[Chem. Pharm. Bull. 34(1) 345—348 (1986)]

Studies of Platelet Activating Factor (PAF) Antagonists from Microbial Products. II.[1] Pharmacological Studies of FR-49175 in Animal Models

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(Received May 14, 1985)

Platelet activating factor (PAF) antagonistic action of FR-49175 was studied using *in vivo* models designated as PAF-induced bronchoconstriction, hypotension and vascular permeability increase. This compound (0.1 mg/kg, *i.v.*) significantly inhibited PAF-induced bronchoconstriction in guinea-pigs. However, it did not prevent PAF-induced hypotension in rats or vascular permeability increase in mice. Furthermore, the effect of this compound against a systemic anaphylaxis model in guinea-pigs was examined, but it did not show any inhibition of antigen-induced mortality at the dose of 10 mg/kg, *i.v.*

Keywords——FR-49175; platelet activating factor (PAF); PAF antagonist; PAF-induced bronchoconstriction; PAF-induced hypotension; PAF-induced vascular permeability increase; systemic anaphylaxis

FR-49175 was isolated from the fermentation products of *Penicillium terlikowskii* No. 5348, and identified as bisdethiobis(methylthio)gliotoxin (Fig. 1).[1] This compound inhibited platelet activating factor (PAF)-induced rabbit platelet aggregation with an IC_{50} of 8.4 μM. It was much less active against collagen-, arachidonic acid- or adenosine diphosphate (ADP)-induced aggregation.[1]

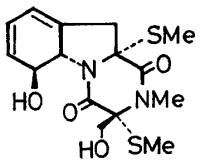

Fig. 1. Chemical Structure of FR-49175

PAF has been considered as a potential mediator of both allergic[2,3] and non-allergic[4] inflammation. Currently, there are many experimental data[5] showing that inflammation is related to PAF generation. The availability of FR-49175, a selective PAF antagonist, will allow critical testing of this association.

This report describes the anti-PAF effects of FR-49175 in some animal models.

Experimental

Materials——PAF (alkyl chain: C_{18}) was synthesized in our laboratories as reported previously,[1] and was dissolved in 0.15 M NaCl containing 2.5 mg/ml bovine serum albumin (Sigma). FR-49175 was dissolved in polyethyleneglycol-400 : ethanol (1 : 1) and diluted with 0.15 M NaCl for *in vivo* use. Tiaramide (Fujisawa) was dissolved in saline.

PAF-Induced Bronchoconstriction——Male Hartley strain guinea-pigs weighing 300—500 g were immobilized with gallamine triethiodide (Sigma, 20 mg/kg, *i.p.*). The resistance of the lung to inflation imposed by artificial respiration was measured by the Konzett–Rossler method.[6] The trachea was cannulated for artificial respiration which was performed with a miniature respiration pump (Harvard, 5 ml/stroke, 60 strokes/min). The side-arm of the

cannula was connected to a pressure transducer (Nihon Koden, LPU-0.1A) coupled to a Biophysiograph 180 system (San-Ei Instrument). A catheter was introduced into the right jugular vein for the administration of PAF (3.2 μg/kg) and drugs. Drug or vehicle was administered 1 min before the PAF injection.

PAF-Induced Hypotension——Seven-week-old, male Sprague-Dawley JCL rats were anesthetized with urethane (Ishizu, 700 mg/kg, *i.p.*). Catheters were introduced into the femoral artery and vein for the measurement of arterial pressure and for drug administration, respectively. Blood pressure was recorded from the femoral artery through a canula connected to a pressure transducer (Nihon Koden, MPU-0.5A) coupled to a Biophysiograph 180 system. Inhibitory activity of test drugs was expressed as inhibition percentage of the hypotension induced by intravenously injected PAF (1 μg/kg). FR-49175 or vehicle was administered 1 min before the PAF injection.

PAF-Induced Vascular Permeability Increase——PAF (50 ng) was injected intradermally to the depilated back of 6-week-old male ddY mice. Evan's blue (1 mg) was injected *i.v.* 5 min before the PAF injection, and the test drug was administered intravenously 2 min before the Evan's blue injection. Thirty minutes later, the animals were killed and the area of vascular leakage (maximum X minimum length in diameter) was measured.

Systemic Anaphylaxis——Male Hartley strain guinea-pigs weighing 250—300 g were used. The animals were passively sensitized with an *i.v.* injection of 32-fold diluted immunoglobulin G (IgG)-antiserum (0.5 ml). This antiserum against egg albumin was prepared in Japanese white rabbit (2.5—3.0 kg). Twenty-four hours later, the guinea-pigs were individually placed in plastic chambers, and an aerosol of 5% egg albumin was sprayed into the chamber with a conventional nebulizer (Omron NE-U10B) at a rate of 5.5 l/min for 3 min. The control animals died in a few minutes under these conditions. When the animals survived for 2 h, they were regarded as being protected from anaphylaxis. Drug or vehicle was administered *i.v.* 1 min before 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

PAF-Induced Bronchoconstriction Model

Intravenously injected PAF induced bronchoconstriction in guinea-pigs. The maximum ventilatory pressure increased was 385.3 ± 2.3 mm H_2O (mean $\pm$ SEM, $n=5$) with 3.2 μg/kg dosage of PAF. To test the PAF antagonistic activity of FR-49175 and tiaramide on the bronchoconstriction, each drug was administered *i.v.* 1 min before the PAF injection. As shown in Table I, FR-49175 significantly inhibited the bronchoconstriction in a dose-dependent manner at doses from 0.1 to 10 mg/kg. The half-inhibitory dose (ID_{50}), calculated by the Probit method was 0.26 mg/kg. Tiaramide also inhibited (59.4%) the bronchoconstriction significantly at a dose of 10 mg/kg, *i.v.*

PAF-Induced Hypotension Model

PAF (1 μg/kg, *i.v.*) induced hypotension in urethane-anesthetized rats. The average value of initial mean arterial blood pressure (MABP) was 110 mmHg. The decrease of MABP was 58.5 ± 1.87 mmHg (mean $\pm$ SEM, $n=5$) for the control group. FR-49175 at a dose of 10 mg/kg *i.v.* given 1 min before the PAF injection did not inhibit the hypotension. The decrease of MABP in the treated group ($n=5$) was 54.0 ± 2.31 mmHg (7.69% inh.). This value is not

Table I. Effects of FR-49175 and Tiaramide on PAF-Induced Bronchoconstriction in Guinea-pigs

Drug	Dose (mg/kg)	*n*	Ventilatory pressure increase (mm H_2O)	Inhibition (%)
Vehicle	—	5	385.3 ± 2.3	—
FR-49175	0.1	5	$237.3 \pm 32.5^{a)}$	38.3
	1.0	5	$120.0 \pm 36.9^{b)}$	68.9
	10.0	5	$89.7 \pm 18.8^{b)}$	76.8
Tiaramide	10.0	5	$228.9 \pm 34.0^{b)}$	59.4

Each value represents the ventilator pressure increase (mean $\pm$ SEM); n = number of guinea-pigs in each group. *a, b)* Indicates statistical significance compared to the control, $p<0.05$, $p<0.01$ respectively. Each drug was administered 1 min before PAF (3.2 μg/kg) injection *i.v.*

TABLE II. Effects of FR-49175 and Tiaramide on Systemic Anaphylaxis in Guinea-pigs

Drug	Dose (mg/kg)	n	Systemic anaphylactic shock[a] Time of death after antigen challenge (min)	Protective effect (survival/tested)
Vehicle	—	6	5.85±0.13	0/6
FR-49175	0.1	6	6.18±0.19	0/6
	1.0	6	5.38±0.22	0/6
	10.0	6	5.76±0.41	0/6
Tiaramide	10.0	6	(7.53)[b]	5/6

a) Each value represents the time of death due to systemic anaphylactic shock after antigen challenge (mean±SEM). *b*) This value expresses the time of the single death in this group. Sample or vehicle was administered 1 min before the antigen challenge *i.v.*

significantly different from the control ($p>0.05$).

PAF-Induced Vascular Permeability Increase Model

Intradermally injected PAF induced vascular permeability increase, resulting in blueing after systemic injection of Evan's blue. PAF was dissolved in 0.15 M NaCl containing 2.5 mg/ml albumin at the concentration of 1 μg/ml and 50 μl of the solution was injected into the skin of a mouse. The average area of vascular leakage induced by PAF was 77.0±4.5 mm^2 (mean±SEM, $n=5$). The albumin solution did not induce vascular leakage. FR-49175 at doses of 10, 1 mg/kg *i.v.* given 7 min before the PAF injection did not inhibit the vascular permeability increase. The average areas in treated groups were 59.8±7.1 mm^2 ($n=5$, 22.3% inh.) and 68.4±3.6 mm^2 ($n=5$, 11.2% inh.) at doses of 10 and 1 mg/kg, respectively. These values are not significantly different from the control ($p>0.05$ for both values).

Systemic Anaphylaxis Model

To examine the effects of FR-49175 and tiaramide on systemic anaphylaxis in guinea-pigs, each drug was injected *i.v.* 1 min before an antigen challenge, and the time from antigen challenge to the death of animals due to anaphylactic shock (min) and the survival ratio (survival/tested) were observed. As shown in Table II, FR-49175 did not prevent anaphylactic shock at doses of 10, 1 and 0.1 mg/kg. FR-49175 (0.1 to 10 mg/kg) did not affect the time from antigen challenge to death, or the mortality, whereas tiaramide at 10 mg/kg was apparently effective in this model (5 animals survived out of 6 animals tested).

Discussion

FR-49175 specifically inhibits PAF-induced platelet aggregation *in vitro*.[1] To examine the *in vivo* effect of FR-49175, some experimental animal models (PAF-induced bronchoconstriction, hypotension and vascular permeability increase) were studied.

In the PAF-induced bronchoconstriction model, this compound (0.1, 1 and 10 mg/kg) significantly prevented the bronchoconstriction in guinea-pigs (ID_{50}; 0.26 mg/kg). However, FR-49175 prevented neither PAF-induced hypotension in rats nor vascular permeability increase in mice, even at high doses (up to 10 mg/kg).

It is known that bronchoconstriction due to PAF is platelet-dependent, as it was suppressed by immune platelet depletion, but the hypotensive effect of PAF was not inhibited after platelet depletion.[7] Furthermore, some reports showed that PAF increased vascular permeability through a mechanism independent of extravasation of platelets or polymorphonuclear leukocytes.[8,9] Moreover, it was reported that the hypotensive effect of PAF was

mainly due to endothelium-dependent vasodilation.[10] These data suggest that PAF elicits its biological effect through at least two different mechanisms, *i.e.* a platelet-dependent mechanism which mediates bronchoconstriction, and a platelet-independent mechanism which elicits hypotension and vascular permeability increases.

The observation that FR-49175 specifically blocked the platelet-dependent action of PAF suggests that there may be different types of PAF receptor, namely platelet type and vasculature type. Although it might be difficult to compare directly the inhibitory effects of FR-49175 given at various times and to different species, it seems reasonable to conclude that FR-49175 is much more effective against the bronchoconstriction model as compared to the other two models.

Tiaramide is a non-steroidal and basic antiinflammatory drug. Anti-anaphylaxis[11] and anti-platelet[12] actions have also been reported. However, the anti-platelet effect of this drug is not PAF-specific. Whether this drug exerts its anti-anaphylactic effect through the PAF antagonistic mechanism has not been elucidated yet.

The novel biological activity of FR-49175 led us to examine its anti-anaphylactic effect on the systemic anaphylaxis model. However, it failed to interfere with anaphylactic shock. A role of PAF in IgE-mediated anaphylaxis has been reported *in vivo*[13] and *in vitro*,[14] whereas in our experiment, the animals were passively sensitized with IgG-antiserum. Whether the failure of FR-49175 to interfere with IgG-mediated anaphylactic shock depends on the type of anaphylaxy is still unclear. However, it seems unlikely that PAF plays a major role in the bronchospasm of IgG-mediated systemic anaphylaxis. The effect of FR-49175 on IgE-mediated anaphylaxis is under investigation.

Acknowledgement The authors are very grateful to Mrs. Setsuyo Kohda and Miss Hiromi Sawamoto for their assistance in the experimental work.

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