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# Comparison of antiplatelet effects of FK409, a spontaneous nitric oxide releaser, with those of TRK-100, a prostacyclin analogue

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**Abstract**

The anti-platelet effects of FK409 ((±)-(*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexeneamide), a new spontaneous nitric oxide releaser, and TRK-100 (sodium *dl*-4-[(1*R*,2*R*,3a*S*,8b*S*)-1,2,3a,8b-tetra-hydro-2-hydroxy-1-[(3*S*,4*RS*)-3-hydroxy-4-methyl-oct-6-yen-(*E*)-1-enyl]-5-cyclopenta[*b*]benzofuranyl]butyrate), a stable prostacyclin analogue, were studied both in vivo and in vitro. FK409 and TRK-100 inhibited ADP-induced platelet aggregation in rat platelet-rich plasma at 1.0 and 0.032 μM, respectively. In a rat extracorporeal shunt model, FK409 suppressed thrombus formation dose dependently and significantly at 1.0 mg/kg and showed the maximum inhibition (52% inhibition) at 10 mg/kg. TRK-100 showed 79% inhibition of thrombus formation at 1.0 mg/kg, but not at less than 1.0 mg/kg. At the doses required for antiplatelet effects, TRK-100 decreased mean blood pressure significantly but FK409 did not alter the blood pressure. These data suggest that FK409 shows more selective activities on platelets than TRK-100 in these experiments.

*Keywords:* FK409; TRK-100; Antiplatelet activity

## 1. Introduction

FK409 ((±)-(*E*)-ethyl-2-[(*E*)-hydroxyimino]-5-nitro-3-hexeneamide) is a structurally unique vasodilator isolated from the fermentation products of *Streptomyces griseosporeus* and shows a vasorelaxant effect on rat isolated aorta and antiplatelet aggregation activity on rabbit platelet-rich plasma (Hino et al., 1989). FK409 produces a potent vasorelaxation mediated by the elevation of cyclic GMP (cGMP) in isolated dog coronary artery (Yamada et al., 1991) and rabbit aorta (Sibata et al., 1991). Recently, we have reported that FK409 decomposes and releases nitric oxide (NO) spontaneously, which is generally accepted as the endothelium-derived relaxing factor (Palmer et al., 1987), in solution (Kita et al., 1994). Previously it has been reported that organic nitrates elevate cGMP level through the activation of guanylate cyclase via NO released from the compounds (Feelish and Noack, 1987). These biological actions of FK409 are therefore thought to be due to NO released from the compound, like those of organic nitrates.

On the other hand, prostacyclin is a vasodilator and an inhibitor of platelet aggregation and acts through elevation of intracellular cyclic AMP (cAMP). Various prostacyclin derivatives have been developed in an attempt to dissociate vasorelaxant and antiplatelet effects and to improve the stability. TRK-100 (sodium *dl*-4-[(1*R*,2*R*,3a*S*,8b*S*)-1,2,3a,8b-tetra-hydro-2-hydroxy-1-[(3*S*,4*RS*)-3-hydroxy-4-methyl-oct-6-yen-(*E*)-1-enyl]-5-cyclopenta[*b*]benzofuranyl]butyrate) is one of the chemically stable prostacyclin analogues. It has been shown that TRK-100 has a potent antiplatelet effect in both in vitro and ex vivo experiments through elevation of platelet cAMP (Nishio et al., 1988) and is orally and intravenously effective in various thrombosis models (Umetsu et al., 1987).

We have now examined the antiplatelet and hypotensive effects of FK409 and TRK-100 in rats and compared the selectivity of the antiplatelet and hypotensive actions of FK409 with those of TRK-100.

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## 2. Materials and methods

### 2.1. In vitro platelet aggregation

Blood from male Sprague-Dawley rats weighing 255–360 g, which were anesthetized with diethyl ether, was collected from the abdominal aorta into plastic vessels containing 2.2% sodium citrate (1/10 volume), as described previously (Nishizawa et al., 1983). Platelet-rich plasma was obtained from the supernatant fraction after centrifugation of the blood at $200 \times g$ for 10 min. The effect of FK409 or TRK-100 on platelet aggregation was determined by Born's turbidimetric method (Born and Cross, 1968) using an aggregometer (Hema Tracer 801, Niko Bioscience Co., Tokyo, Japan). To 225 $\mu$l of platelet-rich plasma in a cuvette, 25 $\mu$l of each drug solution of Tris-acetate (25 mM) and NaCl (120 mM) buffer adjusted to pH 7.4 or vehicle was added and incubated at 37°C for 2 min. After the incubation, platelet aggregation was induced by the addition of 5 $\mu$l of ADP (final concentration: 2.0 $\mu$M). In order to evaluate platelet aggregation, the maximum increase in light transmission was determined from the aggregation curve for 7 min after the addition of ADP. The effects of each drug were expressed as percent inhibition of ADP-induced platelet aggregation compared with the effect of vehicle treatment.

### 2.2. In vivo tests

Male Sprague-Dawley rats were fasted for 24 h and were given orally each drug, which was suspended with 0.5% methylcellulose, in a volume of 5 ml/kg.

#### 2.2.1. Rat extracorporeal shunt model

The procedure was performed as described previously (Umetsu and Sanai, 1978). Briefly, male Sprague-Dawley rats weighing 305–405 g were anesthetized with sodium pentobarbital (50 mg/kg i.p.). A silk thread (5 cm; Tire No. 50, Fujix Co., Osaka, Japan) was placed in a polyethylene tube (6 cm; Hibiki Size 7, Kunii Co., Tokyo, Japan). Both ends of this tubing were connected to other polyethylene tubings (10 cm; Hibiki Size 4, Kunii Co.). An extracorporeal shunt filled with heparin solution (50 IU/ml) was introduced between the right carotid artery and the left jugular vein, and after this, blood circulation was established. After 20 min, the blood flow was stopped on the artery side with a pinch-cock. The middle tubing was then taken away and the thread coated with thrombus was carefully pulled out of the tubing. The wet weight of the thrombus was measured immediately. Another new polyethylene tubing containing a silk thread and filled with the heparin solution was inserted between the arterial and venous tubing ends. Blood circulation was re-established by opening the pinch-cock. After 20 min, the wet weight of the silk thread was measured as before. The third blood circulation was then performed. Thrombus weight was determined by subtracting the weight of a silk thread immersed in heparinized blood from that of the thread coated with thrombus. The total thrombus weight obtained from three blood circulations was taken as net thrombus weight in one experiment. FK409, TRK-100 or vehicle was given orally 60 min before the first blood circulation. The effects of each drug were expressed as percent inhibition of thrombus formation compared with vehicle treatment.

#### 2.2.2. Measurement of mean blood pressure in conscious rats

After male Sprague-Dawley rats weighing 235–310 g were anesthetized with diethyl ether, a polyethylene cannula, filled with heparin solution, was inserted into the left femoral artery of each rat to measure mean blood pressure. The rats were anesthetized with diethyl ether only during the operation. After 120 min from the end of anesthsia, mean blood pressure was measured and then each drug was administered orally. Mean blood pressure was measured with a pressure transducer (TR-400T, Nihon Koden Co., Tokyo, Japan) connected to an amplifier and was recorded on a polygraph (Recti-Horiz-8K, Sanei Co., Tokyo, Japan). The effect of each drug on mean blood pressure was observed 60 min after the administration. Changes in mean blood pressure were expressed as percentage of the pre-administration level.

### 2.3. Drugs

The drugs used were as follows: FK409 was synthesized by Fujisawa Pharmaceutical Co. (Osaka, Japan); TRK-100 was synthesized by Teijin Co. (Tokyo, Japan); sodium citrate and ADP were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and heparin sodium was purchased from Takeda Pharmaceutical Co. (Osaka, Japan).

### 2.4. Statistical analysis

The data are presented as the means + S.E.M. for the number of experiments indicated. For multiple comparisons, the data were analyzed using a one-way analysis of variance followed by Dunnett's test.

## 3. Results

### 3.1. Effects on in vitro platelet aggregation

Fig. 1 shows the inhibitory effects of FK409 and TRK-100 on ADP-induced platelet aggregation in rat

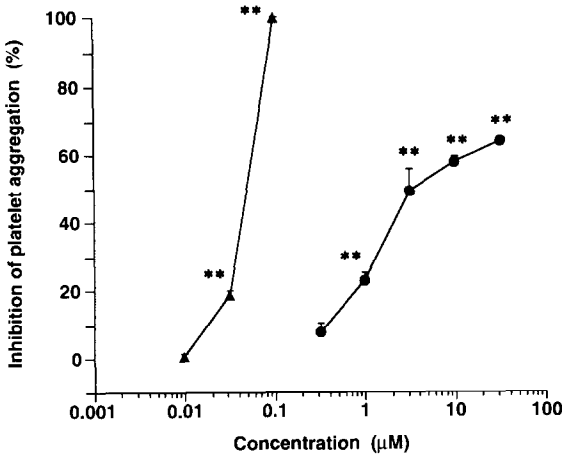


Fig. 1. Inhibitory effects of FK409 (●) and TRK-100 (▲) on ADP-induced platelet aggregation in rat platelet-rich plasma. The effects of each drug were expressed as percent inhibition of ADP-induced platelet aggregation compared with vehicle treatment. Each value represents the mean + S.E.M. for 3–5 experiments. * * $P < 0.01$ compared with platelet aggregation in the vehicle-treated group.

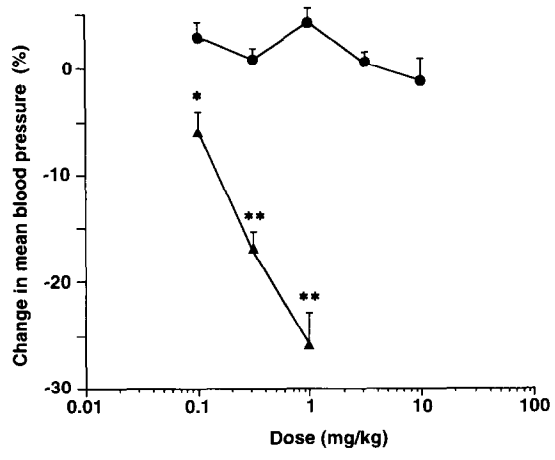


Fig. 3. Effects of FK409 (●) and TRK-100 (▲) on mean blood pressure at 60 min after oral administration. Changes in mean blood pressure were expressed as percentage of pre-administration level. Each value represents the mean + S.E.M. for 5–6 experiments. * $P < 0.05$, * * $P < 0.01$ compared with the vehicle-treated group.

platelet-rich plasma. FK409 (0.32–32 μM) and TRK-100 (0.01–0.1 μM) suppressed aggregation dose dependently and showed significant inhibition at 1.0 and 0.032 μM, respectively. At the maximum concentration tested, FK409 inhibited platelet aggregation by 64% while TRK-100 inhibited it completely.

### 3.2. Effects on thrombus formation in a rat extracorporeal shunt model

To examine the in vivo antiplatelet action of oral FK409 and TRK-100, we evaluated these drugs in a rat extracorporeal shunt model. We did not observe a significant difference in thrombus weights among the first, second and third thrombi in both vehicle-treated and drug-treated groups. Therefore, we used total thrombus weights in three circulations as described earlier (Umetsu and Sanai, 1978). Fig. 2 shows the inhibitory effects of FK409 and TRK-100 in this model. FK409 significantly inhibited thrombus formation at 1.0 mg/kg and showed 52% inhibition at 10 mg/kg. TRK-100 inhibited thrombus formation at 1.0 mg/kg and the inhibition value was 79%.

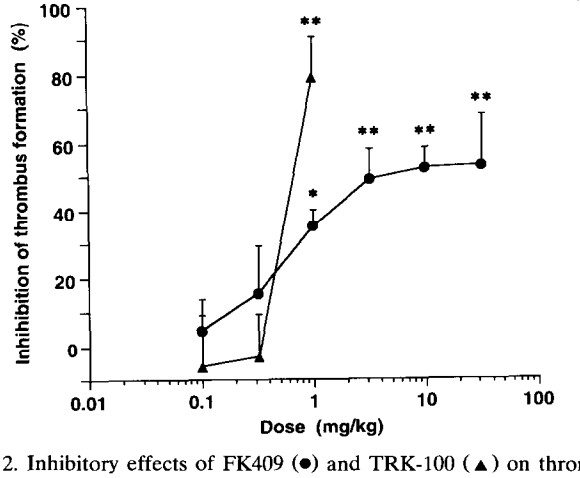


Fig. 2. Inhibitory effects of FK409 (●) and TRK-100 (▲) on thrombus formation in the rat extracorporeal shunt model. The effects of each drug were expressed as percent inhibition of thrombus formation compared with vehicle treatment. Each value represents the mean + S.E.M. for 6 experiments. * $P < 0.05$, * * $P < 0.01$ compared with thrombus formation in the vehicle-treated group.

### 3.3. Measurement of mean blood pressure

The influence of FK409 and TRK-100 on mean blood pressure was examined 60 min after oral administration of each drug in conscious rats (Fig. 3). FK409 (0.1–10.0 mg/kg) did not influence the mean blood pressure at this time. On the other hand, TRK-100 (0.1–1.0 mg/kg) decreased the mean blood pressure dose dependently and significantly with a 26% decrease at 1.0 mg/kg.

## 4. Discussion

Recently we have reported that FK409 decomposes and releases NO spontaneously and subsequently shows biological actions such as vasorelaxant and antiplatelet effects (Kita et al., 1994). TRK-100 is a novel, chemically stable and orally active prostacyclin analogue synthesized by Ohno et al. (Ohno et al., 1985) which was found to possess preferentially antiplatelet activity in comparison to its vasodilating activity (Nishio et al., 1985; Sim et al., 1985).

In in vitro experiments, FK409 and TRK-100 significantly inhibited rat platelet aggregation at 1.0 μM and 0.032 μM, respectively. TRK-100 was 30 times more potent than FK409 in vitro. In in vivo experiments, both FK409 and TRK-100 significantly inhibited thrombus formation in a rat extracorporeal shunt model at 1.0 mg/kg. Thus, the potency of FK409 in vivo was much greater than that expected from the antiplatelet activities of these compound in vitro. FK409 showed dose-dependent antiplatelet effects in both in vitro and in vivo experiments but could not inhibit completely. In in vitro rat platelet aggregation, the inhibition value of FK409 was 64% at the maximum concentration tested (32 μM). In the extracorporeal shunt model, the inhibition value of FK409 was 52% at the maximum dose tested (10 mg/kg). In contrast with FK409, TRK-100 significantly inhibited in vitro rat platelet aggregation at 0.032 μM and achieved complete inhibition of platelet aggregation at 0.1μM. In the extracorporeal shunt model, although TRK-100 could not inhibit thrombus formation at the doses less than 1.0 mg/kg, the compound showed 79% inhibition at 1.0 mg/kg. In addition, the slopes of both in vitro and in vivo dose-response curves of TRK-100 were steeper than those of FK409. It is unclear why the FK409 inhibition of in vitro and in vivo antiplatelet activities is different from that caused by TRK-100.

FK409 did not show any significant effect on mean blood pressure at 10 mg/kg. On the other hand, TRK-100 caused hypotension dose dependently at the minimum dose tested (0.1 mg/kg). Fig. 4 shows the relationship between the decrease in mean blood pressure and the inhibition of thrombus formation in the extracorporeal shunt model. FK409 did not decrease blood pressure at the doses required for antiplatelet effects in vivo, while TRK-100 caused a significant decrease in blood pressure at the doses at which the compound did not show an antiplatelet effect. These results are different from those in previous reports by Nishio et al. (1985) and Sim et al. (1985) in that TRK-100 possesses more potent antiplatelet activity than vasodilating activity. We think that the difference between our results and the conclusion of the previous reports by Nishio et al. (1985) and Sim et al. (1985) is due to the difference between experimental systems. To examine the antiplatelet effect of TRK-100, Nishio et al. (1985) evaluated the drug in ex vivo platelet aggregation experiments in rabbits and Sim et al. (1985) evaluated the drug in experiments on thrombus disaggregation in the microcirculation of the hamster cheek pouch, while we evaluated TRK-100 and FK409 in the rat extracorporeal shunt model.

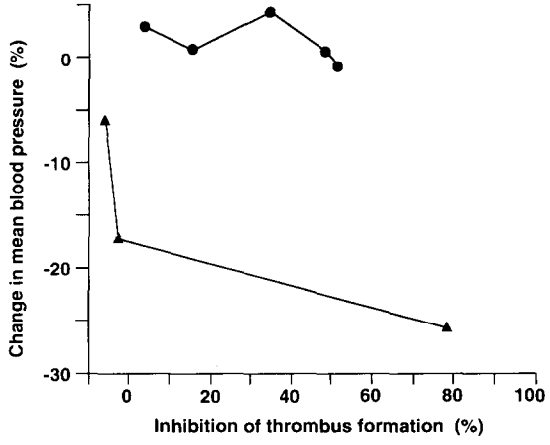


Fig. 4. Relationship between the change in mean blood pressure depression and the inhibition of thrombus formation in the extracorporeal shunt model by FK409 (●) and TRK-100 (▲). The vertical axis shows changes in mean blood pressure. The horizontal axis represents inhibition of thrombus formation.

FK409 and TRK-100 have antiplatelet activity. Evidence suggests that platelets play an important role in the pathogenesis of the acute coronary syndromes of unstable angina (Fitzgerald et al., 1986; Hjemdahl-Monsen et al., 1986; Vejar et al., 1990), myocardial infarction (Tofler et al., 1987; Trip et al., 1990), sudden death (Davies et al., 1984), vasospastic angina (Lam et al., 1987) and hypertensive disorders (Naftilan et al., 1986; Lindner et al., 1987). The inhibition of platelet function seems to be one of the most promising approaches to prevent these syndromes. FK409 and TRK-100 have also been shown to be vasodilators. Although the vasodilation may be desired in some situations, the hypotensive effect associated with vasodilation seems to be unfavorable for the treatment of cardiovascular diseases with only antiplatelet activity expected (Folts, 1991). In the present study, FK409 showed the antiplatelet effect without the decrease in mean blood pressure. Therefore, FK409 may be useful for the treatment of various thrombotic disorders.

In conclusion, we have shown that FK409, a spontaneous NO releaser, inhibited rat platelet aggregation in vitro and thrombus formation in the rat extracorporeal shunt model. In addition, oral FK409 was shown to have differential selectivity between antiplatelet action and hypotensive action in rats, and was superior to oral TRK-100, a prostacyclin analogue, as to selectivity of antiplatelet activity.

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