Further Studies on the Pharmacological Effect of the Anti-inflammatory Compound, Bis[2-(*E*-2-octenoylamino)ethyl] Disulfide

Tsutomu MIMURA,*^a Hiroshi NAKAJIMA,^a Kazutake TSUJIKAWA,^a Byoung Gag LEE,^a Takumi IMAI,^a Yasuhiro KOHAMA,^a Isao KOHDA^b and Kazumasa YOKOYAMA^b

Faculty of Pharmaceutical Sciences, Osaka University,^a Yamadaoka 1-6, Suita, Osaka 565, Japan and Central Research Laboratories, Green Cross Corporation, Ltd.,^b 2-1180-1, Shodai Ohtani, Hirakata, Osaka 573, Japan. Received July 27, 1988

Further studies on the pharmacological effect of orally administered bis[2-(*E*-2-octenoyl-amino)ethyl] disulfide (compd. I-3) was examined by using several experimental models *in vivo*. Compound I-3 showed analgesic activity, inhibiting both acetic acid- and acetylcholine-induced writhings in mice. This compound also showed antipyretic activity against yeast-induced fever in rats, and significantly inhibited arachidonic acid-induced mortality in mice. However, it had no effect on serotonin-induced paw edema formation or platelet activating factor-acether-induced mortality in mice. The effects of compd. I-3 are suggested to be due to inhibition of prostaglandin biosynthesis.

Keywords bis[2-(*E*-2-octenoylamino)ethyl] disulfide; acetic acid-induced writhing; acetylcholine-induced writhing; antipyretic activity; arachidonic acid-induced mortality

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of various diseases involving inflammation. Inhibition of prostaglandin (PG) synthetase has been proposed to be a common mechanism of the anti-inflammatory activity of NSAIDs.¹⁾ We have already reported that a newly synthesized compounds, bis[2-(*E*-2-octenoylamino)ethyl] disulfide (compd. I-3), showed a wide anti-inflammatory spectrum²⁾ and had cyclooxygenase inhibitory activity *in vitro*.³⁾ It is well known that inhibition of PG synthesis produces various pharmacological effects *in vivo*. In the present study, we investigated the pharmacological effects of compd. I-3 by using 6 experimental models *in vivo*.

Experimental

Materials Compound I-3 was synthesized as described previously.⁴⁾ Platelet activating factor (PAF)-acether, dexamethasone, phenidone and arachidonic acid were the products of Sigma. Acetic acid and aspirin were obtained from Nacalai Tesque Inc. Dry yeast and serotonin creatinine sulfate were from Wako Pure Chemical Ind. Ltd., and acetylcholine was from Daiichi Pure Chemical Co., Ltd.

Analgesic Activity i) Acetic Acid-Induced Writhing in Mice: According to the method of Koster *et al.*,⁵⁾ writhing syndrome was induced by an intraperitoneal injection of 10 ml/kg of 0.7% acetic acid in male ddY mice (Shizuoka Lab. Animals) weighing 20–30 g. The number of writhes was counted for 10 min, beginning from 10 min after the acetic acid injection. The test compounds were administered orally 1 h prior to the acetic acid injection and mice were fasted for 12 h before the examination.

ii) **Acetylcholine-Induced Writhing in Mice:** According to the method of Amanuma *et al.*,⁶⁾ male ddY mice weighing 20–30 g were used and the number of writhes was counted for 10 min following the intraperitoneal injection of 10 mg/kg of a 0.05% acetylcholine chloride solution. The test compounds were administered orally 1 h prior to the injection of acetylcholine. The mice showing no writhing response were regarded as positive for analgesic activity. The mice were fasted for 12 h before the examination.

Antipyretic Activity According to the method of Smith *et al.*,⁷⁾ male Wistar rats weighing 250–350 g were injected subcutaneously with 15 ml/kg of a 15% sterilized dry yeast aqueous suspension. Seventeen hours later, the test compounds were administered orally to the animals showing an increase of 2°C or more in rectal temperature. Rectal temperature was measured by a thermistor probe 1 h before and at 1 h intervals for 6 h after drug administration.

Serotonin-Induced Paw Edema Formation in Mice By the method of Ōyanagui,⁸⁾ male ICR mice weighing 25–35 g received s.c. 5 µl of serotonin solution (containing 0.3 µg of serotonin) in the right hindpaw. The equal volume of saline was injected into the left hindpaw and the

thickness of the paws was measured with a dial thickness gauge (Mitsutoyo Mfg. Co., Ltd.) at 15 min after the injection of serotonin. The difference in thickness between the right and left hindpaws was taken. Compd. I-3 was administered orally 1 h prior to the serotonin injection. Dexamethasone was injected subcutaneously 3 h before the serotonin injection. The mice were fasted for 12 h before the examination.

PAF-Acether-Induced Mortality in Mice According to the method of Young *et al.*,⁹⁾ male ICR mice weighing 25–30 g were injected with PAF-acether (150 µg/kg) through the tail vein. The mortality in each group was observed until 45 min after the PAF-acether injection. The test compounds were administered orally 1 h prior to the PAF-acether injection. The mice were fasted for 12 h before the examination.

Arachidonic Acid-Induced Mortality in Mice According to the method described by Kohler *et al.*,¹⁰⁾ male ddY mice weighing 25–30 g were injected intravenously with arachidonic acid (100 mg/kg) and the mortality of each group was observed until 2 min after the arachidonic acid injection. The test compounds were administered orally 1 h prior to the arachidonic acid injection. The mice were fasted for 12 h before the examination.

Results

Analgesic Activity i) Acetic Acid-Induced Writhing in Mice As shown in Table I, compd. I-3 significantly inhibited the writhing syndrome at doses of 25, 50 and 100 mg/kg. The inhibitory activity was dose-dependent in the dose range from 10 to 100 mg/kg. Aspirin, used as a positive control drug, inhibited the writhing syndrome by 33.3% at an oral dose of 250 mg/kg.

ii) **Acetylcholine-Induced Writhing in Mice** The inhibitory effect of compd. I-3 on the acetylcholine-induced

TABLE I. Analgesic Effect of Compd. I-3 on Acetic Acid-Induced Writhing in Mice

Treatment	Dose (mg/kg, p.o.)	No. of mice	Number of writhes	Inhibition ^{a)} (%)
Control ^{b)}	—	10	38.2 ± 2.4	—
Compd. I-3	10	10	32.7 ± 2.4	14.4
	25	10	24.9 ± 3.4 ^{c)}	34.8
	50	10	20.6 ± 3.4 ^{c)}	46.1
	100	10	13.0 ± 1.8 ^{d)}	66.0
Control ^{b)}	—	10	38.1 ± 3.0	—
Aspirin	250	10	25.4 ± 2.3 ^{c)}	33.3

a) The inhibition percent was calculated as compared with the number of writhes in the control group. b) 10% acacia in saline. Each compound was administered 1 h prior to the intraperitoneal injection (10 ml/kg) of 0.7% acetic acid solution. All values represent mean ± S.E. Significantly different from the control group: c) $p < 0.01$, d) $p < 0.001$.

TABLE II. Analgesic Effect of Compd. I-3 on Acetylcholine-Induced Writhing in Mice

Treatment	Dose (mg/kg, <i>p.o.</i>)	No. of mice	No. of mice not responded	Inhibition (%)
Control ^{a)}	—	10	0	—
Compd. I-3	1	10	1	10
	5	10	6	60 ^{b)}
	10	10	9	90 ^{c)}
Aspirin	25	10	4	40
	50	10	10	100 ^{c)}

a) 10% acacia in saline. Each compound was administered 1 h prior to the injection of 0.05% acetylcholine chloride solution. Significantly different from the control group: (x²-test) b) *p* < 0.05, c) *p* < 0.005.

Fig. 1. Effect of Compd. I-3 on Yeast-Induced Fever in Rats

○, Control; □, Compd. I-3 25 mg/kg; ●, Aspirin 100 mg/kg. Each point shows mean ± S.E. (*n* = 6). Significantly different from the control group: a) *p* < 0.05, b) *p* < 0.01, c) *p* < 0.001.

TABLE III. Effect of Compd. I-3 on Serotonin-Induced Paw Edema Formation in Mice

Treatment	Dose (mg/kg)	No. of mice	Swelling (mm)
Control ^{a)}	—	10	1.02 ± 0.04
Compd. I-3 ^{a)}	100	10	0.96 ± 0.08
Dexamethasone ^{b)}	1	10	0.43 ± 0.06 ^{c)}

a) Oral administration. b) Subcutaneous administration. All values represent mean ± S.E. Significantly different from the control group: c) *p* < 0.001.

writhing model is summarized in Table II. Inhibition was 60% at 5 mg/kg and 90% at 10 mg/kg. Inhibition with compd. I-3 was dose-dependent in the dose range of 1 to 10 mg/kg. Aspirin inhibited the writhing 40% at 25 mg/kg and 100% at 50 mg/kg.

Antipyretic Activity As shown in Fig. 1, compd. I-3 significantly inhibited the fever 3 to 6 h after oral administration. Aspirin was effective 1 to 6 h after oral administration.

Serotonin-Induced Paw Edema Formation in Mice Compound I-3 had no inhibitory effect on serotonin-induced paw edema formation at a dose of 100 mg/kg. Dexamethasone, used as a positive control, inhibited the serotonin-induced paw edema at 1 mg/kg s.c. (Table III).

PAF-Acether-Induced Mortality in Mice This model is reported to be useful for demonstrating systemic activity of

TABLE IV. Effect of Compd. I-3 on PAF-Acether-Induced Mortality in Mice

Treatment	Dose (mg/kg, <i>p.o.</i>)	No. of mice used	Dead/alive	Mortality rate (%)
Control ^{a)}	—	12	9/3	75
Compd. I-3	100	12	7/5	58
Phenidone	200	12	0/12	0 ^{b)}

a) 10% acacia in saline. Each compound was administered 1 h prior to the injection of 150 µg/kg PAF-acether. Significantly different from the control group: (x²-test) b) *p* < 0.005.

TABLE V. Effect of Compd. I-3 on Arachidonic Acid-Induced Mortality in Mice

Treatment	Dose (mg/kg, <i>p.o.</i>)	No. of mice used	Dead/alive	Mortality rate (%)
Control ^{a)}	—	10	10/0	100
Compd. I-3	5	10	9/1	90
	10	10	6/4	60
	25	10	5/5	50 ^{b)}
	50	10	2/8	20 ^{c)}
Aspirin	100	10	1/9	10 ^{c)}

a) 10% acacia in saline. Each compound was administered 1 h prior to the injection of arachidonic acid (100 mg/kg, i.v.). Significantly different from the control group: (x²-test) b) *p* < 0.05, c) *p* < 0.005.

novel lipoxigenase inhibitors, leukotriene antagonists and other anti-anaphylactic compounds.⁹⁾ As shown in Table IV, compd. I-3 was ineffective at 100 mg/kg. The lipoxigenase inhibitor, phenidone, completely prevented the mortality at 200 mg/kg.

Arachidonic Acid-Induced Mortality in Mice The intravenous administration of arachidonic acid to mice offers a simple, convenient and useful model for the study of platelet aggregation and thrombosis *in vivo*.¹⁰⁾ Compound I-3 was significantly effective in protecting mice from death at 25 and 50 mg/kg. A dose-response relationship was seen at 5 to 50 mg/kg. Aspirin was also effective at 100 mg/kg (Table V).

Discussion

It has been reported that both acetic acid- and acetylcholine-induced writhings are related to stimulation of PGs biosynthesis.^{6,11)} Compound I-3 inhibited both writhings and was more potent in inhibiting acetylcholine-induced writhing than acetic acid-induced writhing. The report of Amanuma *et al.*⁶⁾ showed that acetylcholine-induced writhing is more closely correlated with PGs biosynthesis than acetic acid-induced writhing.

It is thought that when the leucocytic pyrogen (LP) enters the central nervous system, the LP produces fever through the production and release of PGs within the hypothalamus.¹²⁾ PGE₁ and PGE₂ are considered to be potent chemical mediators for the production of fever and regulation of body temperature.¹³⁾ Compound I-3 showed an antipyretic activity in the yeast-induced fever model.

The arachidonic acid-induced mortality test is used to study platelet aggregation *in vivo*.¹⁰⁾ When platelets are stimulated by aggregating agents, arachidonic acid is liberated from the membrane phospholipids and converted by

cyclooxygenase to PG endoperoxides (PGG₂ and PGH₂), which are further converted by thromboxane (TX) synthetase to TXA₂ which plays an important role in mediating the platelet aggregation and release reaction.¹⁴⁾ Intravenously administered arachidonic acid is also converted to TXA₂.¹⁰⁾ This converted TXA₂ mediates the platelet aggregation and release reaction. Compound I-3 showed an inhibitory effect in this model.

Thus, it is assumed that compd. I-3 showed antipyretic, analgesic and platelet anti-aggregant activities by inhibiting PG biosynthesis *in vivo*.

This compound did not influence serotonin-induced paw edema formation or PAF-acether-induced mortality. It has been reported that the former model is due to the generation of superoxide radicals¹⁵⁾ and the latter is related to the production of leukotrienes.¹⁶⁾

These results indicated that the pharmacological characteristics of compd. I-3 resemble those of most NSAIDs, because they have antipyretic, analgesic,¹⁷⁾ anti-inflammatory¹⁸⁾ and platelet anti-aggregant¹⁹⁾ activities by inhibiting PG biosynthesis, but have no effect on serotonin-induced paw edema formation⁸⁾ or PAF-acether-induced mortality.⁹⁾

However, compd. I-3 is completely different in chemical structure from NSAIDs. Compound I-3 has a long straight chain, while most NSAIDs have an aromatic ring. The inhibitory effect on cyclooxygenase activity may be rationalized in terms of the structure of compd. I-3, which can presumably adopt a conformation like arachidonic acid because of the long straight chain.

Damage to the gastric mucosa is the most important problem in the use of NSAIDs.²⁰⁾ The reason for this is that NSAIDs inhibit PGE₂ and PGI₂ generation that seem to play an important role in maintaining the integrity of the gastric mucosa.²¹⁾ Compound I-3 caused no gastric damage, unlike NSAIDs.⁴⁾ This might be explained as follows. 1) Compound I-3 is very lipophilic. Most lipophilic compounds are absorbed at the small intestine but not at the stomach.²²⁾ 2) Cystamine used to synthesize compd. I-3 is reported to have a protective effect on the gastric mucosa.²³⁾ 3) There is evidence to show that inhibition of PG biosynthesis by NSAIDs varies between tissues.²⁴⁾ Flufenamic acid and azapropazone reduce PG levels in pig plasma without affecting levels in the gastric mucosa.²⁵⁾ Compound I-3 might have an effect like flufenamic acid. Moreover, the low toxicity of this compound²⁾ might be ascribed to its component materials (cystamine and free fatty acid). Both cystamine and free fatty acid can be metabolized, and compd. I-3 may be metabolized quite easily.

It is noteworthy that compd. I-3 had similar pharmacological characteristics to NSAIDs, but it had minimal side effects,^{2,4)} differing from NSAIDs.

Acknowledgement We are grateful to Ms. Ikuko Izumi and Ms. Madoka Murata for their technical assistance.

References

- 1) J. R. Vane, *Nature New Biology* (London), **231**, 232 (1971).
- 2) T. Mimura, H. Nakajima, Y. Kohama, K. Tsujikawa, S. Itoh, T. Ohmura, M. Iwai and K. Yokoyama, *Chem. Pharm. Bull.*, **35**, 4579 (1987); T. Mimura, H. Nakajima, Y. Kohama, M. Okabe, K. Tsujikawa, S. Itoh, T. Ohmura, M. Iwai and K. Yokoyama, *ibid.*, **35**, 4585 (1987).
- 3) T. Mimura, H. Nakajima, K. Tsujikawa, N. Yasuda, M. Haruyama, M. Okabe, M. Iwai and K. Yokoyama, *Chem. Pharm. Bull.*, accepted.
- 4) M. Iwai, I. Kohda, C. Fukaya, Y. Naito, K. Yokoyama, N. Nakajima, K. Tsujikawa, M. Okabe and T. Mimura, *Chem. Pharm. Bull.*, **35**, 4616 (1987).
- 5) R. Koster, M. Anderson and E. J. De Beer, *Fed. Proc.*, **18**, 412 (1958).
- 6) F. Amanuma, C. Wakaumi, M. Tanaka, M. Muramatsu and H. Aihara, *Nippon Yakurigaku Zasshi*, **84**, 543 (1984).
- 7) A. E. W. Smith, E. Frommel and S. Radouco-Thomas, *Arzneim.-Forsch.*, **13**, 338 (1963).
- 8) Y. Oyanagui, *Biochem. Pharmacol.*, **30**, 1791 (1981).
- 9) J. M. Young, P. J. Maloney, S. N. Jubb and J. S. Clark, *Prostaglandins*, **30**, 545 (1985).
- 10) C. Kohler, W. Wooding and L. Ellenbogen, *Thromb. Res.*, **9**, 67 (1976).
- 11) R. Deraedt, S. Jouquey, F. Delevallee and M. Flahaut, *Eur. J. Pharmacol.*, **61**, 17 (1980).
- 12) H. Nakamura, Y. Yokoyama, Y. Seto, T. Kadokawa and M. Shimizu, *Agents Actions*, **15**, 606 (1984).
- 13) W. G. Clark and J. M. Lipton, *Neurosci. Biobehav. Rev.*, **9**, 479 (1985).
- 14) A. J. Marcus, *J. Lipid Res.*, **19**, 793 (1978).
- 15) Y. Oyanagui and S. Suzuki, *Agents Actions*, **17**, 270 (1985).
- 16) B. Samuelsson, *Science*, **220**, 568 (1983).
- 17) R. J. Flower and J. R. Vane, "Prostaglandin Synthetase Inhibitors," ed. by H. J. Robinson and J. R. Vane, Raven Press, New York, 1974, pp. 9—18.
- 18) R. J. Gryglewski, "Anti-inflammatory Drugs," ed. by J. R. Vane and S. H. Ferreira, Springer-Verlag, Berlin, Heidelberg, New York, 1979, pp. 3—43.
- 19) R. L. Vigdahl, R. H. Ferber and S. L. Parrish, *Thromb. Res.*, **16**, 117 (1979).
- 20) H. W. Davenport, *Gastroenterology*, **46**, 245 (1964).
- 21) A. Robert, J. E. Nezamis, C. Lancaster and A. J. Hanchar, *Gastroenterology*, **77**, 433 (1979).
- 22) T. Murata and T. Arita, "Seibutsuyakuzaigaku," 2nd ed., Nankoudou, Ltd., Tokyo, 1982, pp. 18—21.
- 23) S. Szabo, J. S. Trier and P. W. Frankel, *Science*, **214**, 200 (1981).
- 24) A. Dembinska-Kiec, A. Zmuda and J. Krupinska, *Adv. Prost. Thromb. Res.*, **1**, 9 (1976).
- 25) K. D. Rainsford, B. M. Peskar and K. Brune, *J. Pharm. Pharmacol.*, **33**, 127 (1981).