JPP Journal of Pharmacy and Pharmacology

JPP 2009, 61: 1051–1056

Received August 9, 2008
Accepted May 12, 2009
DOI 10.1211/jpp/61.08.0009
ISSN 0022-3573

Research Paper

Relative inhibitory activity of bile acids against 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation, and chenodeoxycholic acid inhibition of tumour promotion in mouse skin two-stage carcinogenesis

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Abstract

Objectives Bile acids are present in Bezoar Bovis and Fel Ursi, traditionally used as antipyretics and antispasmodics. However the anti-inflammatory activity of individual bile acids and related compounds has not yet been investigated. In this paper, we report the structure–activity relationships influencing the anti-inflammatory activity of a variety of structurally different bile acid derivatives and also the inhibitory activity of chenodeoxycholic acid against tumour promotion.

Methods Fifty derivatives of bile acids were examined for their inhibitory activity against the induction of oedema in mouse ear by application of 12-*O*-tetradecanoylphorbol-13-acetate (TPA). Also, the effect of chenodeoxycholic acid was studied in mouse skin in which tumours had been induced by topical application of 7,12-dimethylbenz[*a*]anthracene (DMBA) and promoted by TPA.

Key findings Many bile acid derivatives had an inhibitory effect against TPA-induced ear oedema at a similar grade to that of indometacin. Chenodeoxycholic acid, methyl 3α,7α,15α-trihydroxy-5β-cholan-24-oate and methyl 3α,7α,15β-trihydroxy-5β-cholan-24-oate showed the most potent activity with an ID50 value of 71–110 nmol/ear, a level corresponding to that of hydrocortisone (69 nmol/ear). Furthermore, chenodeoxycholic acid markedly suppressed tumour-promoting activity by TPA following initiation by DMBA in mouse skin.

Conclusions This is the first report on the anti-inflammatory activity of bile acids on TPA-induced inflammatory ear oedema in mice. Chenodeoxycholic acid, methyl 3α,7α,15α-trihydroxy-5β-cholan-24-oate and methyl 3α,7α,15β-trihydroxy-5β-cholan-24-oate showed the most potent activity, at a level corresponding to that of hydrocortisone. Furthermore, chenodeoxycholic acid markedly inhibited tumour promotion in a two-stage carcinogenesis model in mouse skin.

Keywords anti-inflammatory effect; antitumour-promoting agent; bile acid; TPA; two-stage carcinogenesis

Introduction

It is known that, in general, promoters of carcinogenesis have potent irritant activity.[1] In our series of studies on anti-inflammatory active substances, we have investigated the inhibitory effects of chemical components from plants resources and fungi metabolites against inflammatory ear oedema induced by a strong tumour promoter, 12-*O*-tetradecanoylphorbol-13-acetate (TPA), in mice and reported the related inhibitory activity of flavonoids,[2] sterols and terpenoids,[3–5] on TPA-induced inflammation in mice. Among isoprene derivatives, triterpene acids such as pachymic and poricoic acids showed a relatively strong inhibitory effect on TPA-induced inflammation in mice.[6,7]

Bile acids are major components in Bezoar Bovis and Fel Ursi, used as antipyretics and antispasmodics.[8] However the anti-inflammatory activity of individual bile acids and related compounds has not yet been investigated. In this paper, we report the structure–activity relationships influencing the anti-inflammatory activity of a variety of structurally different bile acid derivatives (Figure 1, Table 1), as well as the inhibitory activity of chenodeoxycholic acid (**9**) against tumour promotion in a two-stage carcinogenesis model in mouse skin.

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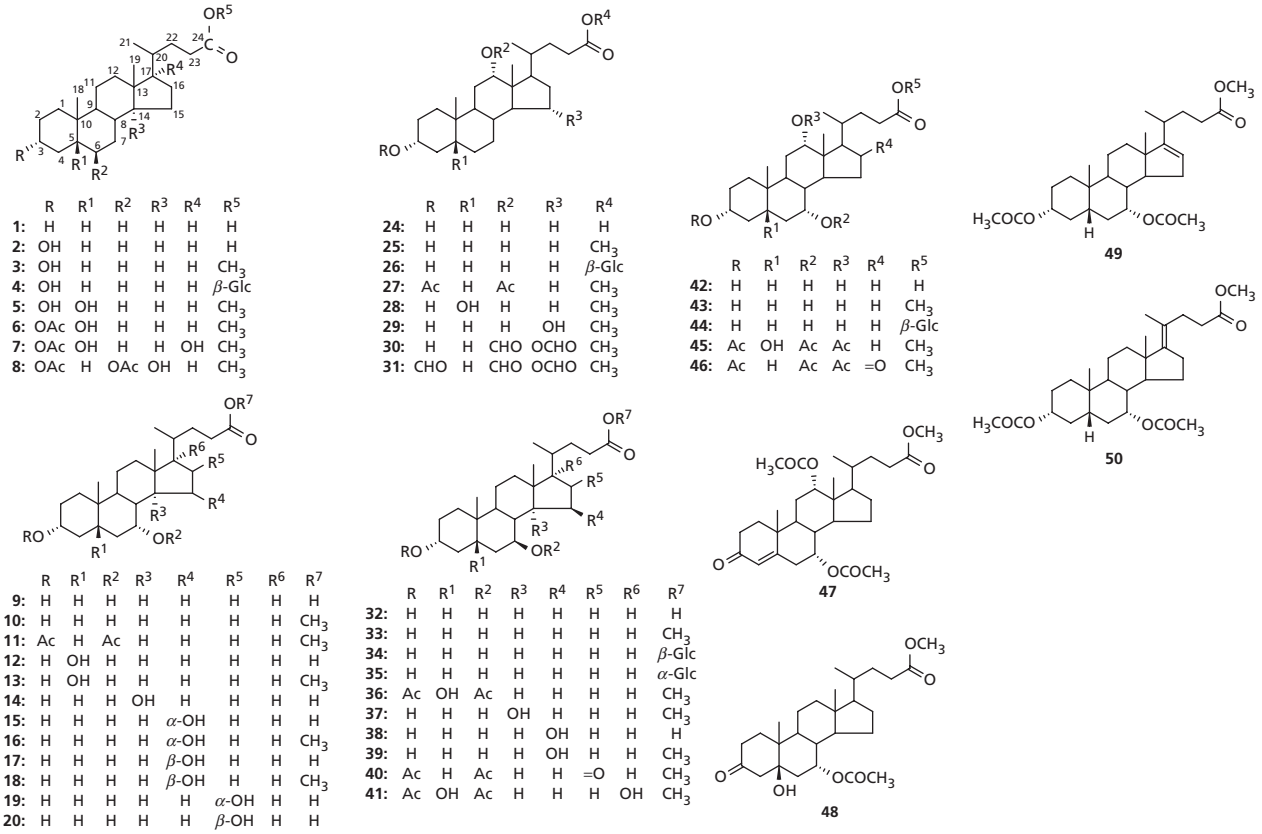

	R	R^1	R^2	R^3	R^4	R^5
1:	H	H	H	H	H	H
2:	OH	H	H	H	H	H
3:	OH	H	H	H	H	CH_3
4:	OH	H	H	H	H	β-Glc
5:	OH	OH	H	H	H	CH_3
6:	OAc	OH	H	H	H	CH_3
7:	OAc	OH	H	H	OH	CH_3
8:	OAc	H	OAc	OH	H	CH_3

	R	R^1	R^2	R^3	R^4
24:	H	H	H	H	H
25:	H	H	H	H	CH_3
26:	H	H	H	H	β-Glc
27:	Ac	H	Ac	H	CH_3
28:	H	OH	H	H	CH_3
29:	H	H	H	OH	CH_3
30:	H	H	CHO	OCHO	CH_3
31:	CHO	H	CHO	OCHO	CH_3

	R	R^1	R^2	R^3	R^4	R^5
42:	H	H	H	H	H	H
43:	H	H	H	H	H	CH_3
44:	H	H	H	H	H	β-Glc
45:	Ac	OH	Ac	Ac	H	CH_3
46:	Ac	H	Ac	Ac	=O	CH_3

	R	R^1	R^2	R^3	R^4	R^5	R^6	R^7
9:	H	H	H	H	H	H	H	H
10:	H	H	H	H	H	H	H	CH_3
11:	Ac	H	Ac	H	H	H	H	CH_3
12:	H	OH	H	H	H	H	H	H
13:	H	OH	H	H	H	H	H	CH_3
14:	H	H	H	OH	H	H	H	H
15:	H	H	H	H	α-OH	H	H	H
16:	H	H	H	H	α-OH	H	H	CH_3
17:	H	H	H	H	β-OH	H	H	H
18:	H	H	H	H	β-OH	H	H	CH_3
19:	H	H	H	H	H	α-OH	H	H
20:	H	H	H	H	H	β-OH	H	H
21:	H	H	H	H	H	β-OH	H	CH_3
22:	H	H	H	H	H	H	OH	H
23:	Ac	OH	Ac	H	H	H	OH	CH_3

	R	R^1	R^2	R^3	R^4	R^5	R^6	R^7
32:	H	H	H	H	H	H	H	H
33:	H	H	H	H	H	H	H	CH_3
34:	H	H	H	H	H	H	H	β-Glc
35:	H	H	H	H	H	H	H	α-Glc
36:	Ac	OH	Ac	H	H	H	H	CH_3
37:	H	H	H	OH	H	H	H	CH_3
38:	H	H	H	H	OH	H	H	H
39:	H	H	H	H	OH	H	H	CH_3
40:	Ac	H	Ac	H	H	=O	H	CH_3
41:	Ac	OH	Ac	H	H	H	OH	CH_3

Figure 1 Chemical structures of bile acids

Materials and Methods

Chemicals

TPA was purchased from Chemicals for Cancer Research Inc. (Minnesota, USA). Compounds **1**, **2**, **3**, **9**, **10**, **11**, **24**, **25**, **27**, **32**, **33**, **42** and **43**, and 7,12-dimethylbenz[*a*]anthracene (DMBA), indometacin and hydrocortisone were obtained from Sigma Chemical Co. (St Louis, MO, USA). Acetone, chloroform and methanol were obtained from Wako Pure Chemical Industries Ltd (Osaka, Japan).

The following bile acid derivatives used in this study were synthesized in our laboratory: **4**, **26**, **34**, **35** and **44**;[9] **5** and **8**;[10] **6**;[11] **7**, **23**, **28**, **36**, **40**, **41**, **45**, **46** and **48**;[12] **14** and **37**;[13] **15**, **16**, **17**, **18**, **19**, **20**, **21**, **22**, **38**, **39**, **49** and **50**;[14] and **47**.[15] The other compounds (**12**, **13**, **29**, **30** and **31**) were from our laboratory collection.

Animals

Experiments were approved by the Committee for Animal Welfare at the College of Pharmacy, Nihon University, and were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the College of Pharmacy, Nihon University, Chiba, Japan. Female ICR mice were purchased from Japan SLC Inc. (Shizuoka, Japan) and housed in an air-conditioned specific pathogen-free room (22–23°C), lit from 0800–2000 h. Food and water were freely available.

Assay of TPA-induced inflammation

TPA (1 μg/ear) dissolved in acetone (20 μl) was applied to the right ear only of ICR mice using a micropipette. A volume of 10 μl was delivered to both the inner and outer surfaces of the ear. The sample or its vehicle (20 μl), chloroform–methanol (1 : 1 v/v), as a control was applied topically about 30 min before each TPA treatment. For thickness determination, a pocket thickness gauge (Mitsutoyo Co. Ltd, Tokyo, Japan) with a range of 0–9 mm, graduated at 0.01-mm intervals and modified so that contact surface area was increased, thus reducing the tension, was applied to the tip of the ear.

The ear thickness was measured before treatment (*a*). Oedema was measured 6 h after TPA treatment (*b*: TPA alone; *b*′: TPA plus sample). The following values were then calculated:

$$\text{Oedema A: oedema induced by TPA alone } (b - a) \quad (1)$$

$$\text{Oedema B: oedema inducedby TPA plus sample } (b' - a) \quad (2)$$

$$\text{Inhibitory ratio } (\%) = [(\text{Oedema A} - \text{Oedema B}) / \text{Oedema A}] \times 100 \quad (3)$$

Table 1 Inhibitory effect of bile acids on TPA-induced inflammation in mice

Scientific name	ID50 (nmol/ear)	95% CI
5β-Cholan-24-oic acid (**1**)	370	347–395
Lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid) (**2**)	327	306–349
Methyl lithocholate (**3**)[a]	1382	1240–1544
1-*O*-(24-Lithocholyl)-β-D-glucopyranose (**4**)	510	448–582
Methyl 3α,5β-dihydroxy-5β-cholan-24-oate (**5**)	>1230	n.a.
Methyl 3α-acetoxy-5β-hydroxycholan-24-oate (**6**)	1384	1232–1550
Methyl 3α-acetoxy-5β,17α-dihydroxycholan-24-oate (**7**)	486	442–537
Methyl 3α,6β-diacetoxy-14α-hydroxy-5β-cholan-24-oate (**8**)	288	252–329
Chenodeoxycholic acid (3α,7α-dihydroxy-5β-cholan-24-oic acid) (**9**)	110	90–137
Methyl chenodeoxycholate (**10**)[a]	303	281–328
Methyl chenodeoxycholate diacetate (**11**)[a]	343	331–352
3α,5β,7α-Trihydroxycholan-24-oic acid (**12**)	1625	1446–1820
Methyl 3α,5β,7α-trihydroxycholan-24-oate (**13**)	315	280–355
3α,7α,14α-Trihydroxy-5β-cholan-24-oic acid (**14**)	587	534–649
3α,7α,15α-Trihydroxy-5β-cholan-24-oic acid (**15**)	367	326–411
Methyl 3α,7α,15α-trihydroxy-5β-cholan-24-oate (**16**)	71	65–77
3α,7α,15β-Trihydroxy-5β-cholan-24-oic acid (**17**)	509	471–548
Methyl 3α,7α,15β-trihydroxy-5β-cholan-24-oate (**18**)	99	91–107
3α,7α,16α-Trihydroxy-5β-cholan-24-oic acid (**19**)	213	190–239
3α,7α,16β-Trihydroxy-5β-cholan-24-oic acid (**20**)	257	221–300
Methyl 3α,7α,16β-trihydroxy-5β-cholan-24-oate (**21**)	336	298–381
Methyl 3α,7α,17α-trihydroxy-5β-cholan-24-oate (**22**)	296	260–334
Methyl 3α,7α-diacetoxy-5β,17α-dihydroxycholan-24-oate (**23**)	304	260–358
Deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid) (**24**)	588	558–616
Methyl deoxycholate (**25**)[a]	209	186–234
1-*O*-(24-Deoxycholyl)-β-D-glucopyranose (**26**)	458	416–503
Methyl 3α-acetoxy-12α-hydroxy-5β-cholan-24-oate (**27**)	672	637–709
Methyl 3α,12α-diacetoxy-5β-hydroxycholan-24-oate (**28**)	541	495–589
Methyl 3α,12α,15α-trihydroxy-5β-cholan-24-oate (**29**)	142	125–159
Methyl 3α-hydroxy-12α,15α-diformyloxy-5β-cholan-24-oate (**30**)	284	250–321
Methyl 3α,12α,15α-triformyloxy-5β-cholan-24-oate (**31**)	261	240–281
Ursodeoxycholic acid (3α,7β-dihydroxy-5β-cholan-24-oic acid) (**32**)	428	402–454
Methyl ursodeoxycholate (**33**)[a]	322	282–369
1-*O*-(24-Ursodeoxycholyl)-β-D-glucopyranose (**34**)	388	355–422
1-*O*-(24-Ursodeoxycholyl)-α-D-glucopyranose (**35**)	555	514–600
Methyl 3α,7β-diacetoxy-5β-hydroxycholan-24-oate (**36**)	320	291–351
Methyl 3α,7β,14α-trihydroxy-5β-cholan-24-oate (**37**)	215	191–241
3α,7β,15β-Trihydroxy-5β-cholan-24-oic acid (**38**)	448	408–495
Methyl 3α,7β,15β-trihydroxy-5β-cholan-24-oate (**39**)	270	238–305
Methyl 3α,7β-diacetoxy-16-oxo-5β-cholan-24-oate (**40**)	262	241–286
Methyl 3α,7β-diacetoxy-5β,17α-dihydroxycholan-24-oate (**41**)	231	206–259
Cholic acid (3α,7α,12α-trihydroxy-5β-cholan-24-oic acid) (**42**)	675	637–714
Methyl cholate (**43**)[a]	163	149–178
1-*O*-(24-Cholyl)-β-D-glucopyranose (**44**)	880	818–946
Methyl 3α,7α,12α-triacetoxy-5β-hydroxycholan-24-oate (**45**)	197	175–221
Methyl 3α,7α,12α-triacetoxy-16-oxo-5β-cholan-24-oate (**46**)	135	127–144
Methyl 7α,12α-diacetoxy-3-oxochol-4-en-24-oate (**47**)	153	135–171
Methyl 7α-acetoxy-5β-hydroxy-3-oxocholan-24-oate (**48**)	992	921–1063
Methyl 3α,7α-diacetoxy-5β-chol-16-en-24-oate (**49**)	1144	1041–1264
Methyl 3α,7α-diacetoxy-5β-chol-17(20)-en-24-oate (**50**)	1766	1654–1885
Indometacin[b]	908	755–1092
Hydrocortisone[b]	69	64–75

ID50, the 50% inhibitory dose; 95% CI, 95% confidence intervals; n.a., not applicable. [a]Trivial name. [b]Standard drug.

Each value used the mean of individual determinations from 5 mice. The 50 % inhibitory dose (ID50) values were determined by probit-graphic interpolation for four dose levels.

Two-stage carcinogenesis experiment

The backs of mice (7 weeks old) were shaved with electric clippers. Tumour initiation was accomplished by a single topical application of DMBA 50 μg. Promotion with TPA 1 μg, applied twice weekly, was begun one week after the initiation. Chenodeoxycholic acid (**9**; 20 nmol), or its vehicle, acetone–dimethyl sulfoxide (9 : 1 v/v; 100 μl), was applied topically 30 min before each TPA treatment. DMBA and TPA were dissolved in acetone and applied to the shaved area in a volume of 100 μl using a micropipette.

The back of each mouse was shaved once a week to remove hair. The number and diameter of skin tumours were measured every other week, and the experiment continued for 20 weeks. Experimental and appropriate control groups each consisted of 15 mice.

Statistical analysis

Statistical differences were verified by using one-way analysis of variance followed by the correction of Tukey–Kramer test. The ID50 values and their 95% confidence intervals (CI 95%) were obtained by nonlinear regression using the GraphPad PRISM v. 5.0 (Intuitive Software for Science, San Diego, USA). The differences between experimental groups were compared by Student's *t*-test and Fischer's exact test.

Results

Bile acid derivatives were tested for their ability to reduce the intensity of TPA-induced ear oedema in mice. As shown in Table 1, among the oxygenated 5β-cholanoic acids examined, chenodeoxycholic acid (**9**), methyl 3α,7α,15α-trihydroxycholan-24-ate (**16**) and methyl 3α,7α,15β-trihydroxycholan-24-ate (**18**) markedly inhibited the TPA-induced inflammation with the ID50 value being 71–110 nmol/ear. By comparison with standard drugs, **9**, **16** and **18** were similar in activity to hydrocortisone, but were more effective than indometacin.

The inhibitory effect of chenodeoxycholic acid (**9**) was found to be stronger than those of lithocholic acid (**2**), deoxycholic acid (**24**), ursodeoxycholic acid (**32**) and cholic acid (**42**) (Figure 2). The 3α-hydroxyl function of 5β-cholanoic acid had either no or little effect. On the other hand, the 7α-hydroxy derivatives (**9**–**23**) of 5β-cholanoic acid were more effective than the analogous β-isomers (**32**–**41**). The hydroxyl functions at the position 5, 12, 15, or 16 of **9** were found to be less effective (**12**, **42**, **15**, **17**, **19**, **20**). The methyl esters at C-24 (**25**, **33**, **43**) seemed to be more active than the corresponding parent free bile acids (**24**, **32**, **42**, respectively). Thus, compound **9**, having the axially-oriented 7α-hydroxyl group, produced a marked inhibitory effect, whereas the corresponding equatorial 7β-epimer (**32**) exhibited only a small activity. In addition, introduction of a hydroxyl group at C-15 (**16**, **18**) in methyl chenodeoxycholanate (**10**) significantly increased the activity to three to four times more than the parent free acids (**15**, **17**).

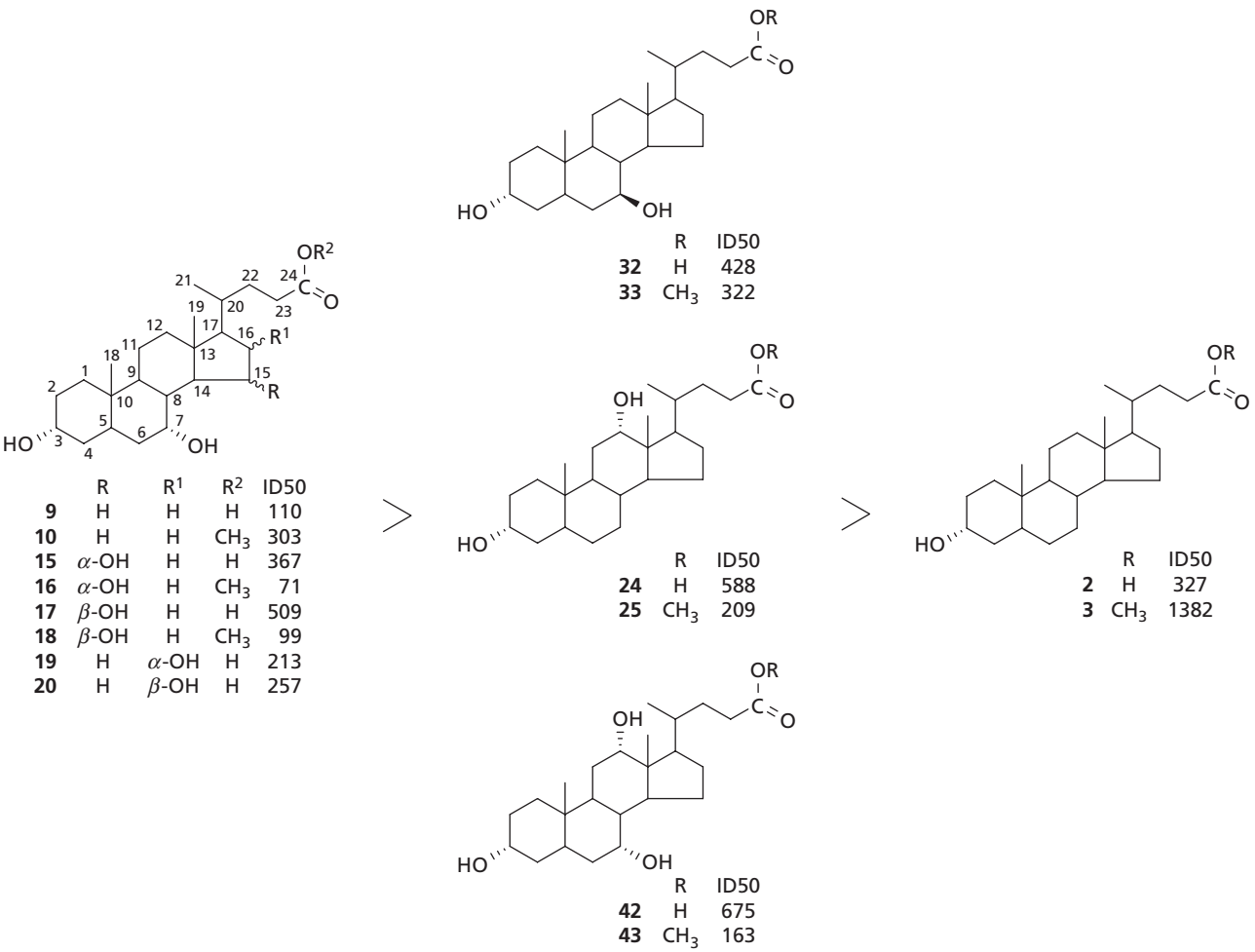

Figure 2 Relative inhibitory activity of bile acid derivatives on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammatory ear oedema in mice. ID50 values are in nmol/ear.

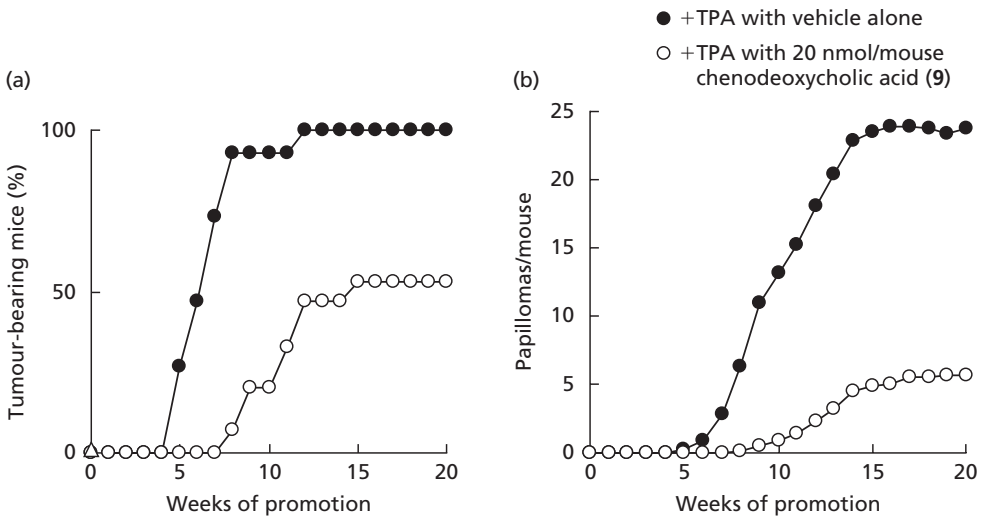

Figure 3 Inhibitory effect of chenodeoxycholic acid (**9**) on the promotion of skin papillomas by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 7,12-dimethylbenz[*a*]anthracene (DMBA)-initiated mice. From 1 week after initiation with a single topical application of DMBA 50 μg, TPA 1 μg was applied twice weekly. Topical application of **9** and vehicle was performed 30 min before each TPA treatment. Data are expressed as percentage of mice bearing papillomas (a) and as average number of papillomas per mouse (b). The chenodeoxycholic acid treated group was statistically different from the control group by Student's *t*-test ($P < 0.05$ = after week 5; $P < 0.01$ = after weeks 6 ~ 20) and Fischer's exact test ($P < 0.05$ = after weeks 6, 9, 10; $P < 0.01$ = after weeks 7, 8).

Chenodeoxycholic acid (**9**) inhibited tumour promotion by TPA following initiation with DMBA. Figure 3a shows the time course of skin tumour formation in the mouse groups treated with DMBA plus TPA and with or without **9**. In the group treated with DMBA plus TPA, the first tumour appeared at week 5 and all 15 mice had tumours at week 12, whereas in the group treated with DMBA plus TPA and 20 nmol of **9**, the first tumour appeared at week 8. The percentage of tumour-bearing mice treated with DMBA plus TPA and **9** was 53%. Figure 3b shows the average number of tumours per mouse at week 20. The group treated with DMBA plus TPA produced 23.7 tumours per mouse, whereas the DMBA plus TPA and **9** group had 5.7 tumours per mouse. Thus, treatment with **9** resulted in a 76% reduction in the average number of tumour per mouse at week 20. Chenodeoxycholic acid (**9**) markedly inhibited the tumour promotion in this two-stage carcinogenesis model in mouse skin, at a level corresponding to that of heliantriol C[16] and pachymic acid.[7]

Discussion

The observation was made during the structure–activity relationship study that among the oxygenated 5β-cholanoic acids examined, **9**, **16** and **18** were similar in activity to hydrocortisone, but were more effective than indometacin in the mouse ear-oedema model of inflammation. The 7α-hydroxy derivatives (**9**–**23**) of 5β-cholanoic acid were more effective than the analogous β-isomers (**32**–**41**). The methyl esters at C-24 (**25**, **33**, **43**) seemed to be more active than the corresponding parent free bile acids (**24**, **32**, **42**, respectively). Thus, compound **9**, having an axially-oriented 7α-hydroxyl group, produced a marked inhibitory effect, whereas the corresponding equatorial 7β-epimer (**32**) exhibited only a small activity. In addition, introduction of a hydroxyl group at C-15 (**16**, **18**) in methyl chenodeoxycholanate (**10**) significantly increased the activity to three-to-four times stronger than that of the parent free acids (**15**, **17**).

We demonstrated that chenodeoxycholic acid (**9**) exhibited a marked activity in inhibiting TPA-induced tumour promotion in a two-stage model of carcinogenicity in mouse skin. Bile acids are present in Chinese traditional crude drugs (Bezoar Bovis and Fel Ursi), which are used in combined Kampo prescriptions and Japanese over-the-counter drugs for nourishment and tonic. Our previous studies demonstrated that extracts from edible plants and mushrooms had inhibited TPA-induced inflammation in mice. Steroids separated from safflowers,[17] an edible mushroom *Hypsigius marmoreus*[18] and edible agar *Chlorella vulgaris*[19] have been identified as the active compounds. Furthermore, these steroids have been shown to inhibit tumour promotion during two-stage carcinogenesis in mouse skin. Many steroids widely distributed in edible and medicinal plants and fungi inhibit the tumour-promoting activity of TPA in mouse skin, and this suggests that they may be important for the chemoprevention of cancer.

Conclusions

This is the first report on the anti-inflammatory activity of bile acids on TPA-induced inflammatory ear oedema in mice. Chenodeoxycholic acid (**9**), methyl 3α,7α,15α-trihydroxy-5β-cholan-24-oate (**16**) and methyl 3α,7α,15β-trihydroxy-5β-cholan-24-oate (**18**) showed the most potent activity, at a grade corresponding to that of hydrocortisone. Furthermore, **9** markedly inhibited tumour promotion by TPA following initiation with DMBA in mouse skin.

Declarations

Conflict of interest

The Author(s) declare(s) that they have ho conflicts of interest to disclose.

Funding

This work was supported, in part, by a Nihon University Multidisciplinary Research Grant for 2007–2008.

References

1. Fujiki H *et al*. Significance of new environmental tumor promoters. *Environ Carcinogen Rev (J Environ Sci Hlth)* 1989; C7: 1–51.
2. Yasukawa K *et al*. Effect of flavonoids on tumor promoter's activity. In: Cody V *et al.* (eds) *Plant Flavonoids in Biology and Medicine II: Biochemical*, *Cellular and Medicinal Properties.* New York: Alan R. Liss, Inc., 1988: 247–250.
3. Akihisa T, Yasukawa K. Antitumor-promoting and anti-inflammatory activities of triterpenoids and sterols from plants and fungi. In: Atta-ur-Rahman (ed.) *Studies in Natural Products Chemistry*, *Vol. 25, Part F*. Amsterdam: Elsevier Science B.V., 2001: 43–87.
4. Akihisa T *et al.* Potentially cancer chemopreventive and anti-inflammatory terpenoids from natural sources. In: Atta-ur-Rahman (ed.) *Studies in Natural Products Chemistry*, *Vol. 29, Part J.* Amsterdam: Elsevier Science B.V., 2003: 73–126.
5. Yasukawa K *et al*. Inhibitory effect of euphol, a triterpene alcohol from the roots of *Euphorbia kansui*, on tumour promotion by 12-*O*-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. *J Pharm Pharmacol* 2000; 52: 119–124.
6. Akihisa T *et al*. Anti-inflammatory and anti-tumor-promoting effects of triterpene acids and sterols from the fungus *Ganoderma tucidum*. *Chem Biodiv* 2007; 4: 224–231.
7. Kaminaga T *et al*. Inhibitory effect of *Poria cocos* on 12-*O*-tetradecanoylphorbol-13-acetate-induced ear oedema and tumor promotion in mouse skin. *Phytother Res* 1996; 10: 581–584.
8. Namba T. *Collored Illastrations of Wakan-Yaku*, *Vol. 2.* Osaka: Hoikusha, 1980: 269–272, 275–293.
9. Iida T *et al*. Potential bile acid metabolites. 24. An efficient synthesis of carboxy-linked glucosides and their chemical properties. *Lipids* 2002; 37: 101–110.
10. Iida T *et al*. Functionalization of unactivated carbons in 3α, 6- and 3α,24-dihydroxy-5β-cholane derivatives by dimethyldioxirane. *Lipids* 2003; 38: 281–287.
11. Iida T *et al*. A comparative study of remote oxy-functionalization of unactivated carbons in 5β-steroids by dimethyldioxirane and 2,6-dichloropyridine *N*-oxide/ruthenium-porphyrin/HBr. *ARKIVOC* 2003; viii: 171–179.
12. Iida T *et al*. A highly efficient, stereoselective oxyfunctionalization of unactivated carbons in steroids with dimethyldioxirane. *J Chem Soc, Perkin Trans* 2001; 1: 2229–2236.
13. Kakiyama G *et al*. Synthesis of 3α,7α,14α-trihydroxy-5β-cholan-24-oic acid: a potential primary bile acid in vertebrates. *Chem Pharm Bull (Tokyo)* 2004; 52: 371–374.
14. Iida T *et al*. Potential bile acid metabolites. 25. Synthesis and chemical properties of stereoisomeric 3α,7α,16- and 3α,7α,15-trihydroxy-5β-cholan-24-oic acids. *Chem Pharm Bull (Tokyo)* 2002; 50: 1327–1334.
15. Ogawa S *et al*. The remote-oxyfunctionalization of unactivated carbons in (5β)-3-oxobile acids by 2,6-di-chloropyridine *N*-oxide catalyzed by ruthenium-porphyrin and HBr: a direct lactonization at C-20. *Org Biomol Chem* 2004; 2: 1013–1018.
16. Yasukawa K *et al*. Inhibitory effect of heliantriol C, a component of edible *chrysanthemum*, on tumor promotion by 12-*O*-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. *Phytomedicine* 1998; 5: 215–218.
17. Kasahara Y *et al*. Carthami Flos extract and its component, stigmasterol, inhibit tumour promotion in mouse skin two-stage carcinogenesis. *Phytother Res* 1994; 8: 327–331.
18. Yasukawa K *et al.* Inhibitory effects of ergosterol isolated from the edible mushroom *Hypsizigus marmoreus* on TPA-induced inflammatory ear oedema and tumour promotion in mice. *Phytother Res* 8: 10–13.
19. Yasukawa K *et al*. Inhibitory effects of sterols isolated from *Chlorella vulgaris* on 12-*O*-tetradecanoylphorbol-13-acetate-induced inflammation and tumor promotion in mouse skin. *Chem Pharm Bull* 1996; 19: 573–576.