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

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Further studies on the pharmacological effect of orally administered bis[2-(E-2-octenoylamino)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 writhing 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-aceteh-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.13 We have already reported that a newly synthesized compounds, bis[2-(E-2-octenoylamino)ethyl] disulfide (compd. I-3), showed a wide anti-inflammatory spectrum24 and had cyclooxygenase inhibitory activity in vitro.25 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.41 Platelet activating factor (PAF)-aceteh, dexamethasone, phenidone and arachidonic acid were the products of Sigma. Acetic acid and aspirin were obtained from Nacalai Tesque Inc. Dry yeast and serotonin creatine sulfate were from Wako Pure Chemical Ind. Ltd., and acetylcholine was from Daiichi Pure Chemical Co., Ltd.

Analytic Activity

i) Acetic Acid-Induced Writhing in Mice: According to the method of Koster et al.,34 writing syndrome was induced by an intraperitoneal injection of 10 ml/kg of 0.57% 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.,45 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.,67 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 Öyanagui,66 male ICR mice weighing 25–35 g received s.c. 5 μl of serotonin solution (containing 0.3 μg of serotonin) in the left 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-Aceteh-Induced Mortality in Mice

According to the method of Young et al.,21 male ICR mice weighing 25–30 g were injected with PAF-aceteh (150 μg/kg) through the tail vein. The mortality in each group was observed until 45 min after the PAF-aceteh injection. The test compounds were administered orally 1 h prior to the PAF-aceteh 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

Analytic 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 writhing was evaluated by the method of Smith et al.67 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.

Table I. Analytic Effect of Compd. I-3 on Acetic Acid-Induced Writhing in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>No. of mice</th>
<th>Number of writhes (% of control)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>10</td>
<td>38.2±2.4</td>
<td>---</td>
</tr>
<tr>
<td>Compd. 1-3</td>
<td>10</td>
<td>10</td>
<td>32.7±2.4</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>10</td>
<td>24.9±3.4</td>
<td>34.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>10</td>
<td>20.6±3.4</td>
<td>46.1</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>13.0±1.8</td>
<td>66.0</td>
</tr>
<tr>
<td>Aspirin</td>
<td>---</td>
<td>10</td>
<td>38.1±3.0</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>10</td>
<td>25.4±2.3</td>
<td>33.3</td>
</tr>
</tbody>
</table>

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.

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Table II. Analgesic Effect of Compd. 1-3 on Acetylcholine-Induced Writhing in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>No. of mice</th>
<th>No. of mice not responded</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Compd. 1-3</td>
<td>10</td>
<td>10</td>
<td>6</td>
<td>60(a)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>100(a)</td>
</tr>
</tbody>
</table>

\(a\) 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: \(a\) \(p\leq 0.05\), \(b\) \(p<0.001\).

Table IV. Effect of Compd. 1-3 on PAF-Acether-Induced Mortality in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>No. of mice used</th>
<th>Dead/alive</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(a)</td>
<td>10</td>
<td>12</td>
<td>9/3</td>
<td>75</td>
</tr>
<tr>
<td>Compd. 1-3</td>
<td>100</td>
<td>12</td>
<td>7/5</td>
<td>58</td>
</tr>
<tr>
<td>Phenindone</td>
<td>200</td>
<td>12</td>
<td>0/12</td>
<td>0(b)</td>
</tr>
</tbody>
</table>

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

Table V. Effect of Compd. 1-3 on Arachidonic Acid-Induced Mortality in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, p.o.)</th>
<th>No. of mice used</th>
<th>Dead/alive</th>
<th>Mortality rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(a)</td>
<td>10</td>
<td>10</td>
<td>10/0</td>
<td>100</td>
</tr>
<tr>
<td>Compd. 1-3</td>
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<td>10</td>
<td>9/1</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>6/4</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>10</td>
<td>5/5</td>
<td>50(b)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>50</td>
<td>10</td>
<td>2/8</td>
<td>20(b)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>10</td>
<td>1/9</td>
<td>10(b)</td>
</tr>
</tbody>
</table>

\(a\) 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: \(a\) \(t\)-test \(b\) \(p<0.005\).

Discussion

It has been reported that both acetic acid- and acetylcholine-induced writhings are related to stimulation of PGs biosynthesis.\(^5,11\) Compound 1-3 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 leucocytes pyrogen (LP) enters the central nervous system, the LP produces fever through the production and release of PGs within the hypothalamus.\(^12\) PGE\(_1\) and PGE\(_2\) are considered to be potent chemical mediators for the production of fever and regulation of body temperature.\(^13\) Compound 1-3 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 novel lipooxygenase inhibitors, leukotriene antagonists and other anti-anaphylactic compounds.\(^9\) As shown in Table IV, compd. 1-3 was ineffective at 100 mg/kg. The lipooxygenase inhibitor, phenindone, 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 1-3 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).

The writhing model is summarized in Table II. Inhibition was 60% at 5 mg/kg and 90% at 10 mg/kg. Inhibition with compd. 1-3 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. 1-3 significantly inhibited the fever from 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 1-3 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 novel lipooxygenase inhibitors, leukotriene antagonists and other anti-anaphylactic compounds.\(^9\) As shown in Table IV, compd. 1-3 was ineffective at 100 mg/kg. The lipooxygenase inhibitor, phenindone, completely prevented the mortality at 200 mg/kg.
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-aggregate 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 radicals¹¹ 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-inflammatory,¹⁸ and platelet anti-aggregate activities by inhibiting PG biosynthesis, but have no effect on serotonin-induced paw edema formation⁹ 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.⁵⁰ The reason for this is that NSAIDs inhibit PG₂ and PG₁ generation that seem to play an important role in maintaining the integrity of the gastric mucosa.¹¹ Compound I-3 caused no gastric damage, unlike NSAIDs.⁹ 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 compound²² 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,²⁴ differing from NSAIDs.

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References