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Inhibition of prostaglandin E_2 production by taiwanin C isolated from the root of *Acanthopanax chiisanensis* and the mechanism of action

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Abstract

Five lignans, *l*-sesamin, savinin, helioxanthin, taiwanin C, and *cis*-dibenzylbutyrolactone, were isolated from the root of *Acanthopanax chiisanensis* (Araliaceae), a Korean medicinal plant, and their inhibitory effects on the production of prostaglandin (PG) E_2 stimulated by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in rat peritoneal macrophages were examined. Among the five lignans, taiwanin C was the most potent (IC_{50} = 0.12 μM), followed by helioxanthin, *cis*-dibenzylbutyrolactone, and savinin. *l*-Sesamin had no effect. Taiwanin C showed no inhibitory effect on the TPA-induced release of radioactivity from [^{3}H]arachidonic acid-labeled macrophages, nor did it inhibit the expression of cyclooxygenase (COX)-2 protein induced by TPA. However, the activities of isolated COX-1 and COX-2 were inhibited by taiwanin C (IC_{50} = 1.06 and 9.31 μM, respectively), reflecting the inhibition of both COX-1- and COX-2-dependent PGE_2 production in the cell culture system. These findings suggest that the mechanism of action of taiwanin C in the inhibition of PGE_2 production is the direct inhibition of COX enzymatic activity.

Keywords: Taiwanin C; Lignans; Cyclooxygenase activity; Prostaglandin E_2; Macrophage

1. Introduction

PGs are important biological mediators that control inflammatory responses, pain, and fever [1]. COX converts arachidonic acids to PGG_2 through oxygenase activity, and this unstable product is reduced to PGH_2 through peroxidase activity [2]. Two isoforms of COX have been identified, COX-1 and COX-2 [3,4]. The former is constitutively expressed in most tissues [5], while the latter is induced by bacterial lipopolysaccharide (LPS) [6], TPA [7], or cytokines such as interleukin-1β and tumor necrosis factor (TNF)-α [8,9] in macrophages [10], fibroblasts [11], and inflamed tissues [12,13]. However, according to recent reports, COX-2 is also constitutively expressed in the brain [14], kidney [15], and stomach mucosa [16]. The various kinds of drugs developed to inhibit these enzymes, non-steroidal anti-inflammatory drugs, are divided into two groups: COX-1/-2 non-selective inhibitors [17–19] and COX-2 selective inhibitors [20–22].

Our efforts have been focused on finding lead anti-inflammatory compounds from natural products, and recently these efforts have led to the isolation of five lignan compounds (*l*-sesamin, helioxanthin, savinin, taiwanin C, and *cis*-dibenzylbutyrolactone) from the roots of *Acanthopanax chiisanensis* [23]. It has been reported that lignan compounds have various biological activities, including anti-viral [24], anti-cancer [25], and anti-inflammatory [26]. They also inhibit the production of cytokines such as TNF-α [27].

In this study, the effects of these five lignan compounds on the production of PGE_2 in rat peritoneal macrophages were examined and attempts were made to clarify their mechanism(s) of action.

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Abbreviations: PG, prostaglandin; COX, cyclooxygenase; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

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2. Materials and methods

2.1. Lignans from the root of A. chiisanensis

The five lignans, *l*-sesamin [3,4:3′,4′-bis(methylenedioxy)-7,9′:7′,9-diepoxylignan], helioxanthin [3,4:3′,4′-bis(methylenedioxy)-2,7′-cycloligna-7,7′-dien-9,9′-olide], savinin [3,4:3′,4′-bis(methylenedioxy)-lign-7-en-9,9′-olide], taiwanin C [3′,4′:4,5-bis(methylenedioxy)-2,7′-cycloligna-7,7′-dien-9,9′-olide], and *cis*-dibenzylbutyrolactone [3-(3,4-dimethoxybenzyl)-2-(3,4-methylenedioxybenzyl)butyrolactone], were isolated from the root of *A. chiisanensis* [23]. The compounds were identified by a comparison of spectral data (NMR, IR, UV) with authentic compounds. Their chemical structures are shown in Fig. 1.

2.2. Preparation of rat peritoneal macrophages

A solution of soluble starch (Wako) and Bactopeptone (Difco), 5% each, was injected intraperitoneally into male Sprague–Dawley rats (400–550 g, specific pathogen-free, Charles River Japan, Inc.) at a dose of 5 mL/100 g of body weight. Four days later, the rats were killed by cutting the carotid artery under anesthesia, and the peritoneal cells were harvested [28]. The experiments were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animals approved by the Animal Ethics Committee of the Graduate School of Pharmaceutical Sciences, Tohoku University.

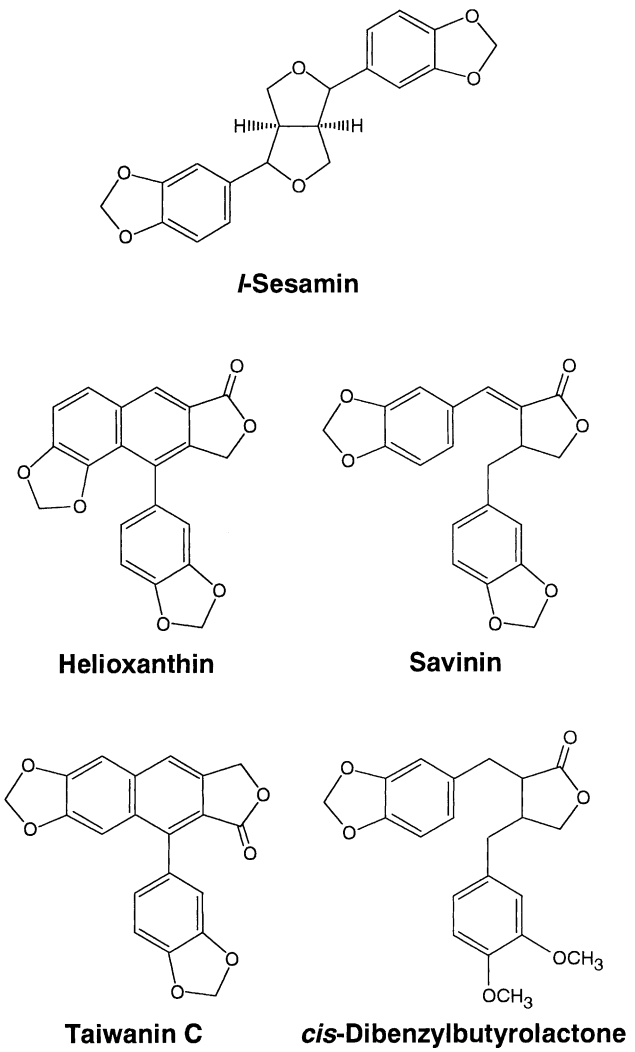

Fig. 1. Chemical structures of five lignan compounds isolated from the root of *A. chiisanensis*.

2.3. Macrophage culture

The peritoneal cells were suspended in Eagle's minimal essential medium (EMEM, Nissui) containing 10% calf serum (Flow Laboratories), penicillin G potassium (18 μg/mL) and streptomycin sulfate (50 μg/mL) (Meiji Seika), then seeded at a density of 7.5×10^5 cells/0.5 mL/well in 24-well plastic tissue culture dishes (Corning Glass Works) or 3.0×10^6 cells/2 mL/well in 6-well plastic tissue culture dishes (Corning Glass Works), and incubated for 2 hr at 37°. Then the wells were washed three times with medium to remove non-adherent cells, and the adherent cells were incubated further for 20 hr at 37°. After three washes, the adherent cells were used for subsequent experiments.

2.4. Incubation of macrophages with drugs

The drugs used were the protein kinase C activator TPA (Sigma) [7], the endomembrane Ca^{2+}-ATPase inhibitor thapsigargin (Wako) [29], the non-specific protein kinase inhibitor staurosporine (Kyowa Medex) [30], the synthetic glucocorticoid dexamethasone (Sigma), the COX-1/COX-2 non-specific inhibitor indomethacin (Wako), the COX-2 specific inhibitor NS-398 (Calbiochem), and arachidonic acid (Sigma). They were dissolved in DMSO or ethanol, and added to the medium. Each lignan was also dissolved in DMSO before being added to the medium. The final concentrations of DMSO and ethanol were adjusted to 0.1% (v/v). The control medium contained the same amount of DMSO. The adherent cells were incubated at 37° for the specified period in 0.5 or 2 mL of medium containing 10% calf serum and various concentrations of each lignan in the presence or absence of drugs.

2.5. Measurement of PGE_2 concentrations

After incubation, the conditioned medium was collected, and centrifuged at 1500 *g* for 5 min at 4°. The concentration of PGE_2 in the supernatant was then radioimmunoassayed [28]. PGE_2 anti-serum was purchased from Assay Designs.

2.6. Macrophage culture for COX-1- and COX-2-dependent PGE_2 production

COX-1- and COX-2-dependent PGE_2 production in rat peritoneal macrophages was determined as described

previously [31]. The peritoneal macrophages (7.5×10^5 cells/well), collected as described in Section 2.2, were incubated at 37° for 2 hr in 24-well plastic tissue culture dishes in 0.5 mL of medium, per well, containing 10% calf serum. The wells then were washed three times to remove non-adherent cells, and the adherent cells were preincubated in 0.5 mL of medium containing dexamethasone (10 μM) for 4 hr at 37°. After three washes, the adherent cells were incubated further for 4 hr at 37° in 0.5 mL of medium containing dexamethasone (10 μM) and the indicated concentrations of indomethacin, NS-398, or taiwanin C in the presence of arachidonic acid (10 μM). After the incubation, the PGE_2 concentration in the conditioned medium was radioimmunoassayed (represents COX-1-dependent PGE_2 production). Another set of macrophages (7.5×10^5 cells) were preincubated for 4 hr at 37° in 0.5 mL of medium containing aspirin (Sigma) (100 μM). After three washes, the adherent cells were incubated further for 4 hr at 37° in 0.5 mL of medium containing the indicated concentrations of indomethacin, NS-398, or taiwanin C in the presence of TPA (30 nM). After the incubation, the PGE_2 concentration in the conditioned medium was radioimmunoassayed (represents COX-2-dependent PGE_2 production).

2.7. Measurement of radioactivity released from [^{3}H]arachidonic acid-labeled macrophages

The peritoneal macrophages (7.5×10^5 cells/well) were incubated in 24-well plastic tissue culture dishes at 37° for 2 hr in 0.5 mL of medium, per well, containing 10% calf serum. The wells were then washed three times to remove non-adherent cells, and incubated further at 37° for 18 hr in 0.5 mL of medium containing 10% calf serum. The wells were again washed three times, and the adherent cells were incubated at 37° for 20 hr in 0.5 mL of medium containing 10% calf serum and 3.7 kBq of [^{3}H] arachidonic acid (2.26 TBq/mmol, Du Pont New England Nuclear). The adherent cells were washed three times with medium to remove free [^{3}H] arachidonic acid, and incubated at 37° for the periods indicated, in 0.5 mL of medium containing 10% calf serum and various concentrations of taiwanin C in the presence or absence of TPA (30 nM). The conditioned medium was collected at 1, 2, and 4 hr, centrifuged at 1500 *g* for 5 min at 4°, and the radioactivity in the supernatant fraction was determined [32].

2.8. Western blot analysis of COX-1 and COX-2 proteins

The peritoneal macrophages (3×10^6 cells/well) were incubated in 2 mL of medium containing 10% calf serum in each well of a 6-well plastic tissue culture dish at 37° for 2 hr. The wells then were washed three times to remove non-adherent cells, and the adherent cells were incubated further at 37° for 20 hr. After three washes, the cells were incubated at 37° for 6 hr in 2 mL of medium containing 10% calf serum in the presence or absence of TPA (30 nM) and various concentrations of taiwanin C. After incubation, the cells were washed three times with PBS (pH 7.4), dipped in 150 μL of ice-cold lysis buffer (20 mM HEPES, 1% Triton-X 100, 10% glycerol, 1 M sodium fluoride, 2.5 mM *p*-nitrophenylene phosphate, 10 μg/mL of phenylmethylsulfonyl fluoride, 1 mM Na_3VO_4, 5 μg/mL of leupeptin, and 1 mM EDTA, pH 7.4) for 15 min, and disrupted with a Handy Sonic Disrupter (UR-20P, TOMY). The lysis buffer containing the disrupted cells was centrifuged at 13,000 *g* and 4° for 20 min. The supernatant fraction obtained was boiled for 5 min in 3 × sample buffer (50 mM Tris, 4% SDS, 10% glycerol, 4% 2-mercaptoethanol, and 0.05 mg/mL of bromophenol blue, pH 7.4) at a ratio of 2:1 (v/v), loaded on an SDS–polyacrylamide gel (8%), and subjected to electrophoresis (150 min at 125 V). Western blotting for COX-1 and COX-2 proteins was carried out as described previously [33]. The levels of COX-1 and COX-2 protein were quantified by scanning densitometry, and the individual band density value for each point was expressed as the relative density signal.

2.9. Determination of COX-1 and COX-2 activities in a cell-free system

Activities of COX-1 and COX-2 in a cell-free system were determined according to the method described by Mancini *et al*. [34] and Kim *et al*. [33]. One unit of COX-1 (isolated from sheep seminal vesicles, purity 95%, Cayman) or COX-2 (isolated from sheep placenta, purity 70%, Cayman) was dissolved in 210 μL of Tris–HCl (100 mM, pH 7.4) containing 10 mM EDTA, 1 mM reduced glutathione, 1 μM hematin, and 0.5 mM phenol. The reaction mixture was preincubated with various concentrations of indomethacin, NS-398, or taiwanin C at 37° for 3 min. After the addition of arachidonic acid (0.1 μM), the mixture was incubated further at 37° for 3 min. Indomethacin and arachidonic acid were dissolved in ethanol, and NS-398 and taiwanin C in DMSO. An equivalent volume (2 μL) of drug was added to the reaction buffer. The final concentration of both DMSO and ethanol was adjusted to 2% (v/v). To terminate the reaction, 20 μL of 1 M HCl was added to the reaction mixture. An equivalent volume of 1 M NaOH was then added to neutralize the mixture, and the amount of PGE_2 was measured by radioimmunoassay.

2.10. Statistical analysis

The statistical significance of the results was analyzed by Dunnett's test for multiple comparisons and Student's *t*-test for unpaired observations.

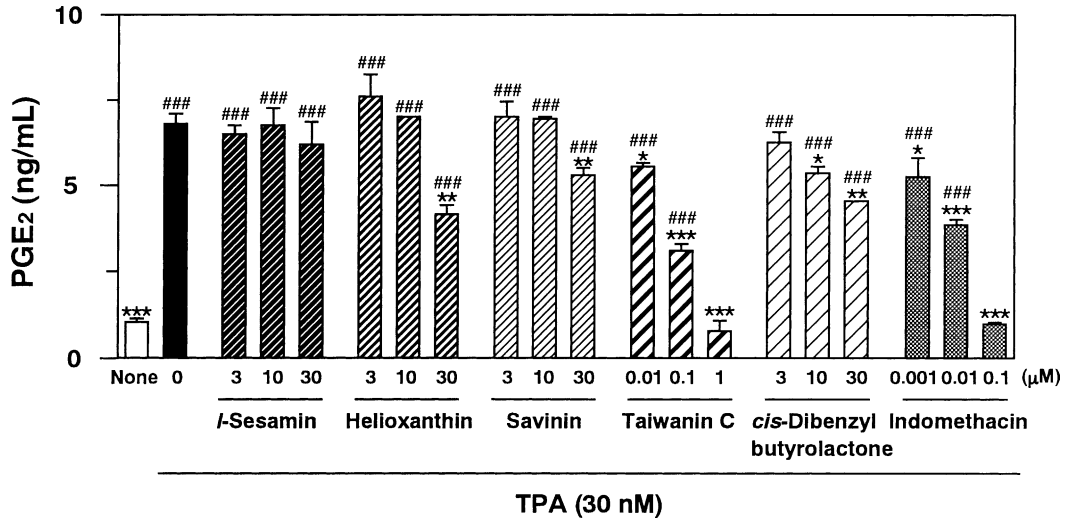

Fig. 2. Effects of various concentrations of lignans on TPA-induced PGE_2 production in rat peritoneal macrophages. Rat peritoneal macrophages (7.5×10^5 cells) were incubated at 37° for 8 hr in 0.5 mL of medium in the presence of TPA (30 nM) and the indicated concentration of each lignan compound. The PGE_2 concentration in the conditioned medium was radioimmunoassayed. Values are the means from four samples with the SEM shown by vertical bars. Statistical significance: (###) $P < 0.001$ vs. none; (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ vs. TPA control.

3. Results

3.1. Effects of five lignans on TPA-induced PGE_2 production in rat peritoneal macrophages

The incubation of rat peritoneal macrophages at 37° for 8 hr in the presence of TPA (30 nM) induced a prominent increase in PGE_2 production (Fig. 2). Among the five lignans, taiwanin C showed the most potent inhibitory effect on the TPA-induced production of PGE_2 with an IC_{50} value of 0.12 μM. At 30 μM, the production was suppressed 46.2, 38.9, and 26.2% by helioxanthin, *cis*-dibenzylbutyrolactone, and savinin, respectively; *l*-sesamin showed no significant effect (Fig. 2). Under the conditions, indomethacin as a positive control inhibited the TPA-induced production of PGE_2 with an IC_{50} value of 0.01 μM (Fig. 2). TPA (30 nM) stimulated the production in a time-dependent manner from 4 to 24 hr, and taiwanin C at 1 μM showed almost complete inhibition up to 24 hr as did indomethacin at 0.1 μM (Fig. 3).

3.2. Effects of taiwanin C on COX-1- and COX-2-dependent PGE_2 production

In the dexamethasone (10 μM)-pretreated macrophages, PGE_2 production was prominently increased by exposure

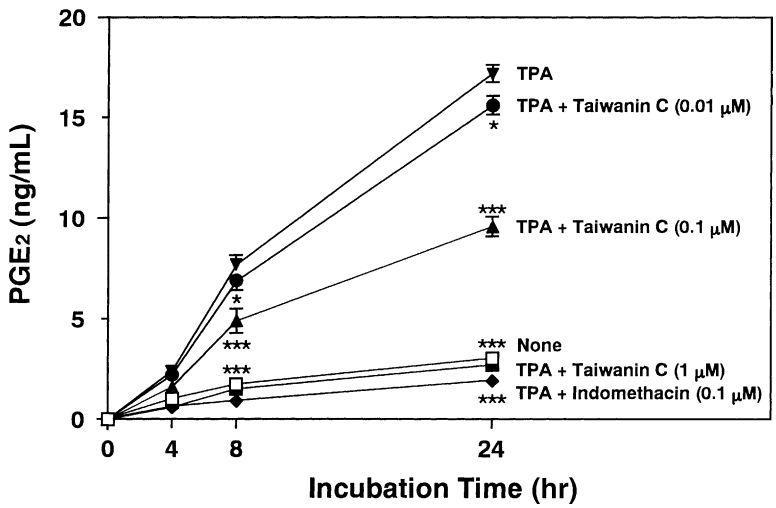

Fig. 3. Time course of the effect of taiwanin C on TPA-induced PGE_2 production. Rat peritoneal macrophages (7.5×10^5 cells) were incubated at 37° for the periods indicated in 0.5 mL of medium in the presence of TPA (30 nM) and the indicated concentration of taiwanin C or indomethacin (0.1 μM). PGE_2 concentrations in the conditioned medium were radioimmunoassayed. Values are the means from four samples with the SEM shown by vertical bars. Symbols without the SEM bars mean that the SEM is within the symbol. Statistical significance: (*) $P < 0.05$, TPA vs. TPA + taiwanin C (0.01 μM) at 4, 8, and 24 hr; (***) $P < 0.001$, TPA vs. TPA + taiwanin C (0.1 μM), TPA + taiwanin C (1 μM), TPA + indomethacin (0.1 μM), and none at 4, 8, and 24 hr.

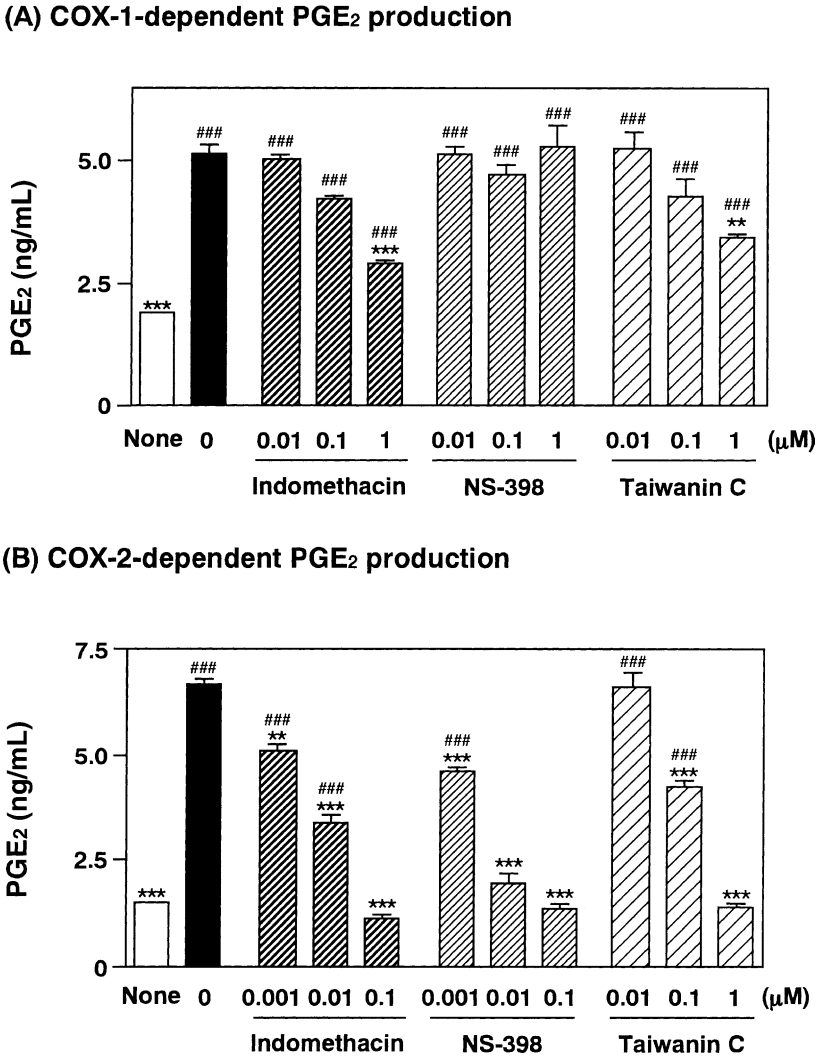

Fig. 4. Effects of taiwanin C on COX-1- and COX-2-dependent PGE_2 production. (A) Rat peritoneal macrophages (7.5×10^5 cells) were preincubated in 0.5 mL of medium containing dexamethasone (10 μM) at 37° for 4 hr. After three washes, the cells were incubated in 0.5 mL of medium containing dexamethasone (10 μM) and the indicated concentration of indomethacin, NS-398, or taiwanin C in the presence of arachidonic acid (10 μM) for 4 hr, and the PGE_2 concentration in the conditioned media was radioimmunoassayed (represents COX-1-dependent PGE_2 production). (B) Another set of macrophages (7.5×10^5 cells) were preincubated in 0.5 mL of medium containing aspirin (100 μM) at 37° for 4 hr. After three washes, the cells were incubated in 0.5 mL of medium containing the indicated concentration of indomethacin, NS-398, or taiwanin C in the presence of TPA (30 nM) for 4 hr at 37°, and the PGE_2 concentration in the conditioned media was radioimmunoassayed (represents COX-2-dependent PGE_2 production). Values are the means from four samples with the SEM shown by vertical bars. Statistical significance: (###) $P < 0.001$ vs. none; (**) $P < 0.01$, and (***) $P < 0.001$ vs. corresponding control.

to arachidonic acid (10 μM) for 4 hr in the presence of dexamethasone (represents COX-1-dependent PGE_2 production). Indomethacin inhibited the arachidonic acid-induced increase in PGE_2 production with an IC_{50} value of 0.33 μM (Fig. 4A). However, NS-398 showed no inhibitory activity at concentrations of 0.01–1 μM. In the aspirin (100 μM)-pretreated macrophages, PGE_2 production increased markedly following treatment with TPA (30 nM) for 4 hr (represents COX-2-dependent PGE_2 production). Indomethacin and NS-398 inhibited the TPA-induced increase in the production of PGE_2 with an IC_{50} value of 0.01 and 0.005 μM, respectively (Fig. 4B). At 1 μM, taiwanin C inhibited the COX-1-dependent PGE_2 production by 46.9% (Fig. 4A). The COX-2-dependent PGE_2 production also was inhibited by taiwanin C, with an IC_{50} value of 0.23 μM (Fig. 4B).

3.3. Effects of taiwanin C on the TPA-induced release of radioactivity from [^{3}H]arachidonic acid-labeled macrophages

TPA (30 nM) stimulated the release of radioactivity from [^{3}H]arachidonic acid-labeled macrophages into the medium at 1–4 hr, and taiwanin C at concentrations of 0.01–1 μM showed no effect on the TPA-induced release of radioactivity (Fig. 5). These findings suggest that the inhibition of PGE_2 production by taiwanin C is not due to the inhibition of phospholipase A_2.

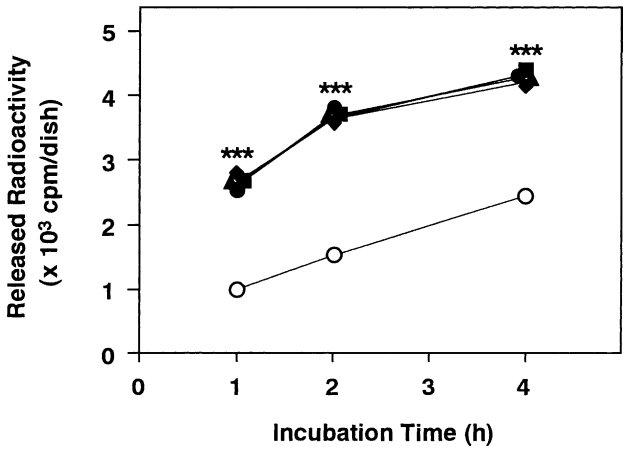

Fig. 5. Effects of taiwanin C on the release of radioactivity from [^{3}H]arachidonic acid-labeled macrophages. Rat peritoneal macrophages (7.5×10^5 cells) were incubated at 37° for 20 hr in medium containing 10% calf serum and 3.7 kBq of [^{3}H]arachidonic acid. After three washes, the cells were incubated at 37° for the periods indicated in 0.5 mL of medium in the presence of TPA (30 nM) and various concentrations of taiwanin C (◆, 0 μM; ●, 0.01 μM; ▲, 0.1 μM; and ■, 1 μM). The amount of radioactivity released into the conditioned medium was determined. The radioactivity released from non-stimulated macrophages is shown by the open circles. Values are the means from four samples. The SEM of each value is too small to depict. Statistical significance: (***) $P < 0.001$ vs. non-stimulated control.

3.4. Effects of taiwanin C on the protein levels of COX-1 and COX-2 in macrophages

After treatment with TPA (30 nM) for 6 hr, the COX-2 protein level increased, while the COX-1 protein level did not change (Fig. 6A). Treatment with taiwanin C at 0.01–1 μM in the presence of TPA (30 nM) did not affect the protein levels of COX-1 or COX-2. These findings indicate that the inhibition of PGE_2 production by taiwanin C is not due to the inhibition of the expression of COX-2 protein.

3.5. Effects of taiwanin C on the enzymatic activities of isolated COX-1 and COX-2

Indomethacin, a COX-1/COX-2 non-selective inhibitor, inhibited the activity of COX-1 and COX-2 in a concentration-dependent manner at 0.01–1 μM, the IC_{50} values for COX-1 and COX-2 being 0.09 and 0.16 μM, respectively. Taiwanin C also inhibited the activity of COX-1 (Fig. 7A) and COX-2 (Fig. 7B); the IC_{50} values for COX-1 and COX-2 were 1.06 and 9.31 μM, respectively. NS-398, a COX-2 specific inhibitor, inhibited COX-2 activity in a concentration-dependent manner at 0.01–1 μM (IC_{50} value: 0.11 μM), but showed no inhibitory effect on COX-1 at such concentrations. These findings suggest that the inhibition of PGE_2 production by taiwanin C is due to the direct inhibition of the activities of both the COX-1 and COX-2 enzymes.

3.6. Effects of taiwanin C on PGE_2 production induced by thapsigargin and staurosporine

To show that the inhibitory activity of taiwanin C is not specific to the TPA-induced production of PGE_2, the effects of taiwanin C on PGE_2 production stimulated by other drugs including thapsigargin, an endomembrane

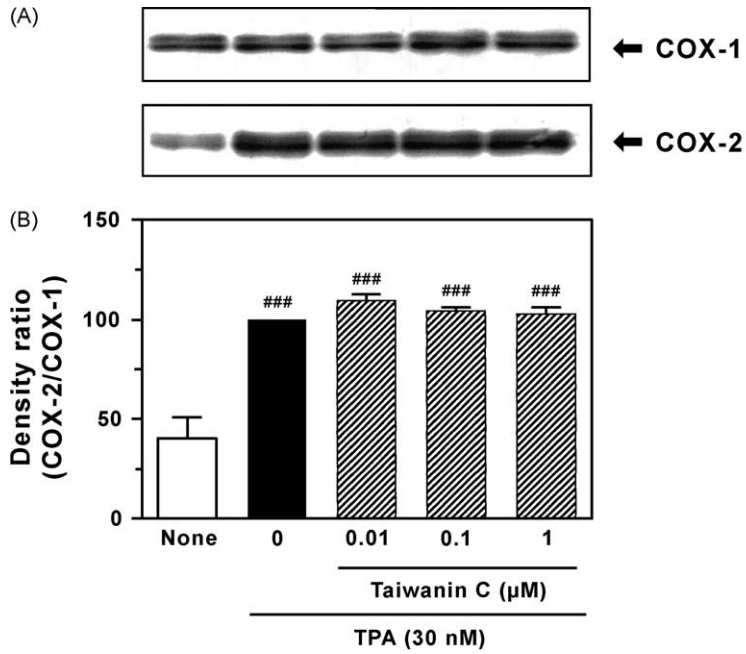

Fig. 6. Effects of taiwanin C on the protein levels of COX-1 and COX-2 in TPA-stimulated rat peritoneal macrophages. Rat peritoneal macrophages (3×10^6 cells) were incubated at 37° for 6 hr in 2 mL of medium containing TPA (30 nM) and the indicated concentration of taiwanin C. The protein levels of COX-1 and COX-2 were determined by western blot analysis (A). The density ratios of COX-2 protein to COX-1 protein were calculated, and the mean value of the density ratio in the TPA-treated control group was set to 100 (B). Values are the means from four samples with the SEM shown by vertical bars. Statistical significance: (###) $P < 0.001$ vs. non-stimulated control.

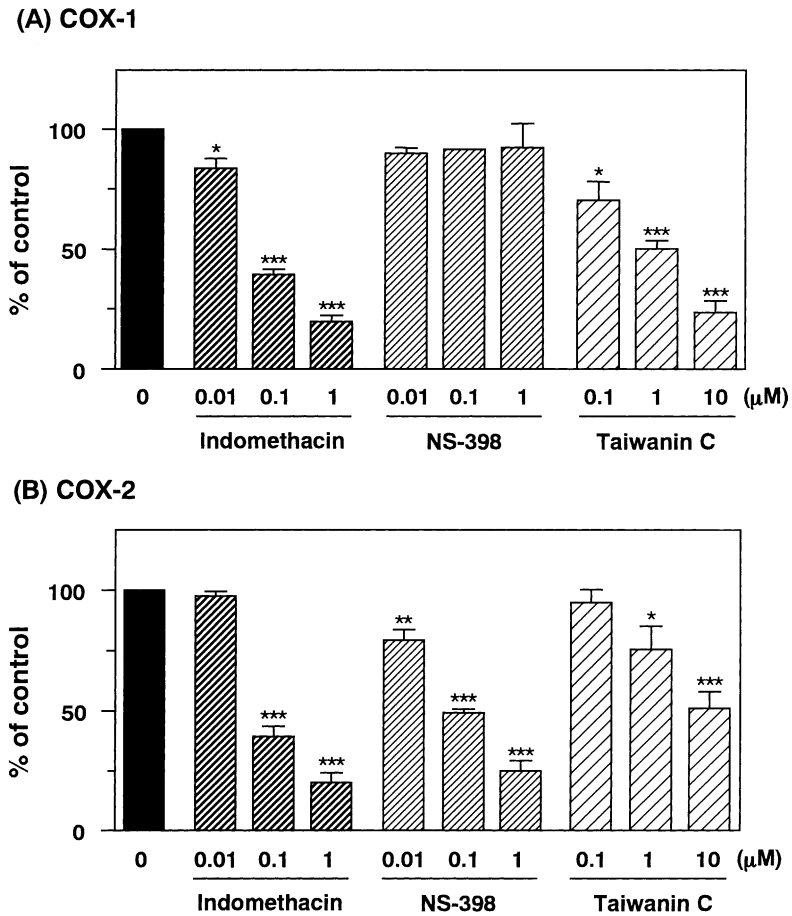

Fig. 7. Effects of taiwanin C on the activity of isolated COX-1 and COX-2. One unit of COX-1 (isolated from sheep seminal vesicles) (A) and one unit of COX-2 (isolated from sheep seminal placenta) (B) dissolved in 210 μL of 100 mM Tris–HCl (pH 7.4) containing 10 mM EDTA, 1 mM reduced glutathione, 1 μM hematin, and 0.5 mM phenol were incubated for 3 min at 37° in the presence of the indicated concentrations of taiwanin C, indomethacin, or NS-398. Arachidonic acid (0.1 μM) was then added, and the incubation continued at 37° for another 3 min. The PGE_2 concentration in the reaction mixture then was radioimmunoassayed, and the mean PGE_2 concentration in the control group was set to 100. Values are the means from four samples with the SEM shown by vertical bars. Statistical significance: (∗) $P < 0.05$, (∗∗) $P < 0.01$, and (∗∗∗) $P < 0.001$ vs. corresponding control.

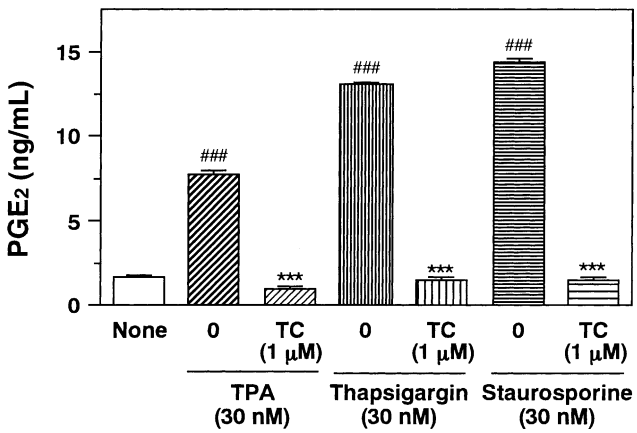

Fig. 8. Effects of taiwanin C on PGE_2 production induced by TPA, thapsigargin, and staurosporine. Rat peritoneal macrophages (7.5×10^5 cells) were incubated at 37° for 8 hr in 0.5 mL of medium containing TPA (30 nM), thapsigargin (30 nM), or staurosporine (30 nM) in the presence or absence of taiwanin C (TC, 1 μM). The PGE_2 concentration in the conditioned medium was radioimmunoassayed. Values are the means from four samples with the SEM shown by vertical bars. Statistical significance: (∗∗∗) $P < 0.001$ vs. corresponding control; and (###) $P < 0.001$ vs. none.

Ca^{2+}-ATPase inhibitor, and staurosporine, a non-specific protein kinase inhibitor, were examined. In the presence of thapsigargin (30 nM) or staurosporine (30 nM), PGE_2 production was increased prominently at 8 hr (Fig. 8). Upon treatment with taiwanin C (1 μM), the thapsigargin- and staurosporine-induced PGE_2 production was strongly inhibited as in the case of the TPA-induced production of PGE_2 (Fig. 8).

4. Discussion

In this study, the effects of the five lignans, *l*-sesamin, helioxanthin, savinin, taiwanin C, and *cis*-dibenzylbutyrolactone (isolated from the roots of *A. chiisanensis*), on the TPA-induced production of PGE_2 in rat peritoneal macrophages were examined. In addition, the mechanism of action of taiwanin C, which showed the most potent inhibitory effect (Fig. 2), was clarified. Previously, we reported that thapsigargin, an endomembrane Ca^{2+}-ATPase inhibitor [29], and staurosporine, a non-specific

protein kinase inhibitor [30], induce the production of PGE_2 in rat peritoneal macrophages [28,32,35,36]. As shown in Fig. 8, taiwanin C also inhibited thapsigargin- and staurosporine-induced PGE_2 production, indicating that its mechanism of action is not through the inhibition of protein kinase C. Taiwanin C showed no effect on the TPA-induced release of arachidonic acid from membrane phospholipids (Fig. 5) or on the expression of COX-2 protein (Fig. 6), but did show a direct inhibitory effect on the activity of the isolated COX-1 and COX-2 enzymes (Fig. 7). These findings indicate that taiwanin C suppresses TPA-induced PGE_2 production by inhibiting the activities of COX-1 and COX-2 directly. Taiwanin C also inhibited COX-1- and COX-2-dependent PGE_2 production (Fig. 4), but with slightly different IC_{50} values. It is reported that IC_{50} values change depending on the assay system [37], probably reflecting differences in protein binding and distribution across cell membranes [38]. The mechanism of action of taiwanin C for the inhibition of PGE_2 production is similar to that of acidic non-steroidal anti-inflammatory drugs such as indomethacin, ibuprofen, and meclofenamate.

Acidic non-steroidal inhibitors commonly have a carboxylic acid residue that binds to Arg^{120} in COX-1 and blocks the approach of arachidonic acid to the catalytic subunit of COX-1 [34]. It is well known that gastrointestinal injury, a major side-effect of acidic non-steroidal anti-inflammatory drugs [39], is caused by the inhibition of prostanoid production via the inhibition of COX-1 in the gastrointestinal mucosa. Recently, it has been reported, however, that the most abundant phospholipid on the surface of the gastric mucus gel layer is phosphatidylcholine, a surface protectant, and that acidic non-steroidal anti-inflammatory drugs can interact chemically with phosphatidylcholine to detach it from the surface and induce gastrointestinal injury by exposing the surface to luminal acid [40]. Because taiwanin C has no carboxylic acid residue, it may have less of a gastrointestinal side-effect than acidic non-steroidal anti-inflammatory drugs.

To inhibit the activity of COX-2 selectively, a methylsulfonylphenyl or sulfonamoylphenyl group at the *para* position of the aryl ring must interact with the specific residues within the side-pocket of COX-2 [37,41]. Using this approach, several COX-2 inhibitors such as celecoxib [42] and rofecoxib [43] have been developed recently. However, these present findings indicate that taiwanin C, which has no methylsulfonylphenyl or sulfonamoylphenyl substituent, inhibits COX-2 activity. Therefore, we suggest that a lactone moiety in the taiwanin C molecule participates in the inhibition of COX-2 activity. In the five-membered ring of some COX-2 inhibitors such as rofecoxib and celecoxib, atoms containing lone pair electrons seem to interact with some residues in COX-2. It is reported that an oxygen atom of a ketone in the lactone moiety makes a hydrogen bond with residues lining the primary COX-2 channel, particularly the Arg^{120} residue, and this interaction helps to inhibit the activity of COX-2 [44]. The inhibition of COX-2 activity by taiwanin C might involve the same mechanism.

Helioxanthin, savinin, and *cis*-dibenzylbutyrolactone, which also contain a lactone moiety, inhibited the TPA-induced production of PGE_2 less potently than taiwanin C (Fig. 2). The position of the lactone moiety in these compounds is different from that in taiwanin C, and this might be the reason for their weak inhibitory effect. In contrast, *l*-sesamin having no lactone moiety showed no inhibitory effect on TPA-induced PGE_2 production.

It has been reported that the lignan glycoside phillyrin isolated from the leaves of *Phillyrea latifolia* (Oleaceae) inhibits PGE_2 production in calcium ionophore A23187-stimulated mouse peritoneal macrophages, and thromboxane B_2 production in A23187-stimulated human platelets with IC_{50} values of 45.6 and 168 μM, respectively [45], although the mechanism of action has not been clarified. In the present report, it was elucidated that the arylnaphthalide lignan taiwanin C has direct inhibitory activity on isolated COX-1 and COX-2 enzymes with IC_{50} values of 1.06 and 9.31 μM, respectively (Fig. 7). In addition, it has been reported that the lignans neojusticin A and justicidin B isolated from *Justicia procumbens* L. (Acathaceae), which structurally resemble taiwanin C, inhibit arachidonic acid-induced rabbit platelet aggregation with IC_{50} values of 1.1 and 8.0 μM, respectively [46]. Considering the present findings, it is possible that the arachidonic acid-induced aggregation of rabbit platelets is inhibited by these lignans through the inhibition of COX activity, resulting in the inhibition of the production of thromboxane A_2 that induces platelet aggregation [47].

In conclusion, among the five lignans (*l*-sesamin, helioxanthin, savinin, taiwanin C, and *cis*-dibenzylbutyrolactone) isolated from the medicinal plant *A. chiisanensis*, taiwanin C is the most potent inhibitor of TPA-induced PGE_2 production in rat peritoneal macrophages. It also inhibits the thapsigargin- and staurosporine-induced production of PGE_2. The mechanism of its action is the direct inhibition of COX-1 and COX-2 activity. Taiwanin C might be a lead compound for a COX inhibitor having no carboxylic acid. It is suggested that the anti-inflammatory activity of the extract of *A. chiisanensis* is partly due to the inhibition of PGE_2 production by the lignans.

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