Effect of a Novel Thromboxane A2 Receptor Antagonist, S-145, on Collagen-Induced ECG Changes and Thrombocytopenia in Rodents

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Abstract—The effects of S-145, a newly synthesized thromboxane A2 (TXA2) receptor antagonist, were studied on collagen-induced changes of electrocardiograms (ECG) in rats and thrombocytopenia in rats and mice. Intravenous injection of collagen induced abnormal ECG changes such as elevation or depression of the ST segment, arrhythmia and in severe cases, cardiac arrest. These changes peaked at 3-5 min and lasted for 10 min. S-145 showed remarkable improvement of the ECG changes by both intravenous and oral administration, and the action lasted over 4 hr with 10 mg/kg, p.o. Reference compounds ONO-3708, dazoxiben and aspirin also improved the ECG changes significantly, but ticlopidine was ineffective. S-145 prevented the collagen-induced thrombocytopenia in rats but did not affect the increase in plasma TXB2 levels. S-145 also prevented collagen-induced thrombocytopenia in mice after either intravenous or oral administration in a dose-dependent manner. The efficacy of S-145 was 4-13 times greater than those of the reference compounds, and the duration of action was over 4 hr with 10 mg/kg, p.o. These results indicate that S-145 is a potent, orally active and long-lasting TXA2 receptor antagonist, which will be promising as a drug for thromboembolism and ischemic heart disease caused by platelet activation.

Thromboxane A2 (TXA2), an arachidonic acid metabolite produced mainly by platelets, induces platelet aggregation and vasoconstriction (1). Much has been reported on the possible involvement of TXA2 in ischemic heart diseases such as angina pectoris (2, 3) and myocardial infarction (4, 5).

In order to alleviate disease states possibly mediated by TXA2, a number of selective inhibitors of TXA2 synthetase have recently been developed. However, they may induce the accumulation of endoperoxide prostaglandin H2 (PGH2) (6) and do not block the effects of PGH2 which is a potent inducer of platelet aggregation and vasoconstriction by acting on the same receptors as TXA2 (7). This has been claimed to result in the phenomena of “responders and non-responders” in the inhibition of platelet aggregation induced by arachidonic acid in vitro (8, 9). These findings lead to the suggestion that in disease states in which TXA2 is implicated, PGH2/TXA2 receptor antagonists may be preferable to TXA2 synthetase inhibitors (10).

S-145, (±)-(S)-7-[3-endo-[(phenylsulfonyl)amino]bicyclo-[2.2.1.]hept-2-exo-yl]-heptenoic acid, is a newly synthesized TXA2 receptor antagonist with a structure of the carba analog of TXA2 (Fig. 1). S-145 has been found to antagonize platelet aggregation induced by arachidonic acid or 9,11-methanoepoxy-PGH2 (U-48619) (11) and to

Fig. 1. Chemical structure of S-145.
displace \textsuperscript{3}H\textsuperscript{-}U-46619 binding to rat, rabbit and human platelets (12). It also antagonizes vasoconstriction induced by U-46619 in rat aorta (11) and cat and monkey arteries in vitro (13). In this study, we selected collagen-induced changes of the electrocardiogram (ECG) as an in vivo model of ischemic heart disease (14) and examined the effect of S-145 on collagen-induced ECG changes in rats and collagen-induced thrombocytopenia in rats and mice. For comparison, the following were used as reference compounds: ONO-3708, a TXA\textsubscript{2} receptor antagonist (15); dazoxiben, a TXA\textsubscript{2} synthetase inhibitor (16); aspirin, a cyclooxygenase inhibitor and ticlopidine, an antithrombotic drug (17).

**Materials and Methods**

**Collagen-induced ECG changes in rats:** Experiments were performed according to the methods of Matsumura et al. (14). Male Sprague-Dawley rats (8–10 weeks, 260–380 g) were anesthetized with pentobarbital (45 mg/kg, i.p.). Ten minutes later, about 2 mg/kg of a calf skin type III collagen was injected into a penile vein for 3–4 sec, and the ECG (leads I, II and III) was recorded every minute for 5 sec at each lead over 10 min using a polygraph (Cardiofax ECG-6601, Nihon Kohden). Changes of the ST segment in the ECG were analyzed before and after collagen and were graded as follows: grade 0, less than 0.05 mV of changes in ST or T; grade 1, 0.05–0.1 mV of changes in ST or T; grade 2, more than 0.1 mV of changes in ST or T; grade 3, arrhythmia or cardiac arrest. Since the dose of collagen to induce ECG changes varied from 1.4 to 2.6 mg/kg, i.v., every saline or vehicle control was followed by two or three doses of S-145 or reference compounds.

**Collagen-induced thrombocytopenia in rats and mice:** Male Sprague-Dawley rats (9 weeks, 280–300 g) were anesthetized with pentobarbital (45 mg/kg, i.p.). At 5 min after the intravenous injection of calf skin type III collagen (1 mg/kg), 5 ml of blood was drawn from the abdominal artery through a 20G needle into a plastic syringe containing 1/10 volume of 77 mM EDTA containing 100 \textmu g/ml of indomethacin. We used EDTA as an anticoagulant to measure the plasma prostanoid levels from the same blood sample. One milliliter of blood was diluted to 3 ml with ISOTON\textsuperscript{®} II consisting of 0.8% sodium chloride, 0.04% potassium chloride and 0.5% sodium phosphate and then allowed to stand for 1 hr. The remaining blood was centrifuged at 2000 g for 10 min at 4°C. The plasma samples were frozen with dry-ice/acetone immediately after the separation and then stored at −80°C until the measurement of TXB\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha}.

Male ddY mice (4 weeks, 23–26 g) were anesthetized with pentobarbital (65 mg/kg, i.p.). Five min after the intravenous injection of horse tendon collagen (1 mg/kg), 0.5 ml of blood was collected by cardiac puncture through a 24G needle with a plastic syringe containing 1/10 volume of 3.8% sodium citrate. The blood of three mice was pooled and diluted to 3 ml with ISOTON\textsuperscript{®} II and left standing for 1 hr.

The platelet-rich plasma from rats and mice was collected and diluted with ISOTON\textsuperscript{®} II, and the platelet number was counted using a Coulter counter.

**Radioimmunoassay of TXB\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha}**: TXB\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha} in rat plasma were extracted by the modified method of Powell (18). The sample was deproteinized by adding 4 volumes of ethanol and centrifuged at 2000 g for 10 min at 4°C. The supernatant was diluted to 10% ethanol concentration by adding water, acidified to pH 3.0 with 1 N HCl, and loaded on a SEP-PAK C\textsubscript{18} cartridge (Waters Associates, Milford, U.S.A.), which was washed with 100% ethanol and water before use. Next, the cartridge was washed successively with 20 ml of 10% ethanol and 20 ml of petroleum ether. Prostanoids were finally eluted with 8 ml of ethylacetate. The eluant was evaporated under a stream of nitrogen gas at room temperature, and the residue was dissolved in assay buffer solution which consisted of 0.9% NaCl, 0.01 M EDTA, 0.3% bovine gamma-globulin, 0.005% Triton X-100 and 0.05% sodium azide in 50 mM phosphate buffer (pH 6.8).

From the preliminary experiments, the recoveries of \textsuperscript{3}H\textsuperscript{-}labeled TXB\textsubscript{2} and 6-keto-PGF\textsubscript{1\alpha} (each 5000 dpm, New England Nuclear, Boston), which had been added to
the sample immediately before extraction, were found to be 72±1 and 79±3% (mean±S.D., n=3), respectively. Therefore, only [3H]-TXB2 was added to assess the recovery. TXB2 and 6-keto-PGFα were estimated by radioimmunoassay using kits from New England Nuclear: TXB2 (No. NEK-024A, threshold of detection=12.5 pg/ml plasma) and 6-keto-PGFα (No. NEK-025A, threshold of detection=25 pg/ml plasma).

Chemicals: Calf skin type III collagen (100 mg) (acid-soluble, lot No. 93F-81 60, Sigma) was dissolved in 40 ml saline by stirring at 4°C for 15 hr and then centrifuged at 2000 g for 10 min at 4°C. The supernatant was collected and used for the induction of ECG changes and thrombocytopenia in rats. The dose of the collagen was expressed as mg protein determined by Lowry’s method (19). Horse tendon collagen (1 mg/ml) (Collagenreagent Horm®, Hormon-Chemie, München) was diluted with saline and used for the induction of thrombocytopenia in mice. S-145, ONO-3708, dazoxiben, aspirin and ticlopidine were synthesized in our laboratories. All drugs were suspended in gum arabic solution and administered orally. For intravenous injection, sodium salts of S-145 and ONO-3708 were used as saline solution.

Data analysis: Statistical significance was determined by Mann-Whitney’s U-test (ECG changes), the x²-test (ECG change), the paired t-test (ECG change), Dunnett’s t-test (thrombocytopenia) or Student’s t-test (prostanoid level). The ED50 value was calculated by regression line analysis.

Results

Effects on collagen-induced ECG changes in rats: The typical ECG changes induced by intravenous injection of collagen are shown in Fig. 2A. About 40 sec after the injection of collagen, abnormal ECG patterns appeared, which mainly consisted of an ST-fall in lead I, an ST-fall or -elevation in lead II, and an ST-elevation in lead III. These changes peaked at 3–5 min after the collagen injection, coinciding with intermittent apnea, and disappeared almost 15 min later. About 20% of the rats injected with collagen died within 15 min due to atrioventricular block and subsequent cardiac arrest.

Figure 2B shows the typical records of the effect of S-145 on collagen-induced ECG changes. Intravenous injection of S-145 (1 mg/kg) 10 min prior to collagen administration had no effect on normal ECG patterns, but it potently improved the abnormal ECG patterns elicited by collagen. These results are graded and summarized for 6–8 rats in Fig. 3. Intravenous injection of S-145 (1 mg/kg) and ONO-3708 (5 mg/kg) significantly improved collagen-induced ECG changes (Fig. 3A). Improvement of ECG changes was also observed after the oral administration of S-145 (10 mg/kg) and ONO-3708 (50 mg/kg) (Fig. 3B). As shown in Table 1, the oral administration of S-145 and ONO-3708 30 min before collagen potently reduced the ECG changes of high grade from all leads, indicating a marked decrease of the incidence of arrhythmia or cardiac arrest elicited by collagen.

Dose-response curves of S-145 for inhibiting collagen-induced ECG changes are shown in Fig. 4. S-145 inhibited the ECG changes dose-dependently after either intravenous or oral administration. Significant inhibition was observed from 0.1 mg/kg, i.v. and 1 mg/kg, p.o.

Besides TX receptor antagonists, a selective thromboxane synthetase inhibitor, dazoxiben (30 mg/kg, p.o.), and a cyclooxygenase inhibitor, aspirin (30 mg/kg, p.o.), significantly improved collagen-induced ECG changes (Fig. 5). In contrast, single (100 mg/kg, p.o.) or repeated (200 mg/kg, p.o. × 3 days) administration of ticlopidine, an anti-thrombotic drug, did not antagonize the ECG changes induced by collagen (Fig. 5).

Figure 6 shows the duration of action of TX receptor antagonists S-145 (10 mg/kg, p.o.) and ONO-3708 (50 mg/kg, p.o.). S-145 significantly improved collagen-induced ECG changes from 30 min to 4 hr with peak effects at 30 min. ONO-3708 was also effective from 30 min to 2 hr with peak effects at 1 hr.

Effects on collagen-induced thrombocytopenia and plasma prostanoid levels in rats: Since collagen is known to induce thrombocytopenia, we preliminarily studied the effects of S-145 and ticlopidine on platelet number immediately after the end of the ECG experiments, namely 10 min after collagen
injection. Platelet number had been decreased by collagen injection by 78% from the normal level (from 123±9 to 27±4x10^4/mm^3, n=5, P<0.001) (data not shown). S-145 (10 mg/kg, p.o.) but not ticlopidine (100 mg/kg, p.o.) significantly prevented the decrease of platelet number induced by collagen (39±3x10^4/mm^3 with S-145, n=6, P<0.05; 27±4x10^4/mm^3 with ticlopidine, n=5) (data not shown). As already shown in Fig. 5, S-145 but not ticlopidine improved the collagen-induced ECG changes. This suggested that there might be a positive relationship between the improvement of collagen-induced ECG changes and the prevention of collagen-induced thrombocytopenia. We therefore examined the effects of S-145 and reference compounds on collagen-induced thrombocytopenia, and also measured the changes of plasma prostanoid levels. In this experiment, 1 mg/kg of collagen was employed to prevent rats from dying of cardiac arrest and to
Fig. 3. Effects of S-145 and ONO-3708 on collagen-induced serial ECG changes in rats. (A) (left panel): (○) saline control injected with collagen (1.4–1.8 mg/kg, i.v.); (●) S-145, 1 mg/kg, i.v., 10 min before collagen (n=8); (■) ONO-3708, 5 mg/kg, i.v., 5 min before collagen (n=8). (B) (right panel): (○) saline control injected with collagen (1.6–1.8 mg/kg, i.v.); (●) S-145, 10 mg/kg, p.o., 30 min before collagen (n=6); (■) ONO-3708, 50 mg/kg, p.o., 30 min before collagen (n=6). Each point indicates the mean ECG change-grade of 6 or 8 rats. *P<0.05 **P<0.01 vs. saline control (Mann-Whitney’s U-test).

Table 1. Effects of orally administered S-145 and ONO-3708 on collagen-induced ECG changes in rats

| Treatment (p.o.) | N×q | Distribution of ECG-change grade (for 10 min) | P     |
|------------------|-----|---------------------------------------------|-------|
|                  |     | 0 | 1 | 2 | 3 |     |
| Lead I           |     |   |   |   |   |     |
| Control          | 6×10| 5 | 1 | 23 | 31 |       |
| S-145, 10 mg/kg  | 6×10| 50 | 6 | 3 | 1 | ***  |
| ONO-3708, 50 mg/kg | 6×10| 36 | 2 | 12 | 10 | ***  |
| Lead II          |     |   |   |   |   |     |
| Control          | 6×10| 7 | 9 | 13 | 31 | ***  |
| S-145, 10 mg/kg  | 6×10| 40 | 17 | 2 | 1 | ***  |
| ONO-3708, 50 mg/kg | 6×10| 39 | 9 | 2 | 10 | ***  |
| Lead III         |     |   |   |   |   |     |
| Control          | 6×10| 8 | 11 | 10 | 31 | ***  |
| S-145, 10 mg/kg  | 6×10| 55 | 2 | 2 | 1 | ***  |
| ONO-3708, 50 mg/kg | 6×10| 32 | 10 | 8 | 10 | ***  |

Compounds were administered 30 min before the injection of collagen (1.6–1.8 mg/kg, i.v.). N: number of animals. q: number of observed points. ***P<0.001 vs. control (2 samples, χ²-test).
Fig. 4. Dose-response curves for the inhibition of collagen-induced ECG changes in rats after the intravenous (○) or oral (▲) administration of S-145. S-145 was dosed intravenously 10 min before or orally 1 hr before the injection of collagen (1.4–2.6 mg/kg, i.v.). C: control. ECG changes were calculated using lead I and expressed as total ECG change-grade for 10 min after collagen injection. Each point indicates the mean±S.E. of 6 rats except for control in which the data from 30 rats are pooled. Statistical significance was determined by comparison with the corresponding controls composed of 6 rats each. *P<0.05, **P<0.01, ***P<0.001 vs. control (paired t-test).

Fig. 5. Effects of S-145 and reference compounds on collagen-induced ECG changes in rats. ECG change was calculated using lead I and expressed as in Fig. 4. C: control; S-145: 30 min after 10 mg/kg, p.o.; DAZ: dazoxiben, 1 hr after 30 mg/kg, p.o.; ASA: aspirin, 2 hr after 30 mg/kg, p.o.; TIC: ticlopidine, 2 hr after 100 mg/kg, p.o. × 1 day or 200 mg/kg, p.o. × 3 days. Each bar indicates the mean±S.E. of 6 rats except for the control in which the data from 24 rats are pooled. **P<0.01, ***P<0.001 vs. control (paired t-test).

As shown in Fig. 7A, S-145 (10 mg/kg, p.o.), ONO-3708 (50 mg/kg, p.o.) and dazoxiben (30 mg/kg, p.o.) significantly antagonized the thrombocytopenia induced by collagen, while ticlopidine (300 mg/kg, p.o.) had no effect. As for the plasma prostaglandin levels, S-145 and ONO-3708 did not affect either the TXB\(_2\) or 6-keto-PGF\(_{1\alpha}\) level (Fig. 7B, 7C). In contrast, dazoxiben, a thromboxane synthetase inhibitor, markedly inhibited the increase of the TXB\(_2\) level and caused a slight but significant increase of 6-keto-PGF\(_{1\alpha}\) level. Ticlopidine did not affect the 6-keto-PGF\(_{1\alpha}\) level, but tended to increase the TXB\(_2\) level.

Effects on collagen-induced thrombocytopenia in mice: The results are summarized in Tables 2 and 3. The intravenous injection of S-145, ONO-3708 and dazoxiben significantly prevented the thrombocytopenia induced by collagen with similar ED\(_{50}\) values (Table 2). Significant prevention was also observed 1 hr after the oral administration of S-145, ONO-3708, dazoxiben and aspirin (Table 3). S-145 was 5 to 13 times more...
Fig. 6. Time course of the improving effects of S-145 and ONO-3708 on collagen-induced ECG changes in rats. (○) control; (●) S-145, 10 mg/kg, p.o.; (■) ONO-3708, 50 mg/kg, p.o. ECG change was calculated and expressed as in Fig. 4. Each point indicates the mean±S.E. of 6 rats. *P<0.05, **P<0.01, ***P<0.001 vs. control (paired t-test).

Fig. 7. Effects of S-145 and reference compounds on platelet count (A) and plasma prostanoid levels (B, C) in rats 5 min after collagen injection. N: normal; C: control; S-145: 10 mg/kg, p.o.; ONO: ONO-3708, 50 mg/kg, p.o.; DAZ: dazoxidin, 30 mg/kg, p.o.; TIC: ticlopidine, 300 mg/kg, p.o. The mean±S.E. of 6 rats is shown. Each compound was administered 1 hr before collagen (1 mg/kg, i.v.) injection. Only ticlopidine was given 3 hr before collagen. *P<0.05, **P<0.01, ***P<0.001 vs. saline control (A: Dunnett’s t-test, B, C: Student’s t-test).
Table 2. Effects of intravenously injected S-145, ONO-3708 and dazoxiben on collagen-induced thrombocytopenia in mice

| Compound       | Dose mg/kg | N  | Platelet count (×10^4/mm³) | % of increase | ED50 mg/kg (95% confidence limits) |
|----------------|------------|----|----------------------------|---------------|----------------------------------|
| Saline         |            | 5  | 42.19±2.30                 |               |                                  |
| S-145 (Na salt)| 0.3        | 5  | 49.66±3.16                 | 17.7          | 2.52                             |
|                | 1          | 5  | 57.13±3.18                 | 35.4*         |                                  |
|                | 3          | 5  | 63.42±3.90                 | 50.3**        | (1.52–4.76)                      |
| Saline         |            | 5  | 31.82±1.32                 |               |                                  |
| S-145 (Na salt)| 10         | 5  | 55.13±2.27                 | 73.2**        |                                  |
| Saline         |            | 5  | 40.70±2.70                 |               |                                  |
| ONO-3708       | 1          | 5  | 51.39±3.18                 | 26.3          | 3.51                             |
|                | 3          | 5  | 62.42±4.08                 | 53.4**        | (1.66–8.95)                      |
|                | 10         | 5  | 66.93±2.72                 | 64.4**        |                                  |
| Saline         |            | 5  | 31.82±1.32                 |               |                                  |
| Dazoxiben      | 0.3        | 5  | 35.50±2.04                 | 11.6          |                                  |
| Saline         |            | 5  | 29.38±0.98                 |               | 2.74                             |
| Dazoxiben      | 1          | 5  | 40.95±2.55                 | 39.5*         | (1.46–6.71)                      |
|                | 3          | 5  | 44.92±2.59                 | 53.0**        |                                  |
|                | 10         | 5  | 49.16±3.89                 | 67.4**        |                                  |

Compounds were given 15 min before collagen (1 mg/kg, i.v.) injection. Platelet number was counted 5 min after collagen. N: the number of samples composed of 3 animals each. Values indicate means± S.E. *: P<0.05, **: P<0.01. vs. saline control (Dunnett’s t-test).

Table 3. Effects of orally administered S-145, ONO-3708, dazoxiben and aspirin on collagen-induced thrombocytopenia in mice

| Compound       | Dose mg/kg | N  | Platelet count (×10^4/mm³) | % of increase | ED50 mg/kg (95% confidence limits) |
|----------------|------------|----|----------------------------|---------------|----------------------------------|
| Saline         |            | 5  | 28.28±2.23                 |               |                                  |
| S-145          | 3          | 5  | 38.85±1.88                 | 37.4**        | 6.34                             |
|                | 10         | 5  | 43.68±2.17                 | 54.5**        | (0.79–13.89)                     |
|                | 30         | 5  | 48.03±2.02                 | 69.8**        |                                  |
| Saline         |            | 5  | 30.85±1.94                 |               |                                  |
| ONO-3708       | 10         | 5  | 42.68±2.17                 | 38.4**        | 33.11                            |
|                | 30         | 5  | 47.03±1.84                 | 52.5**        | (9.09–161.82)                    |
|                | 100        | 5  | 48.90±2.18                 | 58.5**        |                                  |
| Saline         |            | 5  | 28.89±1.47                 |               |                                  |
| Dazoxiben      | 10         | 5  | 37.98±0.91                 | 31.1**        | 23.27                            |
|                | 30         | 5  | 44.42±2.47                 | 53.8**        | (15.34–32.48)                    |
|                | 100        | 5  | 53.38±1.83                 | 84.9**        |                                  |
| Saline         |            | 5  | 26.92±2.28                 |               |                                  |
| Aspirin        | 30         | 5  | 33.82±4.22                 | 25.6          | 82.92                            |
|                | 100        | 5  | 43.44±3.20                 | 61.4**        | (11.78–269.65)                   |
|                | 300        | 5  | 46.41±3.24                 | 72.4**        |                                  |

Compounds were given 1 hr before collagen (1 mg/kg, i.v.) injection. Platelet number was counted 5 min after collagen. N: the number of samples composed of 3 animals each. Values indicate means± S.E. *: P<0.05, **: P<0.01. vs. saline control (Dunnett’s t-test).
Fig. 8. Time course of the effects of S-145 and reference compounds on collagen-induced thrombocytopenia in mice. Percent increase of platelet count from saline control is shown. (●) S-145, 10 mg/kg, p.o.; (■) ONO-3708, 30 mg/kg, p.o.; (▲) dazoxiben, 30 mg/kg, p.o.; (◆) aspirin, 30 mg/kg, p.o.; (◇) ticlopidine, 100 mg/kg, p.o. Each point indicates the mean of 5-10 samples composed of 3 animals each. *P<0.05, **P<0.01 vs. saline control (Dunnett’s t-test).

Discussion

A bolus intravenous injection of collagen caused the abnormal ECG changes comprising elevation or depression of the ST segment, arrhythmia and cardiac arrest in severe cases. These ECG changes have already been reported by Dejana et al. (20) using continual intravenous infusion of ADP in rats and are used to examine the effect of antithrombotic drugs (21). In the present experiments, we required a large amount of collagen to induce ECG changes and thrombocytopenia in rats. We therefore used calf skin collagen, although its potency was weaker than that of horse tendon collagen. In fact, collagen from calf skin and horse tendon respectively induced 56 and 74% decrease of platelet count in mice 5 min after 1 mg/kg, i.v.

S-145 showed a marked improvement of the ECG changes induced by collagen after both intravenous and oral administration, and the duration of action was over 4 hr with 10 mg/kg, p.o. Reference compounds ONO-3708 (15), dazoxiben (16) and aspirin but not ticlopidine (17) also significantly improved the collagen-induced ECG changes, indicating that the compounds which antagonize TXA2 receptors or inhibit TXA2 synthesis were effective in this model.

Matsumura et al. (14) recently reported that ticlopidine and a TXA2 synthetase inhibitor (CV-4151) improved collagen-induced ECG changes, but did not antagonize thrombocytopenia in rats. In the present experiments, the compounds which improved the ECG changes also inhibited the thrombocytopenia in rats. This suggests that the inhibition of thrombocytopenia, i.e., platelet aggregation, could be one of the factors responsible for the improvement of collagen-induced ECG changes. Inhibition of the thrombocytopenia in rats was further substantiated by experiments with mice, in which S-145 and reference compounds, except ticlopidine, significantly prevented collagen-induced thrombocytopenia.
induced thrombocytopenia, and the duration of action of S-145 lasted over 4 hr with 10 mg/kg, p.o.

Ticlopidine is a clinically useful antiplatelet drug whose mechanism of action, at first ascribed to the elevation of cAMP levels (22) and recently to the interaction with platelet fibrinogen receptors or platelet glycoproteins (23), is still unknown. Our results that ticlopidine has no effect in our ECG experiments is inconsistent with the results of Matsumura et al. (14). Although the reason for this discrepancy is not clear, the difference in the lot of collagen used may be responsible because we experienced that the change of lot from 93F-8160 to 58F-8125 resulted in the loss of ECG changes even after 3 mg/kg, i.v., suggesting that the collagen-induced ECG changes critically depend on the source and lot of collagen.

Collagen is known to selectively increase the plasma TXB2 level in rats (24). S-145 did not affect the collagen-induced increase of the plasma TXB2 level, indicating clearly that S-145 is a TXA2 antagonist in vivo. Dazoxiben (16), a thromboxane synthetase inhibitor, markedly inhibited the increase of TXB2 levels and caused a slight but significant increase of 6-keto-PGF1α levels, which was probably caused by shifting the PGH2 from the platelets to the white blood cells and vascular wall for the synthesis of PGI2 (25). This increase of PGI2 synthesis together with the inhibition of TXA2 synthesis might be responsible for the beneficial effects of dazoxiben on the ECG changes and thrombocytopenia. However, in a clinical situation, when patients suffering from ischemic heart disease have reduced PGI2 synthetase activity (26), the above-mentioned shift could not be expected.

In conclusion, S-145 appears to be a potent, orally active and long-lasting PGH2/TXA2 receptor antagonist in vivo. This compound should be effective in the treatment of thromboembolism and ischemic heart disease caused by platelet activation.

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