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INHIBITION OF HISTIDINE DECARBOXYLASE AND TUMOUR PROMOTER-INDUCED ARACHIDONIC ACID RELEASE BY LECANORIC ACID ANALOGUES

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Summary : Lecanoric acid analogues containing benzanilide structure inhibited histidine decarboxylase and arachidonic acid release from the cell membrane phospholipids induced by a tumour promoter, 12-O-tetradecanoylphorbol-13-acetate. But they did not inhibit cellular binding of phorbol-12,13-dibutylate. Lecanoric acid analogues also inhibited prostaglandin synthetase and delayed-type hypersensitivity responses against sheep red blood cells in mice. Thus, lecanoric acid analogues antagonized several enzymic and cellular effects of the tumour promoter.

Lecanoric acid was first isolated from lichen in 1913 (1). Sixty years later it was again isolated from the culture filtrate of a fungus, *Pyricularia* as a potent inhibitor of histidine decarboxylase (2). It inhibited rat embryo histidine decarboxylase competitively at 10^{-6}-10^{-5} M. However, it was not expected to have any effect *in vivo*, because it is rapidly metabolized to orsenillic acid and orcinol when given intraperitoneally, subcutaneously or orally to mice (2). Therefore, to obtain compounds with activity *in vivo* we tested 56 synthetic lecanoric acid analogues with a peptide bond in place of an ester bond.

12-O-Tetradecanoylphorbol-13-acetate (TPA), a skin tumour promoter, was shown to induce histidine decarboxylase activity in

Abbreviations: PDBu, phorbol-12,13-dibutylate; SRBC, sheep red blood cells; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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mouse skin (3). TPA is also known to induce arachidonic acid release and prostaglandin synthesis (4) and to have a synergistic effect with mitogens in lymphocyte activation (5).

We studied the effect of lecanoric acid analogues on histidine decarboxylase, TPA-induced arachidonic acid release, prostaglandin synthetase and delayed-type hypersensitivity in mice.

Materials and Methods:

Materials:

Lecanoric acid was isolated from a strain of *Pyricularia* (2). Fifty-six lecanoric acid analogues were kindly synthesized and supplied by the Central Research Laboratory of Sanraku-Ocean Co., Ltd. TPA was purchased from Consolidated Midland Corporation. [5,6,8,9,11,12,14,15-^{3}H]-Arachidonic acid (78.2 Ci/mmol) and [ring 2-^{14}C]-L-histidine (54.3 mCi/mmol) were purchased from New England Nuclear and Commissariat à l'Energie Atomique, respectively. Female ddY mice were purchased from Funabashi Farm Co., Ltd.

Methods:

Histidine decarboxylase activity was assayed as described before (2). A crude histidine decarboxylase was prepared from rat embryos by the method of Häkanson (6). The reaction mixture (1.0 ml) consisted of 0.06 M potassium phosphate buffer, pH 6.8, 3.7×10^{-5}M pyridoxal phosphate, 2.4×10^{-4}M (0.05 µCi) ^{14}C-L-histidine, the test chemical and 1 mg protein of the enzyme preparation. The reaction was carried out at 37°C for 2 hrs and stopped by heating the tube at 100°C for 5 min. The histamine formed was separated on a column of Amberlite CG-50 (0.5 ml) and its radioactivity was measured.

Arachidonic acid release was assayed as described by Mufson *et al.* (7). About 10^{5} C3H10T1/2 cells were incubated with 1 ml of ^{3}H-arachidonic acid (2 µCi/ml) in a 35 mm dish for 24 hrs. The cells were then washed with cold phosphate-buffered saline and incubated with 50 ng/ml of TPA and test chemicals for 1 hr at 37°C in 1 ml of serum-free Dulbecco's modified Eagle's medium. Then an aliquot (0.05 ml) of the medium was taken for measuring radioactivity. The control value without TPA (400-800 cpm) was subtracted from all assay data.

Prostaglandin synthetase activity was assayed as described by Granström *et al.* (8). The microsomal fraction of sheep seminal vesicles was isolated and used as the enzyme. The reaction mixture (1.5 ml) consisted of 0.05 M potassium phosphate buffer, pH 7.4, ^{3}H-arachidonic acid (0.3 µCi, 10 µg), the test chemical and the enzyme preparation. It was incubated at 37°C for 30 min and then acidified and extracted with chloroform. The chloroform layer was evaporated and prostaglandin E_2 was separated by silica-gel thin-layer chromatography and its radioactivity was counted.

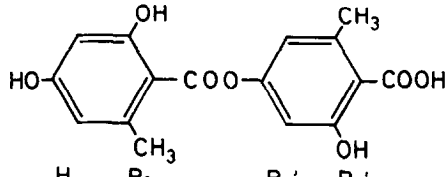
Delayed-type hypersensitivity was assayed as described before (9). Groups of five female ddY mice were used for each assay. They were given a subcutaneous injection of 10^{8} sheep red

blood cells (SRBC) first in the right hind footpad, and 4 days later in the left hind footpad, and 24 hrs later the thickness of left hind footpad was measured. The test chemical (50 mg/kg) was injected intraperitoneally, either on Day 0 when SRBC were first injected or on Day 4 when the immunized mice were challenged with SRBC.

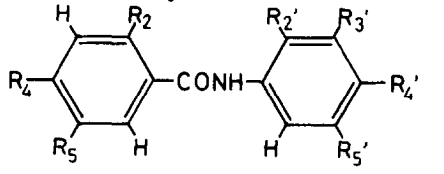
Results:

The structures of lecanoric acid and its analogues are shown in Table 1. Their effects on histidine decarboxylase, TPA-induced arachidonic acid release, prostaglandin synthetase and delayed-type hypersensitivity responses are shown in Table 2. Of the 56 synthetic benzanilide derivatives, all those with an ID_{50} of less than 10 µg/ml inhibition of TPA-induced arachidonic acid release except product number SD-170 were tested further.

Table 1 Structure of lecanoric acid analogues



Lecanoric acid



Lecanoric acid analogues

Product No.	Substitution						
	R_2	R_4	R_5	R_2'	R_3'	R_4'	R_5'
SD - 151	OH	-[1]	Cl	-	OH	COOH	-
SD - 161	OH	-	-	-	-	Cl	-
SD - 166	OH	OH	-	Br	-	COOH	OH
SD - 170	OH	-	-	-	Cl	OH	Cl
SD - 194	OH	-	-	-	CH_3	OH	CH_3
SD - 199	OAc	OAc	-	-	OH	$COOCH_3$	-
SD - 702	OH	CH_3	-	-	-	OCH_3	-
SD - 706	OAc	CH_3	-	-	-	OCH_3	-
SD - 713	OH	CF_3	-	-	Cl	OH	Cl
SD - 714	OH	-	-	OH	NO_2	-	NO_2
SD - 717	OH	F	-	-	Cl	OH	Cl

[1] No substitution (hydrogen atom).

Table 2 Enzymic and biological effects of lecanoric acid analogues

Chemical	ID_{50} (μg/ml)		% inhibition		
	histidine decarboxylase	arachidonic acid release	prostaglandin[1] synthetase	DTH[2] Day 0	DTH[2] Day 4
Lecanoric acid	1.1	>20	-[3]	-	-
SD - 151	0.080	0.78	0	73	53
SD - 161	1.5	1.9	39	0	19
SD - 166	0.033	2.2	53	17	0
SD - 170	0.007	>20	59	20	83
SD - 194	0.27	9.8	62	6	17
SD - 199	0.50	2.3	20	55	55
SD - 702	2.5	0.35	57	7	80
SD - 706	0.74	2.2	62	29	23
SD - 713	0.030	1.1	50	5	5
SD - 714	0.0014	0.59	80	67	84
SD - 717	0.028	1.1	75	9	83

[1] % Inhibition of test chemical at 10 μg/ml.
[2] Delayed-type hypersensitivity. Test chemicals were injected either on Day 0 or on Day 4. % Decrease of footpad thickness is shown.
[3] Not tested.

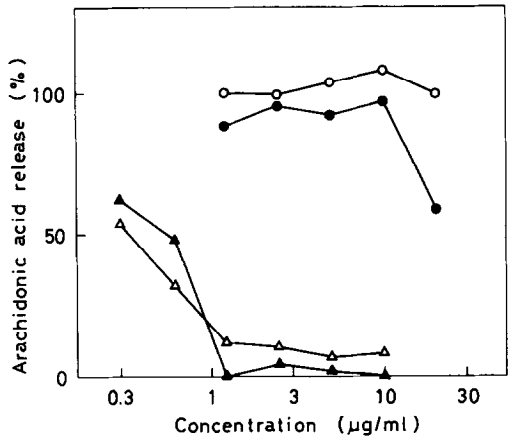


Fig. 1 Inhibition of TPA-induced arachidonic acid release by lecanoric acid analogues. C3H10T1/2 cells prelabelled with ^{3}H-arachidonic acid were incubated with 50 ng/ml of TPA and lecanoric acid (○), SD-170 (●), SD-702 (△) and SD-714 (▲) for 1 hr in 1 ml Dulbecco's modified Eagle's medium. An aliquot (0.05 ml) of the medium was taken for measuring radioactivity. The control value was 3100 cpm.

Most of the benzanilide derivatives listed in Table 2 showed stronger inhibition of histidine decarboxylase than lecanoric acid. Substitution of electron-withdrawing groups such as nitro groups or chlorine atoms at the 3 and 5 positions of an aniline moiety appeared to increase the inhibitory effect on histidine decarboxylase.

Lecanoric acid analogues such as SD-702 and SD-714 inhibited TPA-induced arachidonic acid release at low concentrations, although lecanoric acid showed no inhibitory activity. The dose-effects of lecanoric acid, SD-170, SD-702 and SD-714 on arachidonic acid release are shown in Fig. 1.

Lecanoric acid and SD-170 are specific inhibitors of histidine decarboxylase and do not inhibit dihydroxyphenylalanine decarboxylase or diamine oxidase, a histamine metabolizing enzyme (10). However, SD-170 and other lecanoric acid analogues inhibited prostaglandin synthetase at 10 μg/ml as shown in Table 2.

On intraperitoneal injection lecanoric acid analogues inhibited the delayed-type hypersensitivity reaction against SRBC in

mice. SD-151, SD-199 and SD-714 were active when they were given at the time of sensitization (afferent stage) or of elicitation of the response (afferent stage). SD-170, SD-702 and SD-717 were active only when they were injected at the afferent stage.

Discussion:

Lecanoric acid analogues inhibited histidine decarboxylase, prostaglandin synthetase and TPA-induced arachidonic acid release. However, there appear to be no causative relation between these different effects. It is likely that inhibition of delayed-type hypersensitivity by lecanoric acid analogues is due to their anti-inflammatory effects with inhibition of histamine and prostaglandin syntheses. But it may be due to inhibition of lymphocyte activation, since we found that SD-151 and SD-170 also inhibited antibody formation against SRBC in mice.

Binding of phorbol ester to receptor can be assayed using labelled phorbol-12,13-dibutylate (PDBu) (11). Although lecanoric acid analogues inhibited TPA-induced arachidonic acid release, SD-702 and SD-714 did not inhibit cellular PDBu binding at 20 μg/ml (data not shown). Therefore, they probably bind to a different receptor from that for TPA. Lecanoric acid analogues possibly stabilize the cell membrane, since SD-170 and SD-714 were shown to inhibit heat-induced haemolysis (12).

Although inflammation alone may not be essential for tumour promotion (13), anti-inflammatory steroids inhibited TPA-induced tumour promotion (14). Lecanoric acid analogues antagonize several enzymic and cellular effects of TPA related to inflammation. Unlike lecanoric acid, benzanilide analogues are not easily metabolized *in vivo*, and their intraperitoneal injection inhibited delayed-type hypersensitivity in mice. SD-170 showed low toxicity with LD_{50} of 2000 mg/kg rat by intraperitoneal

injection (10). The *in vivo* anti-carcinogenic effects of lecanoric acid analogues are now being studied.

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