Relative Inhibitory Activity of Berberine-Type Alkaloids against 12-*O*-Tetradecanoylphorbol-13-acetate-Induced Inflammation in Mice

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Forty eight derivatives of berberine-type alkaloids were examined for their inhibition activity against the induction of edema on mouse ear by application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Berberine had an inhibitory effect against TPA-induced ear edema at a grade corresponding to those of quercetin, caffeine and cepharanthine. Berberine derivatives had stronger inhibitory activity than palmatine derivatives. 9-*N,N*-Diphenylcarbamoyl derivatives of both 9-demethylberberine and 9-demethylpalmatine had rather strong activity. These inhibitory activities are about ten times the activity of the respective mother compounds. Furthermore, 9-*N*-monophenylcarbamoyl derivatives and *N,N*-diphenylcarbamoyl chloride are found to have no effect.

Keywords anti-tumor promoter; 12-*O*-tetradecanoylphorbol-13-acetate (TPA); berberine-type alkaloids; anti-inflammation; berberine; isoquinoline alkaloid

Introduction

Since it is known that, in general, promoters of carcinogenesis also have potent irritant activity,¹⁾ we studied the inhibitory effects of chemical components of higher plants against inflammation in mouse skin induced by a strong tumor promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA). We have reported the relative inhibitory activity of flavonoids,²⁾ sterols and triterpenes³⁾ on TPA-induced inflammation in mice, from results of screening tests for antitumor-promoting activity. These studies revealed a certain correlation between the inhibitory effect against the TPA-induced inflammation and the inhibition of TPA-induced tumor promotion in a two-stage carcinogenesis experiment.^{2–5)}

As berberine is a major compound present in *Phellodendri Cortex* (Obaku in Japanese) and *Coptidis Rhizoma* (Oren in Japanese), which are used for treating diarrhea and other gastrointestinal diseases in Japan and other Eastern countries, there are many reports of studies on its anti-inflammatory activity,⁶⁾ anti-hypertensive activity,⁷⁾ and anti-bacterial activity.⁸⁾ The anti-tumor activity^{9,10)} of berberine, and its inhibition of promotion in two-stage carcinogenesis¹¹⁾ have also been reported. Cepharanthine, a dimer of benzylisoquinoline derivatives, is also known to inhibit promotion in two-stage carcinogenesis.¹²⁾ Berberine has almost the same level of inhibitory activity against TPA-induced inflammation as quercetin, which was reported by Sugimura *et al.* to have an inhibitory effect against promotion in two-stage carcinogenesis.^{13,14)}

In the present study, the inhibitory effect of 48 derivatives of berberine and palmatine on TPA-induced inflammation in mice was investigated. It was found that berberine derivatives had stronger inhibitory activity than palmatine derivatives. However, 9-*N,N*-diphenylcarbamoyl derivatives of both 9-demethylberberine and 9-demethylpalmatine had rather strong activity. Of interest may be that *N*-monophenylcarbamoyl derivatives had no influence on the substitution at 9-position of the compounds.

Materials and Methods

Chemicals TPA was purchased from Chemicals for Cancer Research Inc., Minnesota, U.S.A. Berberine chloride (3), berberine sulfate (4),

N-phenylcarbamic acid methyl ester, *N*-phenylcarbamic acid ethyl ester, phenylcarbamide, *N,N*-dimethylcarbamoyl chloride, *N,N*-diethylcarbamoyl chloride, *N,N*-diphenylcarbamoyl chloride, acetone, chloroform and methanol were purchased from Tokyo Kasei Kogyo Co., Ltd., Japan. Palmatine chloride (27) was offered by Alps Pharmaceutical Industrial Co., Ltd., Japan. Other compounds were synthesized by one of the authors (F. Shimada): berberrubine (1),¹⁵⁾ berberrubine hydrochloride (2),¹⁶⁾ 9-ethyl, 9-demethylberberine chloride (5), 12-bromoberberrubine (16),¹⁷⁾ 9-*n*-propyl, 9-demethylberberine bromide (6),¹⁸⁾ 9-acetyl, 9-demethylberberine chloride (7), 9-pivaloyl, 9-demethylberberine chloride (8),¹⁹⁾ 9-cinnamoyl, 9-demethylberberine chloride (9),²⁰⁾ 9-*N*-methylcarbamoyl, 9-demethylberberine chloride (10), 9-*N,N*-dimethylcarbamoyl, 9-demethylberberine chloride (11), 9-*N,N*-diethylcarbamoyl, 9-demethylberberine chloride (12), 9-*N,N*-diphenylcarbamoyl, 9-demethylberberine chloride (13), 9-*N*-methylcarbamoyl, 9-demethyl, 13-methylberberine chloride (24), 9-*N*-phenylcarbamoyl, 9-demethyl, 13-methylberberine chloride (25), 9-*N*-methylcarbamoyl, 9-demethylpalmatine chloride (35), 9-*N,N*-dimethylcarbamoyl, 9-demethylpalmatine chloride (36), 9-*N,N*-diphenylcarbamoyl, 9-demethylpalmatine chloride (37),²¹⁾ 8-methylberberine bromide (14), 8-*n*-propylberberine bromide (15),²²⁾ 13-methylberberrubine hydrogensulfate (17), 13-ethylberberrubine (18), palmatrubine hydrochloride (26), 13-methylpalmatine chloride (38), 2,3,9,10-tetraethyl, 2,3,9,10-tetrademethylpalmatine bromide (40), 2,3,9,10-tetraallyl, 2,3,9,10-tetrademethylpalmatine bromide (41),²³⁾ 13-methylberberine iodide (19), 13-ethylberberine iodide (20), 13-allylberberine bromide (21), 13-ethoxyberberine chloride (22), 13-methyl, 2,9-didemethylpalmatine chloride (39), 9-demethyl, 13-methyltetrahydroberberine (45),²⁴⁾ 9-4'-methoxybenzoyl, 9-demethyl, 13-methylberberine chloride (23),²⁵⁾ 9-acetyl, 9-demethylpalmatine chloride (28), 9-propionyl, 9-demethylpalmatine chloride (29), 9-*n*-butyryl, 9-demethylpalmatine chloride (30), 9-palmitoyl, 9-demethylpalmatine chloride (31), 9-isovaleroyl, 9-demethylpalmatine chloride (32), 9-benzoyl, 9-demethylpalmatine chloride (33), 9-4'-chlorobenzoyl, 9-demethylpalmatine chloride (34),²⁶⁾ tetrahydroberberine (42), 9-propyl, 9-demethyltetrahydroberberine hydrochloride (43), 9-acetyl, 9-demethyltetrahydroberberine methosulfate (46), *N*-methyl, 9-demethyltetrahydroberberine chloride (47), *N*-ethyltetrahydroberberine chloride (48).²⁸⁾

Animals Female ICR mice were obtained from Shizuoka Laboratory Animal Center, Shizuoka, Japan, and housed in an air-conditioned room (22–23°C) lit from 08:00 to 20:00. Food and water were available *ad libitum*.

Assay of TPA-Induced Inflammation TPA (1 µg) dissolved in acetone (20 µl) was applied to the right ear only of ICR mice by means of a micropipette. A volume of 10 µl was delivered to both the inner and outer surfaces of the ear. The sample or its vehicle, chloroform-methanol (1:1, 20 µl), as a control, was applied topically about 30 min before each TPA treatment. The edema was measured 8 h after TPA treatment. Application of the sample (2, 1, 0.5, 0.2, 0.1 or 0.05 mg/ear) completely inhibited TPA-induced inflammation and this inhibitory activity was reduced in a dose-dependent manner. For ear thickness determinations, a pocket

thickness gauge (Mitsutoyo Co., Ltd. Japan) with a range of 0–9 mm, graduated at 0.01-mm intervals and modified so that the contact surface area was increased, thus reducing the tension, was applied to the tip of the ear. Each value was the mean of individual determinations from 5 mice, and ED₅₀ values were determined by method of probit-graphic interpolation for at least 4 dose levels.

Results

Berberine sulfate (4) had an inhibitory effect against TPA-induced inflammation in the ears of mice at a grade corresponding to those of quercetin, caffeine and cepharanthine (Table I). As shown in Tables II and III, berberine and palmatine derivatives were tested for their ability to reduce the intensity of TPA-induced ear edema.

TABLE I. Inhibitory Effect of Anti-tumor Promoters on TPA-Induced Inflammation in Mice

Compound	ED ₅₀	
	μmol/ear	mg/ear
Quercetin	6.6	2.0
Caffeine	8.8	1.7
Cepharanthine	0.9	0.5
Berberine sulfate	1.2	0.9

Compounds were applied 30 min before TPA: ear thickness was determined at 8 h after TPA treatment.

The inhibitory effect of berberine derivatives, berberine chloride (3), 9-acetyl, 9-demethylberberine chloride (7), 9-*N,N*-dimethylcarbamoyl, 9-demethylberberine chloride (11) and 9-*N,N*-diphenylcarbamoyl, 9-demethylberberine chloride (13), were found to be stronger than that of palmatine derivatives, palmatine chloride (27), 9-acetyl, 9-demethylpalmatine chloride (28), 9-*N,N*-dimethylcarbamoyl, 9-demethylpalmatine chloride (36) and 9-*N,N*-diphenylcarbamoyl, 9-demethylpalmatine chloride (37). Berberrubine hydrochloride (2), 12-bromoberberrubine (16) and 13-ethylberberrubine (18) were stronger than the mother compound, berberrubine (1). On the 8-position of berberine, 8-*n*-propyl berberine bromide (15) was stronger than 8-methylberberine bromide (14). On the 13-position of berberine, 13-ethoxycarbonylberberine chloride (22) and 13-methyl, 9-*N,N*-diphenylcarbamoyl, 9-demethylberberine chloride (25) were lower than these 8-hydro derivatives (3) and (11), respectively. When substituted with an *N,N*-diphenylcarbamoyl function at 9-position of both 9-demethylberberine and 9-demethylpalmatine, the compounds had about ten times the activity of the respective mother compound (Tables II and III). Of interest may be that *N*-monophenylcarbamoyl derivatives had no influence on the substitution at 9-position of the compounds. *N*-Phenylcarbamic acid derivatives and *N,N*-diphenylcarbamoyl chloride had no apparent effect (Table IV). Of the

TABLE II. Inhibitory Effect of Berberine Derivatives on TPA-Induced Inflammation in Mice

No.	Substituent					ED ₅₀ μmol/ear	Inhib. ^{a)} (%)
	C ₈	C ₉	C ₁₂	C ₁₃	X		
1	H	O ⁻	H	H	—	6.7	34 ^{d)}
2	H	OH	H	H	Cl ⁻	1.3	52 ^{c,e)}
3	H	OMe	H	H	Cl ⁻	6.5	36 ^{d)}
4	H	OMe	H	H	SO ₄ ²⁻	1.2	53 ^{e)}
5	H	OEt	H	H	Cl ⁻	—	45 ^{e)}
6	H	OC ₃ H ₇	H	H	Br ⁻	2.0	51 ^{e)}
7	H	OAc	H	H	Cl ⁻	—	44 ^{b,e)}
8	H	OCOCMe ₃	H	H	Cl ⁻	3.1	84 ^{b,e)}
9	H	OCOCHCH=CH-C ₆ H ₅	H	H	Cl ⁻	—	26 ^{b)}
10	H	OCO-NHMe	H	H	Cl ⁻	—	9
11	H	OCO-NMe ₂	H	H	Cl ⁻	2.0	51 ^{e)}
12	H	OCO-NEt ₂	H	H	Cl ⁻	1.0	79 ^{e)}
13	H	OCO-N(C ₆ H ₅) ₂	H	H	Cl ⁻	0.6	88 ^{e)}
14	Me	OMe	H	H	Br ⁻	—	16 ^{c)}
15	C ₃ H ₇	OMe	H	H	Br ⁻	3.2	60 ^{b,e)}
16	H	O ⁻	Br	H	—	1.0	86 ^{b,e)}
17	H	OH	H	Me	HSO ₄ ⁻	—	76 ^{b,e)}
18	H	O ⁻	H	Et	—	2.5	69 ^{b,e)}
19	H	OMe	H	Me	I ⁻	—	19 ^{c)}
20	H	OMe	H	Et	I ⁻	—	18
21	H	OMe	H	CH ₂ CH=CH ₂	Br ⁻	3.8	52 ^{b,e)}
22	H	OMe	H	CO-OEt	Cl ⁻	—	12
23	H	OCO-C ₆ H ₄ -4-OMe	H	Me	Cl ⁻	6.3	43 ^{e)}
24	H	OCO-NHMe	H	Me	Cl ⁻	—	22
25	H	OCO-NH(C ₆ H ₅)	H	Me	Cl ⁻	—	32 ^{d)}

Compounds were applied 30 min before TPA: ear thickness was determined at 8 h after TPA treatment. a) Inhibition ratio at 1 mg/ear. b) Inhibition ratio at 2 mg/ear. c) Inhibition ratio at 0.5 mg/ear. d) *p* < 0.05, e) *p* < 0.01 by Student's *t* test as compared with the control group.

TABLE III. Inhibitory Effect of Palmatine Derivatives on TPA-Induced Inflammation in Mice

No.	Substituent						ED ₅₀ μmol/ear	Inhib. ^{a)} (%)
	C ₂	C ₃	C ₉	C ₁₀	C ₁₃	X		
26	OMe	OMe	OH	OMe	H	Cl ⁻	—	22
27	OMe	OMe	OMe	OMe	H	Cl ⁻	—	29 ^{b,d)}
28	OMe	OMe	OAc	OMe	H	Cl ⁻	—	6
29	OMe	OMe	OCOEt	OMe	H	Cl ⁻	—	15
30	OMe	OMe	OCOBu	OMe	H	Cl ⁻	1.9	68 ^{e)}
31	OMe	OMe	OCOC ₁₅ H ₃₁	OMe	H	Cl ⁻	1.7	49 ^{e)}
32	OMe	OMe	OCOCH ₂ CHMe ₂	OMe	H	Cl ⁻	1.7	62 ^{e)}
33	OMe	OMe	OCO-C ₆ H ₅	OMe	H	Cl ⁻	—	32 ^{c,d)}
34	OMe	OMe	OCO-C ₆ H ₄ -4-Cl	OMe	H	Cl ⁻	1.4	71 ^{e)}
35	OMe	OMe	OCO-NHMe	OMe	H	Cl ⁻	—	4
36	OMe	OMe	OCO-NMe ₂	OMe	H	Cl ⁻	2.1	53 ^{e)}
37	OMe	OMe	OCO-N(C ₆ H ₅) ₂	OMe	H	Cl ⁻	1.0	87 ^{e)}
38	OMe	OMe	OMe	OMe	Me	Cl ⁻	—	19
39	OH	OMe	OH	OMe	Me	Cl ⁻	—	10
40	OEt	OEt	OEt	OEt	H	Br ⁻	2.0	81 ^{b,e)}
41	OCH ₂ CH=CH ₂	OCH ₂ CH=CH ₂	OCH ₂ CH=CH ₂	OCH ₂ CH=CH ₂	H	Br ⁻	1.5	77 ^{e)}

Compounds were applied 30 min before TPA; ear thickness was determined at 8 h after TPA treatment. a) Inhibition ratio at 1 mg/ear. b) Inhibition ratio at 2 mg/ear. c) Inhibition ratio at 0.5 mg/ear. d) $p < 0.05$, e) $p < 0.01$ by Student's t test as compared with the control group.

TABLE IV. Inhibitory Effect of Carbamoyl Derivatives on TPA-Induced Inflammation

Compound	Inhib. ^{a)} (%)
<i>N</i> -Phenylcarbamic acid methyl ester	0
<i>N</i> -Phenylcarbamic acid ethyl ester	7
Phenylcarbamide	0
<i>N,N</i> -Dimethylcarbamoyl chloride	0
<i>N,N</i> -Diethylcarbamoyl chloride	0
<i>N,N</i> -Diphenylcarbamoyl chloride	0

Compounds were applied 30 min before TPA; ear thickness was determined at 8 h after TPA treatment. a) Inhibition ratio at 2 mg/ear.

TABLE V. Inhibitory Effect of Tetrahydroberberine Derivatives on TPA-Induced Inflammation

No.	Substituent				ED ₅₀ μmol/ear	Inhib. ^{a)} (%)
	C ₉	C ₁₃	N	X		
42	OMe	H	—	—	—	43 ^{d)}
43	OC ₃ H ₇	H	—	HCl	—	39 ^{d)}
44	OAc	H	—	HCl	1.6	47 ^{c,d)}
45	OH	Me	—	—	—	15 ^{c)}
46	OH	—	Me	MeSO ₄ ⁻	27	—
47	OMe	—	Me	Cl ⁻	—	6
48	OMe	—	Et	Cl ⁻	—	22 ^{b)}

Compounds were applied 30 min before TPA; ear thickness was determined at 8 h after TPA treatment. a) Inhibition ratio at 1 mg/ear. b) Inhibition ratio at 2 mg/ear. c) Inhibition ratio at 0.5 mg/ear. d) $p < 0.01$ by Student's t test as compared with the control group.

various tetrahydroberberines, that in which an acetyl function was substituted for the methyl group at the 9-position of tetrahydroberberine was proved to inhibit TPA-induced inflammation (Table V).

Discussion

It was demonstrated that berberine, a main alkaloid of *Phellodendri Cortex* and *Coptidis Rhizoma*, had an inhibitory effect against TPA-induced inflammation in the ears of mice at a level corresponding to those of quercetin,^{13,14)} caffeine⁵⁾ and cepharanthine¹²⁾ (Table I), which are known to have an anti-tumor promotion effect in two-stage carcinogenesis. In general, the inhibitory effect of berberine derivatives was found to be stronger than that of palmatine derivatives (Tables II and III). This finding suggested that the methylene-dioxy function at the 2,3-position of the alkaloids is more important for production of anti-inflammation activity against TPA-induced ear edema than in the dimethoxyl group. Substitution of the methyl group at the 9-position of both berberine and palmatine to other functions generally augmented the inhibitory activity. In particular, when substituted with an *N,N*-diphenylcarbamoyl function, the compounds had about ten times the activity of the respective mother compound (Fig. 1). However, substitution with *N*-methylcarbamoyl or *N*-phenylcarbamoyl groups did not produce the same augmentation as that by substitution with an *N,N*-diphenylcarbamoyl group. Furthermore, *N,N*-diphenylcarbamoyl chloride had no apparent effect (Table IV). Among the *N,N*-di-substituted carbamoyl derivatives, the diphenyl derivative had the highest activity, followed by the diethyl and dimethyl derivatives in that order. Therefore, it is suggested that introduction of lipophilic functions into the *N*-terminus of carbamoyl in these

R	ED ₅₀	R	ED ₅₀
methyl	6.5 μM	methyl	-
N-methylcarbamoyl	-	N-methylcarbamoyl	-
N,N'-dimethylcarbamoyl	2.0 μM	N,N'-dimethylcarbamoyl	2.1 μM
N,N'-diethylcarbamoyl	1.0 μM		
N,N'-diphenylcarbamoyl	0.6 μM	N,N'-diphenylcarbamoyl	1.0 μM

Fig. 1. Relative Inhibitory Activities of Berberine and Palmatine Derivatives on TPA-Induced Inflammation in Mice

alkaloids augments their anti-tumor promotion activity. Of the various tetrahydroberberines, that in which an acetyl function was substituted for the methyl group at the 9-position of tetrahydroberberine was proved to inhibit TPA-induced inflammation (Table V).

Based on the studies described above, the following structure-activity relationship can be postulated:

1. Berberine derivatives had stronger inhibitory activity than palmatine derivatives.
2. The 9-*N,N*-diphenylcarbamoyl function was substituted for the methyl group at the 9-position of berberine and palmatine had about ten times the activity of the respective mother compounds. In addition, *N,N*-diphenylcarbamoyl chloride proved to have no effect.

As berberine and some of its derivatives are present in Chinese traditional crude drugs, which are used in combined Kampo prescriptions, it is of interest from the viewpoints of both Chinese traditional medicine and pharmacognostical science that such alkaloids have inhibitory activity against promotion of carcinogenesis.

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