series of time–density curves obtained before subarachnoid hemorrhage, Day 0 (A), on Day 2 (B) and on Day 7 (C) from a representative animal in the control group. Left and right curves show the time–density curve of the basilar artery and the straight sinus. The vertical dotted line shows the time of the peak.

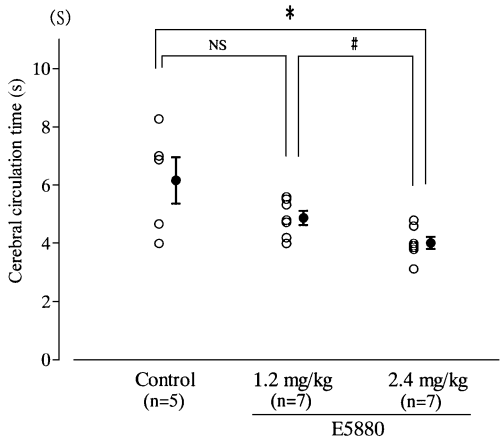

Fig. 2. Effect of E5880 on cerebral circulation time on Day 7 following subarachnoid hemorrhage in a canine model. Open circles indicate individual values of cerebral circulation time for each animal. Closed circles indicate the mean ± S.E.M. of cerebral circulation time in individual groups. *,# $P < 0.05$ versus the control and 1.2 mg/kg groups by Kruskal–Wallis test.

3. Results

3.1. Change in cerebral circulation time

Fig. 1 shows a series of time–density curves in regions of interest of the arterial and venous phase obtained before

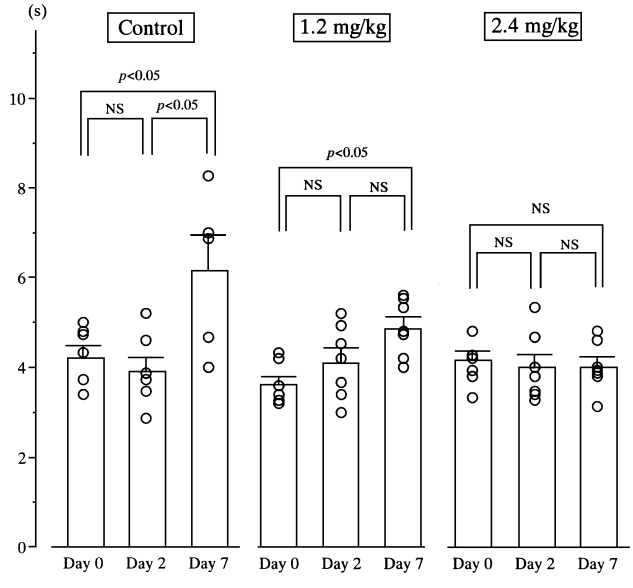

Fig. 3. Time course of cerebral circulation time following subarachnoid hemorrhage in a canine model, in the control (left), E5880 1.2 mg/kg (middle) and 2.4 mg/kg (right). Open circles indicate individual values of cerebral circulation time for each animal. Columns indicate the mean ± S.E.M. of each group on Day 0 (before subarachnoid hemorrhage), Day 2 and Day 7. Statistical analysis were made by Wilcoxon's signed-rank test.

subarachnoid hemorrhage (Day 0), and on Days 2 and 7 from representative animals in the control group.

On Day 7, a straight sinus could not be found in two of seven animals in the control group, over 10 s after the iomeprol injection. However, it was located in all animals of the E5880-treated groups, and cerebral circulation time values were calculated for these animals. In the control group, cerebral circulation time was 6.16 ± 0.79 s in five animals, excluding the two animals mentioned above. Cerebral circulation time was 4.88 ± 0.24 and 4.02 ± 0.21 s in the L and H groups, respectively. There was a significant shortening of cerebral circulation time in E5880 2.4 mg/kg group in comparison to the control and E5880 1.2 mg/kg group (Fig. 2).

The changes in cerebral circulation time with time are shown in Fig. 3. The average cerebral circulation time in the control group was 4.25, 3.92 and 6.16 s (five animals) on Days 0, 2 and 7, respectively. There was a significant prolongation of cerebral circulation time on Day 7 in comparison to those on Days 0 and 2. In E5880 1.2 mg/kg group, cerebral circulation time value was 3.63, 4.13 and 4.88 s, respectively, indicating a significant prolongation on Day 7 in comparison to Days 0 and 2. In contrast, in E5880 2.4 mg/kg group, there was no significant difference in cerebral circulation time among Days 0, 2 and 7; the average cerebral circulation time was 4.16, 3.99 and 4.02 s on Days 0, 2 and 7, respectively.

Cerebral circulation time on Day 0 was 4.25 ± 0.24, 3.63 ± 0.17 and 4.16 ± 0.20 s in the control, E5880 1.2 and 2.4 mg/kg groups, respectively. There were no significant differences in cerebral circulation time among the three groups.

3.2. Diameter of basilar arteries

Fig. 4 shows angiographic images obtained before subarachnoid hemorrhage (Day 0) and on Day 7 from typical animals of the control and E5880 2.4 mg/kg groups.

The average diameter of the basilar artery on Day 0 was 0.96 ± 0.02 mm in the control group, 0.97 ± 0.02 mm in the L group and 1.00 ± 0.02 mm in the H group. There was no significant difference in the diameter of the vessels among these three groups. The results of basilar artery diameter ratio (%) on Day 7 are shown in Fig. 5. The percentage of vasoconstriction of basilar artery diameter on Day 7 was $57.0 \pm 1.9\%$ in the control group, $66.7 \pm 3.3\%$ in E5880 1.2

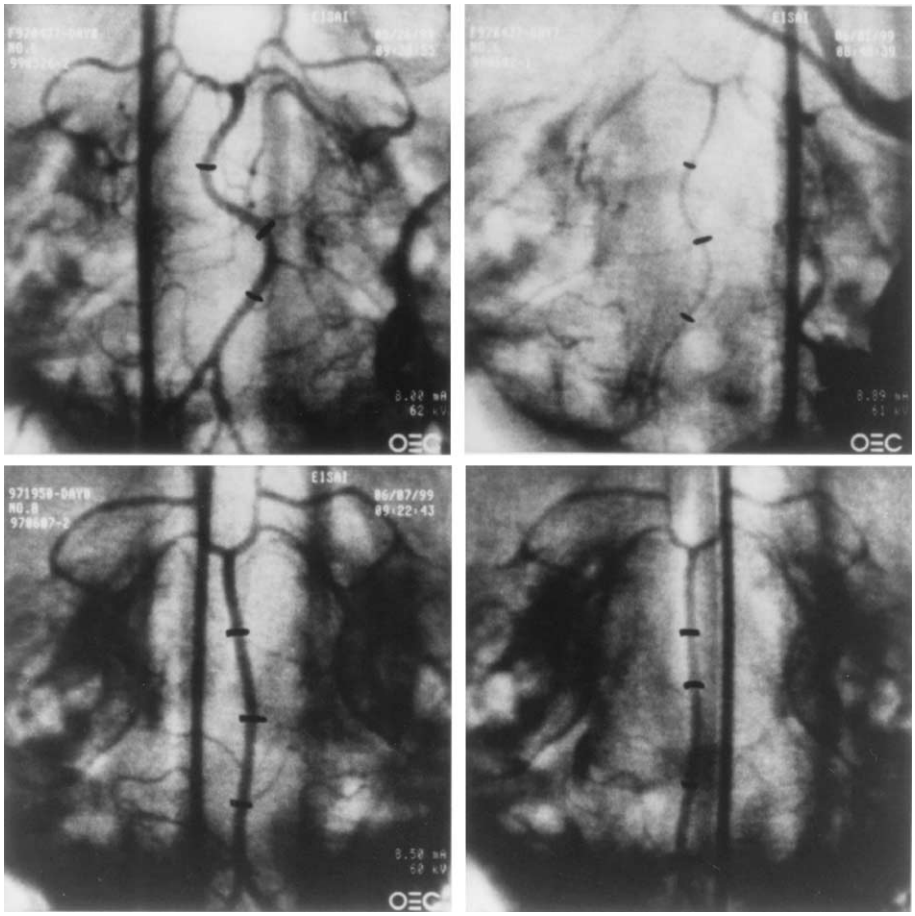

Fig. 4. Typical angiograms of basilar arteries of control animals (upper) and 2.4 mg/kg E5880-treated animals (lower) on Day 0 (left) and Day 7 (right). Basilar artery diameter ratio was reduced to 51.5% and 79.2% in the control and E5880-treated animals.

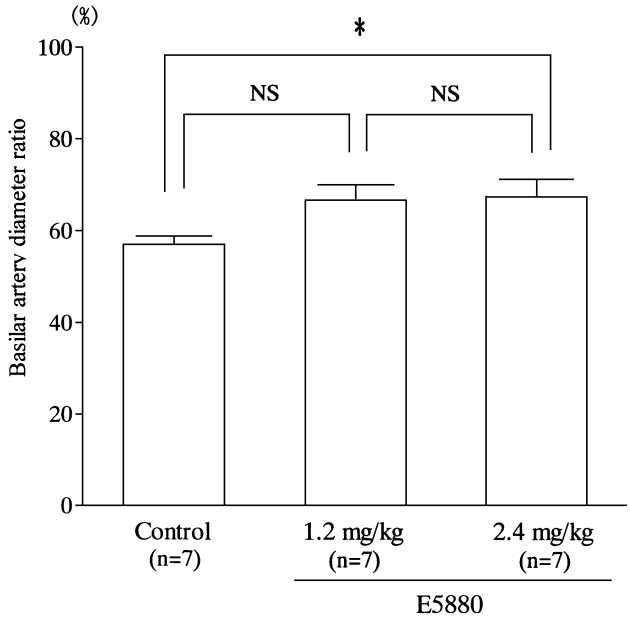

Fig. 5. Effect of E5880 on the basilar artery ratio (%) following subarachnoid hemorrhage. Columns indicate the mean ± S.E.M. for each group of seven animals. *$P < 0.05$ versus the control group by Dunnett's multiple comparison test.

mg/kg group and 67.4 ± 3.7% in E5880 2.4 mg/kg group. A significant inhibition of the vasoconstriction of basilar artery was observed on Day 7 in E5880 2.4 mg/kg group compared to the control group, but there was no significant difference between E5880 1.2 and 2.4 mg/kg groups.

4. Discussion

The major findings in the present study were as follows: (a) cerebral circulation time on Day 7 was shorter in E5880-treated at 2.4 mg/kg group compared to the control group, (b) no change in the mean cerebral circulation time was observed in treated animals in contrast to the prolonged cerebral circulation time in the control group, namely, inhibition of the prolongation of cerebral circulation time with vasospasm was observed and (c) significant inhibition of the basilar artery constriction was observed on Day 7 in E5880 treated at 2.4 mg/kg group compared to the control group.

Cerebral circulation time is one of the physiological parameters used to investigate cerebral blood flow dynamics (Milburn et al., 1997; Okada et al., 1994). Milburn et al. (1997) have reported that the mean cerebral circulation time was 5.9 ± 0.8 s in control patients and 6.8 ± 0.11 s in patients with subarachnoid hemorrhage. Therefore, brain mean transit time is clearly prolonged in patients with infarct. In the present study using the canine subarachnoid hemorrhage model, the average cerebral circulation time was 4.01 ± 0.13 s in normal dog ($n = 21$) (before subarachnoid hemorrhage) and 6.16 ± 0.79 s in the control group on Day 7, indicating a prolongation in cerebral circulation time of about 2 s as compared to normal dog. Although the methods used in this study were different from those of Milburn et al. (1997), the results were in agreement to those reported. Thus, our results in the canine double-hemorrhage model also confirmed the prolongation of cerebral circulation time in cerebrovasospasm.

In this study, we observed animals with a non-visible straight sinus within 10 s after angiografin injection in the control group. In addition, we observed cerebral circulation time prolongation in the most animals in the control and E5880 1.2 mg/kg groups. From these results, we inferred that there was a decrease in the cerebral blood flow in the peripheral cerebral vessel with vasospasm of the main trunk artery and the peripheral vessel, and an increase in coagulation due to blood congestion with vasospasm.

Further, IA-DSA was clearly able to show the preventive effects on cerebral circulation lesions of the micro-arteries, which could not be otherwise known by observing only the morphological changes in cerebral main trunk arteries by angiography. These results suggest that E5880 inhibits the occurrence of cerebral microcirculatory lesions accompanied by the accentuation of coagulation in the microarterial and venous vessels. Regarding the change in basilar artery diameter, Hirashima et al. (1996) reported that in rabbits, on Day 4 post-administration, the degree of vasoconstriction of the basilar artery was 82.5% in the control group (Day 0 baseline as 100%), and 123.1% and 110% in E5880 0.1 and 0.5 mg/kg groups, respectively. The differences in the degree of vasoconstriction between the control group and E5880-treated groups were 40.5% and 27.5%, respectively. In this study, the degree of vasoconstriction of the basilar artery diameter in dogs on Day 7 was 57.0% in the control group and 67.4% in E5880 2.4 mg/kg group. A significant inhibition of vasoconstriction of basilar artery was observed in E5880 2.4 mg/kg group. However, the improvement in the vasoconstriction rate was only 10.4%, and there was also a very slight difference in the actual value of vessel caliber on Day 7 between the control and E5880 2.4 mg/kg groups (Control: 0.55 ± 0.01 mm, H group: 0.67 ± 0.03 mm). The difference between the vasoconstriction rates in rabbit and dog could be due to differences in affinity of PAF receptors to vessel smooth-muscle cells in both species. Taken together, these results suggest that E5880 has an inhibitory effect on vasoconstriction due to its anti-PAF action, but that this effect is weaker in dog compared to rabbit.

Thus, E5880 seems to have a weak preventive action on the vasoconstriction by PAF, which is thought as one of the possible causes of cerebrospasm. Although E5880 apparently inhibited prolongation of cerebral circulation time, these results are thought to be based on its inhibitory action towards PAF-induced platelet aggregation activity (Nagaoka et al., 1991).

The occurrence and progress of cerebral vasospasm following subarachnoid hemorrhage has been associated with vessel smooth-muscle constriction, accumulation of

platelets, vessel angiitis and angiostenosis of vessel with thickening of the intima. The extent of spasm of cerebral main trunk arteries has also been related to the occurrence of delayed ischemic neurological deficits with cerebrovasospasm. Further, accentuation of thrombocyte function and coagulation in vessels (Haining et al., 1988; Juvela et al., 1991; Ohkuma et al., 1991; Ou et al., 1994; Yoshida and Nakamura, 1996) and cerebral microcirculatory lesions have also been associated with delayed ischemic neurological deficits.

Clinical studies have shown high concentrations of PAF in the CSF and plasma of patients after subarachnoid hemorrhage. Further, the concentrations in CSF and plasma of patients with cerebral infarctions caused by cerebral vasospasm and delayed ischemic neurological deficits were higher than in patients without cerebral infarction (Hirashima et al., 1994a). These patients had an accentuated platelet consumption and aggregation, an inducement of procoagulant factors (Hirashima et al., 1997). These results suggest that PAF may be associated with cerebral vasospasm after subarachnoid hemorrhage, and that PAF receptor antagonists may play a role in the prevention of cerebral vasospasm.

In conclusion, the diameter of basilar artery on Day 7 in the control group decreased to 57% with delayed cerebral vasospasm and cerebral circulation time was prolonged from 4.25 to 6.16 s in the canine double-hemorrhage model. E5880 reduced the vasoconstriction of the basilar artery and prevented the prolongation of the effect of cerebral circulation time in this model. Clear efficacy after an intravenous administration of E5880 was seen at a dose of 2.4 mg/kg/day.

These results suggest that E5880 may have a preventive effect on neurological symptoms aggravated by cerebrovascular lesions following subarachnoid hemorrhage.

Acknowledgements

We would like to thank Ms Debora Satoh and Dr. Kailash Sharma for reading drafts of the manuscript and providing suggestions on language and style.

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