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Glycyrrhetinic acid derivatives: anti-nociceptive activity of deoxoglycyrrhetol dihemiphthalate and the related compounds

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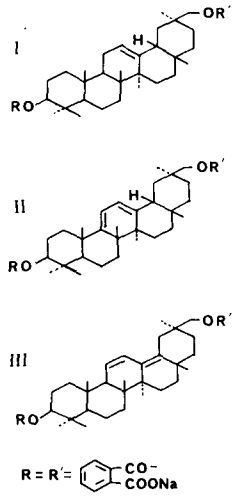
Abstract—The possible inhibitory effect of deoxoglycyrrhetol dihemiphthalate (I) and the related compounds (18β-olean-9(11),12-diene-3β,30-diol) (II) and (olean-11,13(18)-diene-3β,30-diol) III derived from glycyrrhetinic acid has been examined on acetic acid-induced writhing in mice. The compounds inhibited writhing dose-dependently. Their ED50 values were 14, 31 and 22 mg kg^{-1} for I, II, and III, respectively. The compounds like aspirin, also significantly suppressed PGE_2 production in peritoneal fluid together with the writhing response. The results suggests that the analgesic effect of deoxoglycyrrhetol dihemiphthalate and the related compounds is partially due to inhibition of PGE_2 production.

Glycyrrhetinic acid is the aglycone of glycyrrhizin, a pharmacologically active saponin of liquorice (Glycyrrhiza spp.) root. Glycyrrhetinic acid has also been found to be anti-inflammatory (Finney & Somers 1958; Capasso et al 1983), to antagonize tumour promotion (Nishino et al 1986) and to inhibit the growth of mouse melanoma (Abe et al 1987).



Deoxoglycyrrhetol (18β-olean-12-ene-3β, 30-diol) dihemiphthalate (I) and the related compounds (18β-olean-9(11),12-diene-3β,30-diol) (II) and (olean-11,13(18)-diene-3β,30-diol) (III) derived from glycyrrhetinic acid (Shibata et al 1987) were shown to inhibit lipoxygenase and cyclo-oxygenase activities in a cell-free system using mastocytoma cells (Inoue et al 1986) and mouse ear oedema induced by arachidonic acid (Inoue et al 1988) and tetradecanoyl phorbol acetate (Inoue et al 1989). In addition, these compounds were previously reported to strongly suppress the writhing response and vascular permeability induced by acetic acid, whose mechanism, however, has not so far been elucidated (Inoue et al 1987).

The present paper concerns a mode of action of deoxoglycyrrhetol dihemiphthalate and the related compounds on the analgesic effect.

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Materials and methods

Assay for acetic acid-induced writhing response. Male ddY mice, 6 weeks old (Shizuoka Laboratory Animal Center, Japan), were acclimatized under the standard conditions for one week before use, with free access to food and water. Dihemiphthalate compounds (I-III) were prepared according to Shibata et al (1987), and aspirin (Nakarai Chemical Co., Japan) was used as a reference. Test compounds were dissolved in 0·9% NaCl (saline) containing 1% Tween 80 (polyoxyethylene sorbitan monooleate, Tokyo Kasei Chemical Industry, Japan) and given orally 45 min before intraperitoneal injection (10 mL kg^{-1}) of 0·7% acetic acid. Control mice received vehicle only. The number of writhings of each mouse was counted during the first 30 min after injection of acetic acid. Statistical significance of the differences between groups was determined using the unpaired Student's *t*-test.

Assay of PGE_2 production in peritoneal fluid. The mice used in the writhing test were immediately killed 20 min after irritant treatment and injected with 5 mL saline intraperitoneally. The fluid collected from the peritoneal cavity was then centrifuged at 4500 *g* for 15 min at 4°C. Measurement of PGE_2 in the supernatant was based on the method of Kawano et al (1987), the PGE_2 was absorbed by ODS resin, eluted with ethyl acetate, and measured by radioimmunoassay (New England Nuclear, USA). The recovery was 98%. The cross-reactivity of anti-PGE_2 serum was as follows: 100% for PGE_2, 3·7% for PGE_1, 0·4% for PGA_2, 0·03% for PGF_1, 0·02% for TXB_2.

Results and discussion

Following the previous study (Inoue et al 1987), the present experiment showed that deoxoglycyrrhetol dihemiphthalate (I) and compounds (II and III) dose dependently inhibited the writhing response induced by 0·7% acetic acid (10 mL kg^{-1} i.p.) (Fig. 1). The ED50 values were 14, 31 and 22 mg kg^{-1} p.o. for I, II and III, respectively. Glycyrrhetinic acid, the parent compound had little effect at less than 200 mg p.o.

The effect of compounds I and II on acetic acid-induced PGE_2 production in peritoneal fluid was further examined with radioimmunoassay. As shown in Table 1, these compounds significantly inhibited PGE_2 production ($P < 0.05$) at a dose of 25 mg kg^{-1} compared with the control PGE_2 values (vehicle only

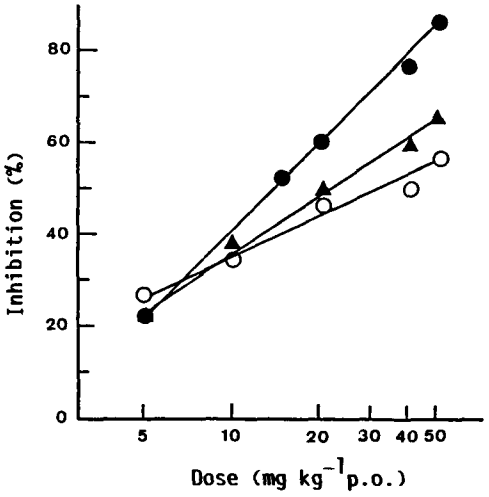

FIG. 1. Dose dependent inhibition of deoxoglycyrrhetol dihemiphthalate and related compounds. Each point represents the percent of control (n = 8). ●: compound I, ○: compound II, ▲: compound III.

Table 1. Inhibition of acetic acid-induced writhing and PGE_2 production by deoxoglycyrrhetol dihemiphthalate and related compounds.

Compound	Dose (mg kg^{-1})	No. of writhings/30 min	PGE_2 content (pg mL^{-1})
Control†		30·5 ± 4·2	200·8 ± 35·3
I	25	9·1 ± 3·4***	91·6 ± 11·8*
II	25	8·8 ± 1·1***	79·0 ± 7·3*
Aspirin	100	9·0 ± 3·0***	6·4 ± 0·8***

PGE_2 was extracted from peritoneal fluid 20 min after irritant treatment. Values are expressed as mean ± s.e. of 7–8 animals.
† Irritant treatment only. *$P < 0.05$, ***$P < 0.001$.

70·5 ± 15·6 pg mL^{-1}). This inhibition could explain why compounds I-III reduce peritoneal vascular permeability (Inoue et al 1987). Aspirin almost completely prevented PGE_2 production ($P < 0.01$) at 100 mg kg^{-1}, while its inhibitory effect on the writhing response was similar to that of compounds I and II. Statistically significant differences between aspirin and the two compounds on PGE_2 inhibition were observed ($P < 0.001$). This suggests that acetic acid-induced writhing is not only mediated by PGs but could also be related to other chemical mediators such as histamine and bradykinin. (Increase of leukotriene C_4 production was confirmed in the peritoneal fluid of mice injected with acetic acid (data not shown.)

Aspirin-like drugs show analgesic effects by suppressing the production of an endogenous pain substance at the site of noxious stimulation (Lim et al 1964). Vane (1971) stated that aspirin-like agents are effective in inhibiting PGE_2 production at the site of inflammation. In the writhing induced after intraperitoneal injection of acetic acid, PGE_1 and PGE_2 were found to be severe irritants (Colliner & Schneider 1972). Deraedt et al (1976) reported that the amount of PGE_2 in the peritoneal fluid increased dramatically on peritoneal injection of acetic acid to rats, and that PG synthetase inhibitors suppressed both the writhing and PG production induced by acetic acid. The effect of agents inhibiting the writhing response is related to the suppression of PG production (Deraedt et al 1980). In contrast, narcotic analgesics, such as morphine, inhibited the writhing response, but had no effect on PG production (Deraedt et al 1980) or vascular permeability (Whittle 1964). The compounds I, II and III inhibited 5-lipoxygenase and cyclo-oxygenase activity of cloned mastocytoma cells at 10^{-5} and $^{-4}$ M (Inoue et al 1986) and suppressed arachidonic acid-induced oedema mediated by leukotrienes and PGE_2 (Inoue et al 1988).

Therefore, the evidence suggests that the inhibitory effect of compounds I, II and III on the writhing response is induced by inhibition of PGE_2 production and not by an action on the central nervous system as with narcotic analgesics.

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