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Studies of Platelet Activating Factor (PAF) Antagonists from Microbial Products. III.[1] Pharmacological Studies of FR-900452 in Animal Models

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1-Methyl-3-[1-[5-methylthiomethyl-6-oxo-3-(2-oxo-3-cyclopenten-1-ylidene)-2-piperazinyl]-ethyl]-2-indolinone (FR-900452), isolated from the fermentation broth of *Streptomyces phaeofaciens* No. 7739, is a specific inhibitor of rabbit platelet aggregation induced by platelet activating factor (PAF) *in vitro*. In order to ascertain the PAF antagonistic activity of FR-900452 *in vivo*, the effects of this compound on PAF-induced bronchoconstriction in guinea-pigs, hypotension in rats and vascular permeability increase in mice were examined. The compound significantly inhibited the bronchoconstriction, the hypotension and the vascular permeability increase at a dosage of less than 10 mg/kg, i.v., and the hypotensive actions induced by i.v. administration of histamine (His, 100 μg/kg), acetylcholine (Ach, 1 μg/kg), bradykinin (Bk, 10 μg/kg) and isoproterenol (Isp, 1 μg/kg) were not altered by FR-900452 (10 mg/kg, i.v.). Therefore, to determine whether endogenous PAF contributes to the pathogenesis of immunoglobulin E (IgE)-mediated anaphylaxis, the effect of FR-900452 on the hypotension induced by IgE-mediated anaphylaxis in rats was tested. The compound significantly prevented the hypotension at a dose of 10 mg/kg, i.v. The results suggest that PAF may play a role in the pathogenesis of the IgE-mediated hypotension in rats.

Keywords——platelet activating factor; PAF inhibitor; FR-900452; IgE-mediated hypotension

Platelet activating factor (PAF) (1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a substance that is secreted by cells that are involved in allergic and inflammatory processes.[2] The pathophysiological role of PAF in these diseases is beginning to be elucidated. Recent reports suggest that endogenous PAF involved in the pathogenesis of endotoxin shock in the rat.[3] However, the role of PAF in allergy and other inflammatory diseases is largely unknown. In order to elucidate the dynamic roles of PAF and to modulate its action in various pathological conditions, clearly it would be highly desirable to have a specific and *in vivo*-active inhibitor.

1-Methyl-3-[1-[5-methylthiomethyl-6-oxo-3-(2-oxo-3-cyclopenten-1-ylidene)-2-piperazinyl]ethyl]-2-indolinone (FR-900452), a new inhibitor of PAF, was isolated from the fermentation broth of *Streptomyces phaeofaciens* No. 7739. The compound potently inhibits PAF-induced rabbit platelet aggregation with an IC_{50} of 3.7×10^{-7} M and it is much less active against collagen-, arachidonic acid- or adenosine diphosphate (ADP)-induced aggregation.[4]

In this study, we examined the PAF-inhibitory activity of FR-900452 in some experimental animal models. Moreover, utilizing FR-900452, we investigated endogenous PAF involvement in immunoglobulin E (IgE)-mediated hypotension in the rat.

Experimental

Material——PAF (alkyl chain; C_{18}) was synthesized in our labolatories as reported previously,[5] and was dissolved in saline containing 0.25% bovine serum albumin (BSA, Sigma) for *in vivo* use. FR-900452 was dissolved in

ethyl alcohol, then diluted 9-fold with saline containing 0.5% methyl cellulose (EtOH–MC) and given i.v. to animals at 2 ml/kg. Tiaramide (Fujisawa Pharm. Co.) was prepared in the same vehicle. Histamine (His) (dihydrochloride, Nakarai Chem. Co.), acetylcholine (Ach) (hydrochloride, Dai-ichi Pharm. Co.), bradykinin (Bk) (Peptide Institute) and isoproterenol (Isp) (hydrochloride, Sigma) were dissolved in saline. In the IgE-mediated hypotension model, FR-900452 was prepared in polyethylene glycol-400 (PEG, Sigma) and 0.5 ml/kg was given to the animals. Disodium cromoglycate (DSCG, Fujisawa Pharm. Co.) was dissolved in saline.

PAF-Induced Bronchoconstriction——The method was described previously.[1] Briefly, male Hartley guinea-pigs weighing 300—400 g were immobilized with gallamine (20 mg/kg i.p., Sigma). The jugular vein was cannulated for administration of PAF and drugs. A catheter was also intubated into the trachea for artificial ventilation. The animal was respirated by means of a miniature respiration pump (60 strokes/min). Resistance to lung inflation was measured by a modification of the Konzett–Rössler overflow technique.[6] Drug (prepared in EtOH–MC) or vehicle was administered i.v. at 2 min prior to i.v. PAF (1 μg/kg). The maximal increase in bronchoconstriction induced by PAF was measured and drug inhibitions were calculated.

PAF-, His, Ach-, Bk- and Isp-Induced Hypotension——Seven-week-old, male Sprague–Dawley JCL rats were anesthetized with urethane (700 mg/kg, i.p., Ishizu Pharm. Co.). Catheters were introduced into the femoral artery and vein for the measurement of arterial blood pressure and for drug administration, respectively. Mean arterial blood pressure (MABP) was recorded from the femoral artery through a canula connected to a pressure transducer (Nihon Kohden, MPU-0.5A) coupled to a Biophysiograph 180 system (San-Ei Instrument). Drug (prepared in EtOH–MC) or vehicle was administered i.v. at different times prior to i.v. PAF (1 μg/kg). To examine the effects of FR-900452 on the hypotension induced by i.v. injection of His (100 μg/kg), Ach (1 μg/kg), Bk (10 μg/kg) and Isp (1 μg/kg), FR-900452 was administered at 10 min prior to each agonist. The maximal decrease in MABP was measured and drug inhibition was calculated.

PAF-Induced Vascular Permeability Increase——The backs of 6-week-old male ddY mice were shaved and Evans blue dye (1 mg/animal, Sigma) was injected intravenously. After 5 min, PAF (50 ng/site) was injected intradermally into the depilated backs of the animals. Thirty minutes later, the animals were killed and the area of dye leakage (maximum × minimum diameter, mm^2) was measured. Drug (prepared in EtOH–MC) or vehicle was administered i.v. at 7 min prior to i.d. PAF.

Preparation of Murine Monoclonal IgE-Antibody——A murine monoclonal IgE-antibody against 1,3,5-trinitrophenyl conjugated bovine serum albumin (TNP–BSA) was prepared by the method of Liu *et al.*[7] Briefly, a murine hybridoma secreting monoclonal IgE-antibody was obtained by fusion of P3-X63-Ag8-U1 myeloma cells and spleen cells from TNP–BSA-hyperimmunized BALB/c strain mouse. The hybridoma cells (1×10^6 per mouse) were injected i.p. into BALB/c mice which had been pretreated with 0.5 ml of pristane (Aldrich) i.p. 1 week before. The tumor cells were allowed to grow as ascites in the peritoneal cavity for approximately 2 weeks, then the ascites fluid was collected in the presence of heparin and centrifuged. The IgE-antibody against TNP–BSA in the supernatant was tested by using passive cutaneous anaphylaxis (PCA) in rats to determine the titer of the antibody.

IgE-Mediated Hypotension——Seven-week-old, Sprague–Dawley rats were adrenalectomized through bilateral incisions under pentobarbital (20 mg/kg, i.p., Dai-Nippon Pharm. Co.) anesthesia. Three days later, the rats were passively sensitized with an i.v. injection of the above described IgE-antibody (PCA titer; × 1500, 1 ml). Twenty-four hours later, the animals were anesthetized with urethane (700 mg/kg, i.p.) and MABP was measured as described above. Drug (prepared in PEG) or vehicle was administered i.v. at 3 min prior to i.v. TNP–BSA (40 mg/animal). The MABP was recorded just before the drug and antigen injection and at 2, 5 and 10 min after the antigen challenge.

Statistical Analysis——Each treated group was compared to a control group injected with the same vehicle. Differences between mean values were analyzed by means of Student's *t*-test.

Results

Effects of FR-900452 and Tiaramide on PAF-Induced Bronchoconstriction in Guinea-Pigs

In order to test the PAF-inhibitory activity of FR-900452 and tiaramide on the bronchoconstriction, each drug was administered i.v. at 2 min prior to the i.v. PAF. In the vehicle-treated group, the increase in maximum ventilatory pressure was 316.7 ± 36.8 mm H_2O (means ± S.E.M., $n=6$) with 1 μg/kg dosage of PAF. As shown in Table I, FR-900452 completely inhibited the bronchoconstriction at a dose of 10 mg/kg and at 1 mg/kg it showed 34% inhibition (not significantly different from the control, $p>0.05$). An antiinflammatory drug, tiaramide, was also tested for comparison. It significantly inhibited the bronchoconstriction by 58% at a dose of 10 mg/kg i.v. ($p<0.01$).

Effects of FR-900452 and Tiaramide on PAF-Induced Hypotension in Rats

Intravenous injection of PAF (1 μg/kg) induced hypotension in urethane-anesthetized

TABLE I. Effects of FR-900452 and Tiaramide on PAF-Induced Bronchoconstriction in Guinea-Pigs

Drug	Dose (mg/kg)	n	Ventilatory pressure increase (mmH_2O)	Inhibition (%)
Vehicle	—	6	316.7±36.8	—
FR-900452	1	4	210.0±73.7	34
	10	4	0	100
Tiaramide	10	4	132.5±26.6[a)]	58

Hartley male guinea-pigs (300—500 g) were immobilized with gallamine (20 mg/kg, i.p.). Each drug was administered i.v. at 2 min prior to PAF (1 μg/kg) injection. Each value expresses the ventilatory pressure increase (means±S.E.M., mmH_2O). *a)* means significantly different from the vehicle control ($p<0.01$).

TABLE II. Effects of FR-900452 and Tiaramide on PAF-Induced Hypotension in Rats

Drug	Dose (mg/kg)	Time of treatment (min)	n	MABP change (mmHg)	Inhibition (%)
Vehicle	—		8	58.1±3.0	—
FR-900452	0.3	−3	4	53.3±4.4	8
	1	−3	4	25.0±7.6[a)]	57
	10	−3	4	2.5±7.6[a)]	96
		−15	5	11.3±4.3[a)]	82
		−60	5	33.8±1.3[a)]	42
Tiaramide	10	−3	4	52.5±2.5	10

S.D. rats (7 weeks) were anesthetized with urethane (700 mg/kg, i.p.). Drug and PAF (1 μg/kg) were administered i.v. Each value expresses the MABP change (means±S.E.M.). *a)* means significantly different from the control ($p<0.001$).

rats. To examine the inhibitory effect of FR-900452 on the hypotension, FR-900452 was administered i.v. at 3, 15 and 60 min prior to the i.v. PAF. As Table II shows, FR-900452 at doses of 1 and 10 mg/kg i.v. given at 3 min prior to the PAF injection significantly prevented the hypotension (57% and 96% inhibitions, respectively). Furthermore, to examine the duration of the anti-hypotensive effect of this compound, FR-900452 was given to the animals at 15 and 60 min prior to the PAF injection. As shown in Table II, pretreatment at 15 and 60 min with 10 mg/kg of FR-900452 significantly inhibited the hypotension by 82% and 42%, respectively. In this model, tiaramide (10 mg/kg) given at 3 min prior to the PAF did not inhibit the hypotension.

Effect of FR-900452 on His-, Ach-, Bk- and Isp-Induced Hypotension in Rats

The i.v. administration of His (100 μg/kg), Ach (1 μg/kg), Bk (10 μg/kg) and Isp (1 μg/kg) to rats reduced blood pressure by 43.1±4.9 (means±S.E.M., $n=5$), 28.1±3.7 ($n=5$), 31.2±1.7 ($n=5$) and 31.5±3.6 ($n=5$) mmHg, respectively. These blood pressure-lowering effects were not influenced by treatment of the rats with FR-900452 (10 mg/kg, i.v.); the blood pressure falls with His, Ach, Bk and Isp at 10 min after administration of FR-900452 were 47.5±1.8, 30.4±4.4, 34.0±4.7 and 33.8±3.6 mmHg, respectively.

Effects of FR-900452 and Tiaramide on PAF-Induced Vascular Permeability Increase in Mice

Intradermal injection of PAF (50 ng/site) induced a vascular permeability increase in mice. The area of dye leakage was 82.9±8.8 mm^2 (means±S.E.M., $n=10$) in the vehicle-treated group. To test the inhibitory effects of FR-900452 and tiaramide on the vascular permeability increase, each drug was administered at 7 min prior to the i.d. PAF. Table III

TABLE III. Effects of FR-900452 and Tiaramide on PAF-Induced Vascular Permeability Increase in Mice

Drug	Dose (mg/kg)	n	Area of dye leakage (mm^2)	Inhibition (%)
Vehicle	—	10	82.9±8.8	—
FR-900452	1	5	39.6±7.7[b]	52
	3	10	37.5±4.9[c]	55
	10	5	22.2±6.6[c]	73
Tiaramide	10	5	46.2±1.7[a]	44

PAF (50 ng) was injected i.d. into the depilated back of mice. Drug was administered i.v. at 7 min prior the i.d. PAF. Each value expresses the area of dye leakage (mean±S.E.M.). *a, b, c*) mean significantly different from the vehicle control ($p<0.05$, $p<0.01$ and $p<0.001$, respectively).

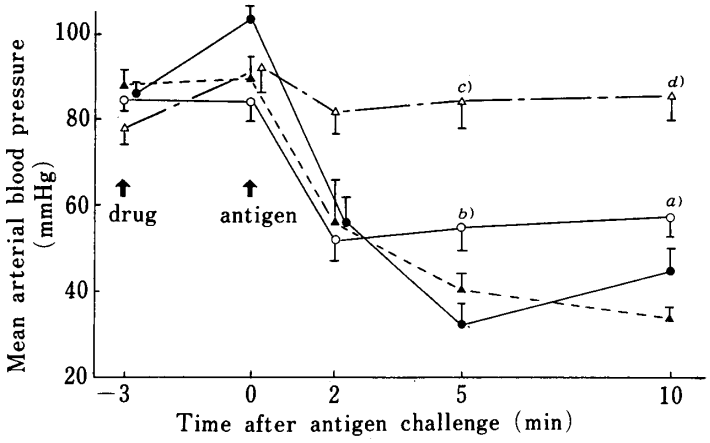

Fig. 1. Effects of FR-900452 and DSCG on Antigen-Induced Hypotension in Murine Monoclonal IgE-Sensitized Rats

—○—, FR-900452 10 mg/kg ($n=12$); —●—, PEG 0.5 ml/kg ($n=6$); —·△—·, DSCG 10 mg/kg ($n=5$); --▲--, saline 1 ml/kg ($n=5$).

Adrenalectomized rats were passively sensitized with murine monoclonal IgE-antibody against TNP–BSA. Hypotension was induced by an i.v. injection of the antigen under urethane (700 mg/kg, i.p.) anesthesia. Each drug or the corresponding vehicle was administered i.v. at 3 min prior to the antigen challenge. Individual points (mmHg) are the mean±S.E.M. *a, b*) mean significantly different from the PEG-treated group (*a*) $p<0.05$, *b*) $p<0.01$). *c, d*) from the saline-treated group (*c*) $p<0.01$, *d*) $p<0.001$).

shows that FR-900452 at doses of 1, 3 and 10 mg/kg significantly inhibited the vascular permeability increase by 52%, 55% and 73%, respectively. Tiaramide (10 mg/kg, i.v.) also inhibited it by 44%.

Effects of FR-900452 and DSCG on Antigen-Induced Hypotension in Murine Monoclonal IgE-Sensitized Rats

To test the inhibitory effects of FR-900452 (prepared in PEG) and DSCG (in saline) on the hypotension, each drug or the corresponding vehicle was administered i.v. at 3 min prior to the i.v. antigen. As shown in Fig. 1, in the control group with saline, the MABP was decreased by 32.0±10.65 mmHg, 47.5±4.47 and 54.0±5.28 (mean±S.E.M., $n=5$) at 2, 5 and 10 min after the antigen, respectively. In the control group with PEG, a slight increase of MABP was observed after the injection of PEG, but the decrease of MABP induced by the antigen was similar to that of the control group with saline. FR-900452 at a dose of 10 mg/kg significantly inhibited the hypotension at 5 and 10 min after the antigen. An anti-allergic drug, DSCG, was also tested in this model, and it (10 mg/kg) apparently prevented the hypotension.

Discussion

PAF is a putative mediator of asthma and inflammatory diseases. This compound induces potent biological effects such as bronchoconstriction,[8] hypotension[9] or increase of vascular permeability[10] in experimental animal models.

We have found that FR-900452 is a potent and specific inhibitor of PAF-induced rabbit platelet aggregation *in vitro*.[4] In addition, Tokumura *et al.* reported that the compound showed selective and competitive inhibition of PAF-induced contraction of rat colon.[11] These observations prompted us to examine the *in vivo* activity of this compound on the PAF-induced bronchoconstriction in guinea-pigs, the hypotension in rats or the vascular permeability increase in mice. FR-900452 at doses of 1—10 mg/kg, injected 2—3 min prior to i.v. PAF, significantly inhibited all of the PAF-induced biological actions in the animal models that we tested. Moreover, in the PAF-induced hypotension model, we found that the inhibitory effect of FR-900452 lasted up to 60 min (Table II). On the other hand, FR-900452 did not show any inhibitory activity on hypotensions induced by His, Ach, Bk and Isp in rats. These observations show that FR-900452 is a specific inhibitor of PAF *in vivo* as well as *in vitro*. Tiaramide is also an inhibitor of rabbit platelet aggregation induced by PAF; this drug at a dose of 10 mg/kg inhibited PAF-induced bronchoconstriction in guinea-pigs and the vascular permeability increase in mice but did not inhibit the hypotension in rats (Tables I, II and III). Some of these data have been discussed in brief previously.[1]

It is well known that PAF is released from several types of inflammatory cells by an IgE-dependent mechanism, and the involvement of PAF in the pathogenesis of IgE-mediated allergy has been suggested.[12] Therefore, utilizing FR-900452, we attempted to prevent the hypotension due to IgE-anaphylaxis in rats. When FR-900452, prepared in PEG was administered at 3 min prior to the antigen challenge, the compound apparently prevented the hypotension at 5 or 10 min after the antigen.

An anti-allergic drug, DSCG, also prevented the hypotension. The effect of DSCG might be mostly due to its mast cell-stabilizing property. In recent studies, DSCG blocked the skin reaction elicited by PAF in man,[13] whereas Lewis *et al.*[8] showed that DSCG did not inhibit the PAF-induced platelet aggregation in rabbit or the bronchoconstriction in guinea-pig. Whether this drug exerts its anti-allergic action partly through a PAF-antagonistic mechanism is still controversial.

In conclusion, from our findings that the *in vivo*-active and PAF-specific inhibitor, FR-900452, prevented the IgE-mediated hypotension, we presume PAF is one of the mediators of IgE-mediated anaphylaxis in the rat.

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