Effect of a Prostaglandin E1 Derivative (OP-1206) and Acetylsalicylic Acid on Electrically Induced Thrombosis in Guinea-Pig Mesenteric Artery and Its Modification by an Inhibitor of Prostacyclin Synthetase, Tranylcypromine

Buichi FUJITANI, Masako WATANABE, Junji KUWASHIMA, Toshimichi TSUBOI, Toshiaki KADOKAWA and *Toshikazu KITAGAWA

Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33-94, Suita, Osaka 564, Japan
*Research Institute, Ono Pharmaceutical Co., Ltd., Sakurai 3-1-1, Shimamoto, Mishima, Osaka 618, Japan

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Abstract—The antithrombotic effect of a prostaglandin E1 derivative, OP-1206 (17S-20-dimethyl-trans-Δ2-PGE1)·α-cyclodextrin clathrate (OP-1206·α-CD), was compared with that of acetylsalicylic acid (ASA) in an electrically induced thrombosis model of guinea-pig mesenteric arteries using intact animals and animals subjected to the superfusion of tranylcypromine (TC, 15 mM) over their mesentery. The drug-effect was assessed by the change of the threshold voltage for the thrombus formation. 1) TC (1.5–15 mM) lowered the threshold voltage, and the effect was comparable to its inhibitory effect on PGI2 formation in vitro, suggesting that PGI2 generated in mesenteric arteries acts to prevent thrombus formation. 2) In intact animals, OP-1206·α-CD at doses of 0.01–0.3 mg/kg, p.o. (as OP-1206), significantly and dose-dependently elevated the threshold voltage. ASA (30–1000 mg/kg, p.o.) significantly elevated the threshold voltage, but the effect reached to its maximum at 100 mg/kg and lessened with further increase of ASA. 3) In TC-treated animals, OP-1206·α-CD elevated the threshold voltage dose-dependently, but the elevation of threshold voltage by ASA reached to its plateau level which was significantly lower than that obtained with OP-1206·α-CD at 0.3 mg/kg, indicating that the antithrombotic effect of ASA is incomplete in this model. 4) Threshold voltages in TC-treated animals given OP-1206·α-CD was significantly lower than those in intact animals at all doses tested, but threshold voltages in TC-treated and intact animals given ASA at 300–1000 mg/kg were not different, suggesting that high doses of ASA inhibits PG12 formation in vivo. Thus, the antithrombotic effect of ASA was restricted by its inhibitory effect on PG12 formation and its incomplete inhibition on thrombus formation, while such differences were not observed in OP-1206·α-CD.

Platelets play an important role in the initiation of arterial thrombosis. Therefore, a variety of compounds inhibiting platelet functions were studied with regards to their antithrombotic potential (1). Among these, acetylsalicylic acid (ASA) has been studied for many years, and it has also been used for the treatment of thrombotic diseases such as transient ischemic attack in man (2). However, the antithrombotic effect of ASA is thought to be restricted by its inhibitory effect on vascular synthesis of prostaglandin I2 (PG12) (3). PG12 is the most potent endogenous inhibitor of platelet functions (4) and may play an important role in the prevention of thrombus formation (5).

A prostaglandin E1 derivative, OP-1206, 17S-20-dimethyl-trans-Δ2-PGE1, also po-
tently inhibits platelet functions in vitro (6). Its oral administration also inhibits platelet aggregation and adhesiveness, and it prevents thrombocytopenia induced by the injection of ADP and collagen (7). However, effects of OP-1206 on thrombus formation and PGI₂ formation have not been known.

In the present study, we compared the effect of OP-1206 and ASA on electrically induced thrombus formation in guinea-pig mesenteric arteries. The influence of a PGI₂ synthetase inhibitor, tranylcypromine (8), was also studied with respect to the anti-thrombotic effect of these compounds in order to examine their possible effect on PGI₂ formation in mesenteric arteries in vivo.

Materials and Methods

Animals: Male guinea-pigs of the Hartley strain, weighing 360–660 g, were used. A commercial diet (RC-4, Oriental Yeast Co., Japan) and water were given ad libitum.

Experimentally induced thrombosis: Guinea-pigs anesthetised with pentobarbital sodium (75 mg/kg, s.c.) were subjected to abdominal incision. The jejunum part of the intestinal loops was extended over a plastic plate, and the mesentery was superfused with saline (37°C). A platinum electrode (200 μm in diameter with sharpened tip) as an anode was touched to the mesenteric artery (30–60 μm in diameter), and a cathodal electrode was fixed to the edge of the incision. Then, the mesenteric artery was electrically stimulated with a single rectangular pulse (pulse duration of 1000 msec). The voltage of the stimulation was changed in a stepwise manner at 2.5 V intervals (5.0–30 V). The thrombus formation was microscopically observed for 10 min after each stimulation, and the lowest voltage causing complete occlusion of the vessel by thrombi (threshold voltage for thrombus formation) was determined. Drug-effect was assessed by the change of the threshold voltage.

PGI₂ formation in aortic tissue in vitro: The formation of PGI₂ in guinea-pig aorta was estimated by a bioassay based on the inhibitory effect of PGI₂ on platelet aggregation as previously described (9). Rings of aorta (10 mg) were incubated with 400 μl of saline buffered with Tris (TBS, pH 7.6) for 5 min at 22°C under the presence or absence of test compounds. Then, an aliquot of the medium was added to platelet-rich plasma preincubated for 1 min at 37°C, and platelet aggregation was induced by ADP (1–2×10⁻⁶ M). The amount of released PGI₂ was calculated by comparison of its antiaggregatory potency with that of a known amount of synthetic PGI₂ sodium salt.

Test compounds: OP-1206-α-cyclodextrin clathrate (OP-1206-α-CD) containing 2.93% of its active principle, OP-1206, was used throughout the experiments, and doses and concentrations of OP-1206-α-CD were expressed as those of OP-1206. OP-1206-α-CD was chemically more stable than OP-1206, but its inhibitory effect on platelet functions in vitro and ex vivo was equipotent to that of OP-1206 (Data are not shown). OP-1206-α-CD dissolved in saline was orally given 4 hr before the electrical stimulation. ASA suspended in 0.5% tragacanth solution was orally given 3 hr before the stimulation. Tranylcypromine dissolved in saline was directly superfused over the mesentery from 10 min before the stimulation. In in vitro experiments, these compounds were dissolved in TBS.

Chemicals: OP-1206-α-CD and PGI₂ sodium salt were synthesized by Ono Pharmaceutical Co., Ltd., Japan. ASA was obtained from Aldrich Chem. Co., U.S.A., and tranylcypromine was from Sigma Chem. Co., U.S.A.

Statistical analysis: Data are shown as the mean±S.E. and analysed with Student’s t-test.

Results

Effects of tranylcypromine on aortic PGI₂ formation in vitro and thrombus formation in mesenteric arteries: Tranylcypromine (TC) at 0.75–7.5 mM inhibited the aortic PGI₂ formation in vitro in a concentration-dependent manner (Fig. 1A). TC (1.5–15 mM) superfused over the mesentery also lowered the threshold voltage for the thrombus formation (Fig. 1B). The concentrations of TC inhibiting PGI₂ formation and lowering the threshold voltage were comparable.
Fig. 1. Effect of tranylcypromine on in vitro PG12 formation and thrombus formation in guinea-pigs. A: Inhibition of PG12 formation in guinea-pig aorta in vitro. Each dot is the mean±S.E. of three experiments. In the control, aortic tissue released 9.7±0.88 ng/10 mg tissue/5 min of PG12. B: Enhancement of thrombus formation in electrically induced thrombosis model in guinea-pig mesenteric artery. Each dot is the mean±S.E. Parentheses indicate the numbers of animals used. *, **: Differences from the control are statistically significant with P<0.05 and P<0.01, respectively. Tranylcypromine was superfused over the mesentery from 10 min before the electrical stimulation.

Antithrombotic effect of OP-1206-α-CD and ASA: The antithrombotic effect was examined both in intact guinea-pigs and guinea-pigs subjected to superfusion of TC at 15 mM over their mesentery.

OP-1206-α-CD (0.01–0.3 mg/kg, p.o.) significantly elevated threshold voltages in intact and TC-treated animals in a dose-dependent manner (Fig. 2). The threshold voltages in TC-treated animals given OP-1206-α-CD were significantly and consistently lower than those in intact animals given OP-1206-α-CD at all doses tested.

ASA (30–1000 mg/kg, p.o.) also elevated threshold voltages in intact and TC-treated animals significantly, although its effect was 1/3000 times less potent than that of OP-1206-α-CD (Fig. 3).

However, the antithrombotic property of ASA was somewhat different from that of OP-1206-α-CD. In intact animals, the elevation of threshold voltage by ASA reached to its maximum of 21 V at 100 mg/kg, and a further increase of ASA caused the reduction of its antithrombotic effect (Fig. 3). In TC-treated animals, the elevation of threshold voltages reached to its plateau of 17 V at 100–1000 mg/kg. The threshold voltage of this plateau level was signifi-
cantly lower than that obtained with OP-1206-α-CD at 0.3 mg/kg in TC-treated animals. The threshold voltages of TC-treated animals given ASA at 10–100 mg/kg were significantly lower than those in intact animals given the same doses of ASA, but threshold voltages of intact and TC-treated animals given ASA at 300–1000 mg/kg were not different (Fig. 3).

**In vitro effect of OP-1206-α-CD and ASA on PGI2 formation in aorta:** ASA at 300 μg/ml inhibited PGI2 formation in guinea-pig aortic tissue, but OP-1206-α-CD at 0.5–1.0 ng/ml did not (Table 1).

**Discussion**

The superfusion of TC over the mesentery enhanced the thrombus formation at concentrations inhibiting PGI2 formation in vitro, suggesting that endogenously formed PGI2 in mesenteric arteries acts to prevent thrombus formation in this model. A similar result has been reported by Bourgain (5). Thus, testing the antithrombotic effect of a certain compound in TC-treated animals may enable us to exclude the indirect effect of the compound through its effect on PGI2 formation, and the difference of threshold voltages between intact and TC-treated animals given a certain compound may reflect its effect on PGI2 formation in vivo.

OP-1206-α-CD and ASA inhibited thrombus formation by oral administration, and the effect of OP-1206-α-CD was more potent than that of ASA. Antithrombotic doses of both compounds are comparable to their inhibitory doses on platelet functions in guinea-pigs reported by us (7, 10), indicating that the antithrombotic effect of these compounds originated from their inhibitory activities on platelet function. However, the dose-effect relationships of these two compounds were somewhat different.

Firstly, in TC-treated animals, the antithrombotic effect of OP-1206-α-CD increased in a dose-dependent manner, whereas that of ASA reached to its plateau level which was significantly lower than the threshold voltage obtained with OP-1206-α-CD at 0.3 mg/kg. The difference may come from the different inhibitory mechanism of OP-1206-α-CD and ASA on platelet functions. A compound that increases platelet cyclic AMP like OP-1206 (6) inhibits all of the activation process of platelets (11), but ASA only inhibits an activation process related to arachidonate-thromboxane pathway (12).

**Table 1. In vitro effect of OP-1206-α-CD and acetylsalicylic acid on PGI2 formation in guinea-pig aorta**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>PGI2 formation (ng/10 mg tissue/5 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>7.60±0.76 (12)</td>
</tr>
<tr>
<td>OP-1206-α-CD</td>
<td>0.5 ng/ml as OP-1206</td>
<td>8.64±0.33 (3)</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>8.61±1.66 (3)</td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>100 μg/ml</td>
<td>6.40±1.21 (3)</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>3.80±0.23** (3)</td>
</tr>
</tbody>
</table>

The values in the table are the means±S.E. Parentheses indicate the numbers of experiments. **: A difference from the control is statistically significant with P<0.01.
Secondly, the consistent differences between threshold voltages in intact and TC-treated animals given OP-1206-α-CD indicate that the compound has little effect on PG\(_{12}\) formation. On the contrary, threshold voltages in intact and TC-treated animals given ASA at 300–1000 mg/kg were not different. Thus, ASA at high doses may inhibit PG\(_{12}\) formation as well as thrombus formation, and this inhibition of PG\(_{12}\) formation may explain a part of the lessened antithrombotic effect of ASA at these doses in intact animals. A similar lessened antithrombotic effect or prothrombotic effect of ASA has been reported earlier (3, 13).

The above results are coincident with the result of in vitro experiments on PG\(_{12}\) formation in aortic tissue. OP-1206-α-CD (0.5–1.0 ng/ml) did not inhibit PG\(_{12}\) formation, although these concentrations of OP-1206 markedly inhibited guinea-pig platelet functions (6). ASA inhibited PG\(_{12}\) formation at 300 μg/ml. This concentration is 30–100 times higher than the concentrations of ASA inhibiting platelet adhesiveness (10), and the ratio is roughly comparable to the ratio of the prothrombotic dose/antithrombotic dose of ASA (ratio: 10–30). The discrepancy may come from the difference of the vascular tissue used, i.e., aorta and mesenteric artery.

In summary, ASA inhibited thrombus formation, but its antithrombotic effect was lessened at its high doses. The reduction of the antithrombotic effect may result from its inhibitory effect on PG\(_{12}\) formation and its incomplete inhibition of the activation process of platelets. In contrast to ASA, OP-1206-α-CD inhibited thrombus formation in a dose-dependent manner. This property of OP-1206-α-CD together with its vasodilating effect (7) may be favorable for the treatment of ischemic vascular diseases.

References


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