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Pharmacological Activities of Glycyrrhetic Acid Derivatives: Analgesic and Anti-Type IV Allergic Effects

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Glycyrrhetic acid (Ia) and fourteen derivatives of glycyrrhetic acid were examined to estimate the antinociceptive activity (tests on writhing and vascular permeability induced by 0.7% acetic acid) and anti-type IV allergic effects in mice. Among them, three dihemipthalate compounds (II_f, III_c, IV_c) derived from, deoxoglycyrrhetol (II_a), showed strong inhibition of writhing and dye leakage at a dose of 6.25 mg/kg (*p.o.*) and type IV allergy at 25 mg/kg (*i.p.*). Among the hemisuccinate compounds, Ib, II_b and III_b, had significant inhibitory effects on both writhing and vascular permeability, whereas II_c and IV_b inhibited only one of the two responses, at 200 mg/kg (*p.o.*). Four of the hemisuccinate compounds (II_b, c, III_b, IV_b) suppressed type IV allergy at 100 mg/kg (*i.p.*). Glycyrrhetic acid (Ia) and deoxoglycyrrhetol (II_a) showed no detectable inhibition in the screening tests at doses of less than 200 mg/kg (*i.p.*).

Keywords—glycyrrhetic acid; antinociceptive activity; type IV allergy; dihemipthalate compound; hemisuccinate compound; deoxoglycyrrhetol; oleanane skeleton

Introduction

Glycyrrhetic acid (Ia), the aglycone of glycyrrhizin, which is obtained from the root of licorice (*Glycyrrhiza* spp.), is an effective anti-inflammatory agent.¹⁾ Nishino *et al.*²⁾ found an inhibitory effect of glycyrrhetic acid on tumor-promoting activity. Glycyrrhetic acid has recently been shown to be an inhibitor of intercellular junctional communication.³⁾ Carbenoxolone sodium (Ib) prepared from glycyrrhetic acid, is clinically employed in the therapy of gastric ulcer.⁴⁾ A glycyrrhetic acid derivative, the sodium salt of glycyrrhetic acid hemipthalate (Ic), was demonstrated to have an anti-inflammatory effect.⁵⁾ It has also been reported that deoxoglycyrrhetol (II_a) inhibits experimental gastric ulcer and allergy.⁶⁾ Furthermore, three dihemipthalate compounds (the disodium salts of 18 β -olean-12-ene-3 β ,30-diol 3 β ,30-di-*O*-hemipthalate (II_f), 18 β -olean-9(11),12-diene-3 β ,30-diol 3 β ,30-di-*O*-hemipthalate (III_c), and olean-11,13(18)-diene-3 β ,30-diol 3 β ,30-di-*O*-hemipthalate (IV_c)) have been shown to prevent gastric ulcer.⁷⁾ Previously we reported that among fifteen derivatives of glycyrrhetic acid so far tested, the dihemipthalate compounds (II_f, III_c and IV_c) showed strong inhibitory effects on the 5- and 12-lipoxygenase and cyclooxygenase activities in cloned mastocytoma.⁸⁾

The present paper describes a further pharmacological study on these compounds in order to examine whether some of them are effective in the writhing and type IV allergy tests.

Data Analysis—Student's *t*-test was used to determine statistical significance.

Results and Discussion

Effects of Glycyrrhetic Acid Derivatives on Writhing and Vascular Permeability

The effects of the compounds tested on the acetic acid-induced writhing and vascular permeability are summarized in Table I. In the control groups, the number of writhes was 26.7 ± 1.4 (S.E.) while the OD value of peritoneal dye-leakage was 0.403 ± 0.03 (S.E.) under our experimental conditions. Some of the test compounds inhibited both writhing and vascular permeability. Three hemiphthalate compounds [the disodium salts of 18 β -olean-12-ene-3 β ,30-diol 3 β ,30-di-*O*-hemiphthalate (II f), 18 β -olean-9(11), 12-diene-3 β ,30-diol 3 β ,30-di-*O*-hemiphthalate (III c) and olean-11,13(18)-diene-3 β ,30-diol 3 β ,30-di-*O*-hemiphthalate (IV c)] exhibited the strongest inhibition of writhing and vascular permeability, and showed significant effects at a dose of 6.25 mg/kg (*p.o.*). The hemisuccinate compounds (II b , c and

TABLE I. Inhibition of Acetic Acid-Induced Writhing and Vascular Permeability by Glycyrrhetic Acid Derivatives

Compound	Dose (mg/kg) (<i>p.o.</i>)	Writhing	Dye-leakage
Ia	200	13	13
Ib	100	3	28
	200	58 ^{a)}	43 ^{b)}
Ic	100	15	14
	200	18	0
IIa	100	21	11
	200	24	22
IIb	100	0	7
	200	66 ^{a)}	32 ^{b)}
IIc	100	7	23
	200	45 ^{b)}	26
IId	100	0	28
	200	28	29 ^{b)}
Ile	100	18	18
	200	36	38 ^{b)}
II f	3.13	20	19
	6.25	32 ^{b)}	34 ^{b)}
	12.5	29 ^{b)}	58 ^{a)}
	25	43 ^{b)}	55 ^{a)}
IIIa	200	13	11
IIIb	100	22	25
	200	40 ^{b)}	37 ^{b)}
IIIc	3.13	21	9
	6.25	45 ^{b)}	50 ^{b)}
	12.5	37 ^{b)}	39 ^{b)}
	25	64 ^{b)}	55 ^{a)}
IVa	200	29	19
IVb	100	6	0
	200	35	39 ^{b)}
IVc	3.13	22	17
	6.25	46 ^{b)}	59 ^{b)}
	12.5	71 ^{a)}	48 ^{a)}
	25	63 ^{a)}	49 ^{a)}
Phthalic acid	200	16	0
Aminopyrine	200	100 ^{c)}	70 ^{a)}

Values of inhibition are expressed as percent of the control ($n=7-8$). *a)* $p < 0.01$; *b)* $p < 0.05$; *c)* $p < 0.001$.

IIIb) including carbenoxolone sodium (Ib), which have a sodium succinate group at position 3 or 30 in the oleanane skeleton, showed activity at 200 mg/kg (*p.o.*), but a dose of less than 100 mg/kg (*p.o.*) exhibited no apparent inhibition. In contrast, glycyrrhetic acid (Ia), deoxyglycyrrhetol (IIa), 18 β -olean-9(11), 12-diene-(IIIa) and 18 β -olean-11,13(18)-diene-3 β ,30-diol (IVa), the skeletal compounds of the above derivatives, were found to have weak inhibitory effects on the writhing response. The compounds having sodium hemiphthalate groups at the 3- and 30- positions of rings A and E in the oleanane skeleton (IIc, IIIc and IVc) showed potent inhibition. The monosodium salt of 18 β -olean-12-ene-3 β ,30-diol 3 β -*O*-hemiphthalate (Ile) showed a slight effect, but it was not statistically significant. Compound Ic was less effective in inhibiting writhing than glycyrrhetic acid (Ia). In addition, we confirmed that phthalic acid itself did not affect writhing or vascular permeability induced by acetic acid. These results suggest that dihemiphthalate substitution on the oleanane skeleton is required for a potent inhibitory effect on writhing and vascular permeability.

Effect of Glycyrrhetic Acid Derivatives on Type IV Allergy

A primary screening of glycyrrhetic acid derivatives was performed for inhibitory effect on PC-induced type IV allergy (Table II). The compounds tested were given intraperitoneally immediately after the second challenge. Each value was expressed as inhibition (percent) of ear swelling at 9 h after challenge. Some compounds (Ib, c, Ile, f, IIIc and IVc) were administered at less than 50 mg/kg since their LD₅₀ (i.p.) values were 60 to 150 mg/kg. As shown in Table II, three dihemiphthalates (IIc, IIIc and IVc) and four hemisuccinates (Ib, c, IIIb and IVb), showed significant inhibition of type IV allergy at 25 to 100 mg/kg. Another hemisuccinate compound, carbenoxolone sodium (Ib), was also found to have inhibitory activity, but Ia, IIa, IIIa and IVa did not have significant effects. The inhibitory effects of the seven derivatives (Ib, c, f, IIIb, c, IVb, c) were further investigated (Table III). These compounds showed potent inhibitory activity, suppressing about 60% of ear swelling when

TABLE II. Inhibition of PC-Induced Contact Dermatitis by Glycyrrhetic Acid Derivatives

Compound	Dose (mg/kg)	Inhibition (%)	Compound	Dose (mg/kg)	Inhibition (%)
Ia	100	15	IIIa	200	6
	200	35	IIIb	50	18
Ib	25	14		100	32 ^{a)}
	50	22 ^{a)}		200	43 ^{b)}
Ic	50	0	IIIc	12.5	4
IIa	100	15		25	42 ^{a)}
	200	18	IVa	100	21
IIb	50	0		200	0
	100	23 ^{a)}	IVb	50	0
	200	33 ^{b)}		100	44 ^{a)}
IIc	50	0		200	43 ^{b)}
	100	55 ^{b)}	IVc	12.5	20
	200	76 ^{b)}		25	45 ^{b)}
IId	100	17	Prednisolone	5	42 ^{b)}
	200	52 ^{b)}		10	77 ^{c)}
Ile	25	16			
IIIf	12.5	13			
	25	19 ^{a)}			

Values of inhibition are expressed as percent of the control at 9 h after challenge (*n* = 7–8). Compounds were given i.p. immediately after challenge. a) *p* < 0.05; b) *p* < 0.01; c) *p* < 0.001.

TABLE III. Inhibition of PC-Induced Contact Dermatitis by Seven Derivatives of Glycyrrhetic Acid

Compound	Dose (mg/kg)	Inhibition (%) after challenge	
		9 h	24 h
IIb	100	33	36
	200	57 ^{a)}	54 ^{b)}
IIc	50	16	34
	100	58 ^{a)}	69 ^{a)}
	200	53	85 ^{b)}
IIf	12.5	23	22
	25	59 ^{b)}	66 ^{b)}
IIIb	100	44	35
	200	58 ^{b)}	70 ^{b)}
IIIc	12.5	41	28
	25	58 ^{b)}	61 ^{b)}
IVb	50	39	25
	100	61 ^{b)}	64 ^{b)}
	200	58 ^{b)}	74 ^{b)}
IVc	12.5	41	23
	25	64 ^{b)}	70 ^{b)}
Prednisolone	5	60 ^{b)}	46 ^{b)}

Values of inhibition are expressed as percent of the control ($n=7-8$). Compounds were given i.p. at 3 and 9 h after challenge. *a)* $p < 0.05$; *b)* $p < 0.01$.

Fig. 1. Inhibitory Effect of Dihemiphthalate Compounds on Type IV Allergy

Each value is the mean $\pm$ S.E. for 8 animals. Test compounds were given i.p. at 3 and 9 h after challenge. The assay was carried out as described in Materials and Methods.

(●), saline; (○), prednisolone 5 mg/kg; (△), compound IIb 25 mg/kg; (▲), compound IIIc 25 mg/kg; (◻), compound IVb 25 mg/kg; (◼), compound IVc 25 mg/kg. *a)* $p < 0.05$; *b)* $p < 0.01$.

administered twice at 3 and 9 h after challenged. In particular, the inhibitory effects at 24 h after challenge were more potent than those of prednisolone (5 mg/kg). The dihemiphthalate compounds (IIb, IIIc and IVc) were found to inhibit the ear swelling significantly at 6 h after challenge (Fig. 1). It is a characteristic of those compounds that the time-course of the ear swelling treated with them was similar to that in the case of prednisolone. However, we do not yet have enough information to discuss the mechanism of inhibitory effect on PC-induced type IV allergy.

We confirmed that some of the glycyrrhetic acid derivatives inhibited writhing and/or

peritoneal dye-leakage, as well as type IV allergy. In particular, the dihemipthalate compounds (IIc, IIId and IVc) strongly inhibited both writhing and peritoneal leakage, suggesting that these compounds have anti-inflammatory and antinociceptive activity. Previously we found that the dihemipthalate compounds (IIc, IIId and IVc) strongly inhibit lipoxygenase and cyclo-oxygenase activities,⁸⁾ and prevent the formation of gastric ulcer.⁷⁾ Therefore, these compounds are candidates as drugs to treat inflammation and allergic disease without unfavorable gastric side effects.⁷⁾

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