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Tryptanthrin inhibits nitric oxide and prostaglandin E_2 synthesis by murine macrophages

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Abstract

Nitric oxide (NO) and prostaglandins have been implicated in the pathogenesis of several inflammatory diseases. In this study, we investigated the effect of tryptanthrin (6,12-dihydro-6,12-dioxoindolo-(2,1-*b*)-quinazoline), an antimicrobial and antitumoral plant compound isolated from *Porigonum tinctorium*, on NO and prostaglandin E_2 production by interferon-γ and lipopolysaccharide-stimulated murine macrophage-like RAW 264.7 cells. Tryptanthrin markedly inhibited both NO and prostaglandin E_2 production in a dose-dependent manner. Tryptanthrin at 20 μM fully inhibited expression of inducible NO synthase, suggesting that the inhibitory effect on NO synthesis was mediated by inhibited expression of the enzyme. On the other hand, tryptanthrin had no effect on the levels of cyclooxygenase-2 protein, but inhibited cyclooxygenase enzyme activity with a ICM_{50} value of 1.5 μM. Thus, tryptanthrin has the dual functions of inhibiting both NO and prostaglandin E_2 production by activated macrophages, suggesting that tryptanthrin exhibits anti-inflammatory properties.

Keywords: Tryptanthrin; Nitric oxide; Inducible NO synthase; Prostaglandin; Cyclooxygenase; Inflammation

1. Introduction

Macrophages synthesize various mediators, which modulate the inflammatory response. Nitric oxide (NO) and prostaglandins are two pleiotropic mediators produced at inflammatory sites by the enzymes NO synthases and cyclooxygenases, respectively (Appleton et al., 1996).

NO synthases can be classified into two major groups (Nathan and Xie, 1994). Neuronal and endothelial NO synthases are in general constitutively expressed, and NO produced by such isoforms is a key regulator of homeostasis. On the other hand, the inducible isoform of NO synthase plays an important role in the cytotoxic activity of activated macrophages (MacMicking et al., 1997). Stimuli such as cytokines and/or bacterial lipopolysaccharide induce inducible NO synthase expression, and once synthesized, inducible NO synthase is responsible for the prolonged, high-output production of NO. Despite its beneficial role in host defense, sustained NO production can be deleterious to the host, and has been implicated in the pathogenesis of various inflammatory diseases such as rheumatoid arthritis (Stefanovic-Racic et al., 1993).

Cyclooxygenase, which catalyzes the conversion of arachidonic acid to prostaglandin H_2, is a rate-limiting enzyme in the biosynthesis of prostaglandins (Smith et al., 1996; Dubois et al., 1998). Two isoforms of this enzyme have been described: the constitutive enzyme cyclooxygenase-1 is present in almost all types of cells and is thought to be involved in homeostatic prostanoid biosynthesis, whereas cyclooxygenase-2 is rapidly induced after stimulation with cytokines and/or lipopolysaccharide. Cyclooxygenase-2 is predominantly expressed in cells involved in inflammatory reactions such as macrophages, endothelial cells, and fibroblasts. In addition to sites of inflammation, cyclooxygenase-2 has also been found to be up-regulated in human colorectal adenomas and adenocarcinomas (Eberhart et al., 1994; Sano et al, 1995). Nonsteroidal anti-inflammatory drugs (NSAIDs) exert their

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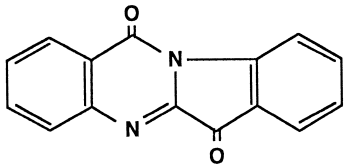

Fig. 1. Chemical structure of tryptanthrin.

therapeutic effects through blocking the synthesis of prostaglandins by inhibiting cyclooxygenase enzyme activity, although the inhibition of homeostatic prostaglandin production causes harmful side-effects such as gastrointestinal ulceration.

Natural plants are potential sources of novel anti-inflammatory drugs. *Polygonum tinctorium* is a well-known plant in Japan. It is a source of indigo to stain cloth, and has been used for the treatment of inflammation in oriental traditional medicine. We have previously reported that the crude extract of *P. tinctorium* suppresses NO production by activated macrophages, although the compounds exhibiting the activity had not been identified (Ishihara et al., 2000). Tryptanthrin, 6,12-dihydro-6,12-dioxoindolo-(2,1-*b*)-quinazoline (Fig. 1), is a naturally occurring compound found in *P. tinctorium*, and has been reported to exhibit antimicrobial (Honda et al., 1980; Hashimoto et al., 1999) and antitumoral properties (Kimoto et al., 1999). In this study, as a part of our search for new anti-inflammatory compounds from *P. tinctorium*, we examined the effect of tryptanthrin on the production of NO and prostaglandin E_2 by activated macrophages. We report here the novel anti-inflammatory activities for tryptanthrin, and its mechanisms of action with emphasis on the suppression of both NO and prostaglandin E_2 synthesis.

2. Materials and methods

2.1. Cell lines and reagents

Murine macrophage RAW 264.7 cells were obtained from the American Type Culture Collection (Manassas, VA). Tryptanthrin was purchased from Wako (Tokyo, Japan). Tryptanthrin was dissolved in dimethyl sulfoxide (DMSO) and further diluted in RPMI 1640 medium. Final concentrations of DMSO were 0.1 % or less, and did not affect the results of the experiments. Lipopolysaccharide (*E. coli* 055:B5) was obtained from Difco Laboratories (Detroit, MI). Murine recombinant interferon-γ was prepared and purified in our laboratories (Kohno et al., 1996). Indomethacin was purchased from Nakarai Chemical (Kyoto, Japan). N^G-monomethyl-L-arginine (L-NMMA) and NS-398 (*N*-[2-(cyclohexyloxy)-4-nitrophenyl]methanesulfonamide) were generously provided by T. Ohtani (Fujisaki Cell Center, Hayashibara Biochemical Laboratories).

2.2. Cell cultures and stimulation

RAW 264.7 cells were cultured in RPMI 1640 medium with 10% fetal calf serum (Life Technologies, Grand Island, NY). Cells were seeded at 5×10^4 cells per well in flat bottomed 96-well microtiter plates and stimulated with interferon-γ (10 IU/ml) and lipopolysaccharide (2 μg/ml) for 16–24 h.

2.3. Determination of NO and prostaglandin E_2

Levels of NO were estimated by the accumulation of the stable NO metabolite, nitrite anion by the Griess assay. Equal volumes of culture supernatants (50 μl) and Griess reagent (50 μl, 1% sulfanilamide/0.1% *N*-[naphthyl]ethylenediamine/2.5% H3PO4) were mixed at room temperature for 10 min, and the absorbance was measured at 570 nm. The amounts of nitrite were calculated against a $NaNO_2$ standard curve. The levels of prostaglandin E_2 accumulated in the supernatants were measured by enzyme immunoassay (Amersham Pharmacia Biotech, Tokyo, Japan).

2.4. Measurement of NO synthase activity in intact cells

RAW 264.7 cells were seeded at 5×10^4 cells per well in flat bottomed 96-well microtiter plates and stimulated with interferon-γ (10 IU/ml) and lipopolysaccharide (2 μg/ml) for 18 h to induce inducible NO synthase. The cells were washed twice with fresh culture medium and then incubated with or without tryptanthrin for a further 18 h in the medium in the absence of interferon-γ and lipopolysaccharide. The supernatants were harvested and the Griess assay was performed as described above.

2.5. Measurement of cyclooxygenase activity

For the assay on intact cells, RAW 264.7 cells were seeded at 5×10^4 cells per well in flat bottomed 96-well microtiter plates and stimulated with interferon-γ (10 IU/ml) and lipopolysaccharide (2 μg/ml) for 16 h to induce cyclooxygenase-2. The cells were washed twice with fresh culture medium and allowed to equilibrate in the absence or presence of tryptanthrin for 10 min. The cells were further incubated for 40 min in medium with 30 μM arachidonic acid in the absence of interferon-γ and lipopolysaccharide. The supernatants were removed and assayed for prostaglandin E_2 as described above.

Cyclooxygenase activities of the purified enzymes were determined by a cyclooxygenase inhibitor screening assay

kit (Cayman Chemical). Briefly, ovine cyclooxygenase-1 or cyclooxygenase-2 were preincubated with or without various concentrations of tryptanthrin in 0.1 M Tris–HCl (pH 8.0) containing 5 mM EDTA, 2 mM phenol and 1 μM heme at 37°C for 2 min. The reactions were initiated by adding arachidonic acid to give a final concentration of 100 μM, and incubated at 37°C for a further 2 min. One twentieth volume of 1 M HCl was added to the reaction mixtures to stop enzyme catalysis, followed by adding one tenth volume of saturated stannous chloride (50 mg/ml). The reaction mixtures were incubated for 5 min at room temperature, and then the amounts of prostaglandin E_2 formed during the reaction were measured by enzyme immunoassay.

2.6. Cell lysate preparation and Western blot analysis

RAW 264.7 cells (5×10^6 cells) were grown in 25 cm^2 culture flasks and were then stimulated with interferon-γ (10 IU/ml) and lipopolysaccharide (2 μg/ml) in the presence or absence of tryptanthrin for 16 h. Cells were lysed on ice for 30 min in extraction buffer containing 0.5% NP-40, 0.15 M NaCl, 2 mM EDTA(4NA), 50 mM Tris, 10 mM iodoacetamide and 1 mM phenylmethylsulfonylfluoride at 2×10^7 cells/ml. Nuclei and cell debris were removed by centrifugation at $500 \times g$ for 10 min. Cell lysates (30 μg) were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Multigel 2/15; Daiichi Pure Chemicals, Tokyo, Japan) under reducing conditions, and were transferred to nitrocellulose membranes (Micron Separations, Westborough, MA) by semi-dry electrophoretic transfer. The nitrocellulose membranes were blocked with a solution containing 10% Block Ace (Dainippon Pharmaceutical, Osaka, Japan) for 1 h and were incubated with anti-mouse inducible NO synthase antibody (Ab) (1/500 dilution) (Wako) or anti-mouse cyclooxygenase-2 Ab (1/250 dilution) (Transduction Laboratories, Lexington, KY) as primary Abs for 1 h. After removing the primary Abs, membranes were washed three times with Tris-buffered saline (TBS) containing 0.05% tween 20, and then incubated with a 1:1000 dilution of secondary Ab conjugated to horseradish peroxidase (Dako, Glostrup, Denmark) for 1 h. The membranes were again washed three times and reaction products were visualized using an enhanced chemoluminescence (ECL) Western blot system (Amersham).

2.7. Statistical analysis

Results were analyzed by the Student's unpaired *t*-test and P values of < 0.05 were taken to be statistically significant.

3. Results

3.1. Effect of tryptanthrin on NO production by the murine macrophage cell line

To investigate the anti-inflammatory effects of tryptanthrin, we examined whether tryptanthrin could modulate NO synthesis in interferon-γ/lipopolysaccharide-stimulated cultures of the murine macrophage cell line, RAW 264.7. Stimulation of RAW 264.7 cells with interferon-γ and lipopolysaccharide resulted in the generation of nitrite, a stable end product of NO metabolism. As shown in Fig. 2, tryptanthrin dose-dependently inhibited NO production by RAW 264.7 cells, as did N^G-monomethyl-L-arginine (L-NMMA), a specific inhibitor of NO synthase enzyme activity. Cell viability was not altered by tryptanthrin at these concentrations as determined by methylthiazol-2-yl-2,5-diphenyl tetrazolium bromide (MTT) assay (data not shown), indicating that inhibition of NO production by tryptanthrin is not simply due to a cytotoxic effect.

To determine whether the reduction of nitrite accumulation by tryptanthrin is a result of direct inhibition of inducible NO synthase enzyme activity or due to inhibition of its expression, we examined whether tryptanthrin could also suppress NO production by RAW 264.7 cells in which inducible NO synthase expression had already been induced. Table 1 shows that addition of tryptanthrin to RAW

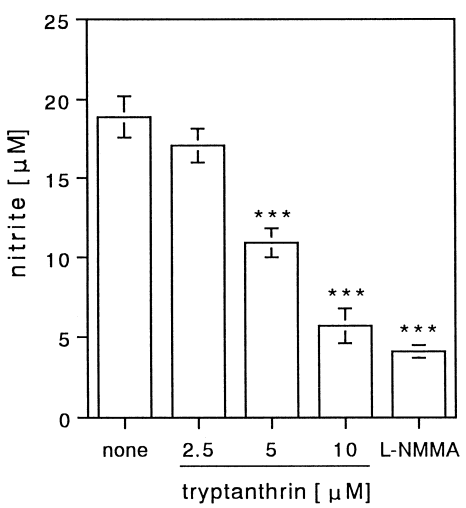

Fig. 2. Effect of tryptanthrin on NO synthesis by interferon-γ/lipopolysaccharide-stimulated RAW 264.7 cells. RAW 264.7 cells were stimulated with interferon-γ (10 IU/ml) and lipopolysaccharide (2 μg/ml) for 18–24 h in the presence or absence of various concentrations of tryptanthrin or L-NMMA (1 mM). The amounts of nitrite accumulated in the supernatant were determined by Griess assay. The data represent the mean ± SD of three independent experiments. The P values represent comparisons with interferon-γ/lipopolysaccharide-stimulated control cells: $^{***}P < 0.005$.

Table 1
Effect of tryptanthrin on NO synthase activity in RAW 264.7 cells pretreated with interferon-γ and lipopolysaccharide

Pretreatment of cells	Addition to pretreated cells	Nitrite [μM]
None	none	0.8 ± 0.8
IFN-γ/LPS (18h)	none	13.8 ± 3.0
	tryptanthrin (5 μM)	14.7 ± 4.1
	tryptanthrin (10 μM)	13.5 ± 3.7
	L-NMMA (1 mM)	2.1 ± 0.7[a]

Raw 264.7 cells were stimulated with interferon-γ (10 IU/ml) and lipopolysaccharide (2 μg/ml) for 18 h. Cells were washed twice with fresh culture medium, and then incubated in the presence or absence of various concentrations of tryptanthrin or L-NMMA for a further 24 h. The levels of nitrite accumulated in the supernatants were determined by Griess assay. The data represent the mean ± SD of three independent experiments. The *P* values represent comparisons with interferon-γ/lipopolysaccharide-pretreated control cells.
[a] $P < 0.01$.

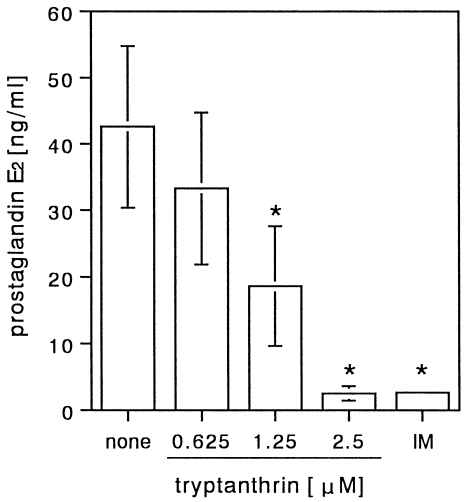

Fig. 4. Effect of tryptanthrin on prostaglandin E_2 synthesis by interferon-γ/lipopolysaccharide-stimulated RAW 264.7 cells. RAW 264.7 cells were stimulated with interferon-γ (10 IU/ml) and lipopolysaccharide (2 μg/ml) for 18–24 h in the presence or absence of various concentrations of tryptanthrin or indomethacin (IM; 0.5 μM). Supernatants were collected, and prostaglandin E_2 concentrations were determined by enzyme immunoassay. The data represent the mean ± SD of three independent experiments. The *P* values represent comparisons with interferon-γ/lipopolysaccharide-stimulated control cells : $^{*}P < 0.05$.

264.7 cells, which had been pretreated with interferon-γ and lipopolysaccharide to induce inducible NO synthase, failed to inhibit NO synthesis. In contrast, L-NMMA inhibited NO synthesis even when added after macrophage activation. The expression of inducible NO synthase protein had reached a plateau level at this time point as determined by Western blot analysis (data not shown). These results suggest that tryptanthrin does not inhibit inducible NO synthase catalytic activity directly, and further suggest that it may block early events in inducible NO synthase expression.

3.2. Effect of tryptanthrin on inducible NO synthase expression

We next investigated whether tryptanthrin might affect levels of inducible NO synthase protein expression. Stimulation of RAW 264.7 cells with interferon-γ and lipopolysaccharide resulted in accumulation of the 130 kDa inducible NO synthase protein as determined by Western blot analysis (Fig. 3). Tryptanthrin reduced the levels of inducible NO synthase protein in a dose-dependent manner. Densitometer scans of the respective blot showed that at concentrations of 5, 10 and 20 μM of tryptanthrin, the levels of inducible NO synthase protein were reduced by 70, 54 and 10 %, respectively, compared with control cells stimulated with interferon-γ/lipopolysaccharide only. Since the amounts of inducible NO synthase protein correlated with the reduced accumulation of nitrite, these results

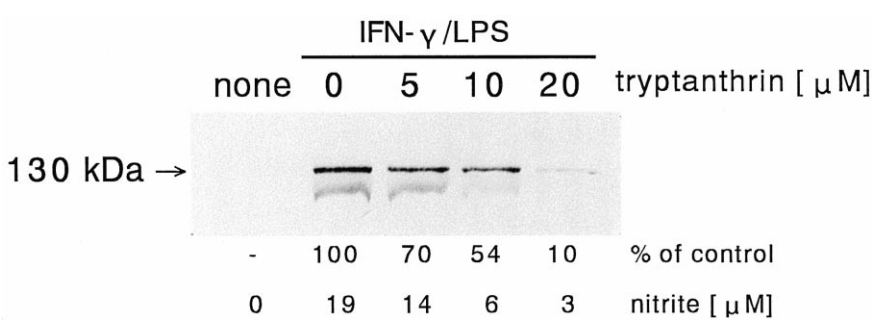

Fig. 3. Western blot analysis of inducible NO synthase protein in interferon-γ/lipopolysaccharide-stimulated RAW 264.7 cells. RAW 264.7 cells were stimulated with interferon-γ (10 IU/ml) and lipopolysaccharide (2 μg/ml) for 16 h in the presence or absence of various concentrations of tryptanthrin. Total lysates (30 μg/lane) were separated on SDS-PAGE and blotted with anti-inducible NO synthase antibody. The relative intensities of the bands were quantified by densitometry and are presented below the respective lanes. The levels of nitrite in the supernatants are also shown. The result shows one of three representative experiments with similar results.

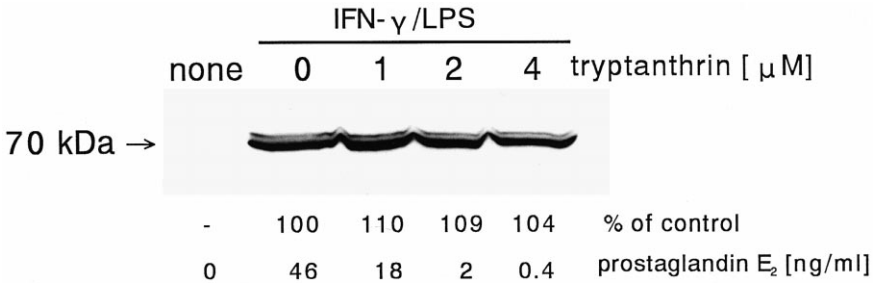

Fig. 5. Western blot analysis of cyclooxygenase-2 protein in interferon-γ/lipopolysaccharide-stimulated RAW 264.7 cells. RAW 264.7 cells were stimulated with interferon-γ (10 IU/ml) and lipopolysaccharide (2 μg/ml) for 16 h in the presence or absence of various concentrations of tryptanthrin. Total lysates (30 μg/lane) were separated on SDS-PAGE and were blotted with anti-cyclooxygenase-2 antibody. The relative intensities of the bands were quantified by densitometry and are presented below the respective lanes. The levels of prostaglandin E_2 in the supernatants are also shown. The result shows one of three representative experiments with similar results.

suggest that tryptanthrin inhibits NO synthesis through a reduction in inducible NO synthase protein expression.

3.3. Effect of tryptanthrin on prostaglandin E_2 synthesis in the macrophage cell line

In addition to nitrite accumulation, stimulation of RAW 264.7 cells with interferon-γ/lipopolysaccharide increased the synthesis of prostaglandin E_2, another inflammatory mediator produced by activated macrophages. As shown in Fig. 4, tryptanthrin suppressed prostaglandin E_2 production in a dose-dependent manner. At a concentration of 2.5 μM, the inhibitory effect of tryptanthrin is comparable to that of indomethacin (0.5 μM), a well-known non-selective cyclooxygenase inhibitor. Furthermore, 2.5 μM of tryptanthrin significantly inhibited prostaglandin E_2 production, while no significant inhibition of NO production was observed at this concentration (Figs. 2 and 4), indicating that the inhibitory activity of tryptanthrin on prostaglandin E_2 production is more effective than that on NO production.

It has been reported that induction of both inducible NO synthase and cyclooxygenase-2 is regulated by common transcription factors such as nuclear factor-κB (NF-κB) (Xie et al., 1994; Crofford et al., 1997). Since tryptanthrin inhibited inducible NO synthase expression, we next examined whether tryptanthrin would also affect the expression of cyclooxygenase-2 protein. Western blot analysis revealed that a 70-kDa cyclooxygenase-2 specific band appeared when cells were stimulated with interferon-γ and lipopolysaccharide (Fig. 5). Tryptanthrin did not change the levels of cyclooxygenase-2 protein even at a concentration of 4 μM, while this concentration of tryptanthrin completely suppressed prostaglandin E_2 generation. This result suggests that tryptanthrin inhibits prostaglandin E_2 formation by mechanisms other than inhibition of cyclooxygenase-2 expression.

3.4. Effect of tryptanthrin on cyclooxygenase enzyme activity

We next investigated whether the inhibitory effect of tryptanthrin on prostaglandin E_2 production would be mediated by the inhibition of cyclooxygenase enzyme activity. For this purpose, RAW 264.7 cells were stimulated with interferon-γ/lipopolysaccharide for 16 h to induce cyclooxygenase-2, then arachidonic acid was added as a substrate with or without tryptanthrin. Western blot analysis revealed that the levels of cyclooxygenase-2 protein had reached a plateau level at this time point (data not shown). As shown in Table 2, tryptanthrin, when added to RAW 264.7 cells after cyclooxygenase-2 expression, significantly inhibited prostaglandin E_2 production with IC_{50} values of approximately 1.5 μM. These results suggest that the inhibition of prostaglandin E_2 production by tryptanthrin is a result of the inhibition of cyclooxygenase enzyme activity. Further confirmation of this result was obtained from experiments to examine the effect of tryptanthrin on purified ovine cyclooxygenase enzyme. As shown in Fig. 6, tryptanthrin markedly inhibited the enzyme activity of both cyclooxygenase-1 and cyclooxygenase-2, as did indomethacin. The potency of inhibition of tryptanthrin on both cyclooxygenase isoforms was al-

Table 2
Effect of tryptanthrin on cyclooxygenase-2 activity in RAW 264.7 cells pretreated with interferon-γ and lipopolysaccharide

Pretreatment of cells	Addition to pretreated cells	Prostaglandin E_2 [ng/ml]
None	none	0.35 ± 0.07
IFN-γ/LPS (16 h)	none	4.33 ± 0.42
	tryptanthrin (1 μM)	2.68 ± 0.94[a]
	tryptanthrin (2 μM)	1.46 ± 0.23[b]
	tryptanthrin (4 μM)	0.68 ± 0.22[b]

RAW 264.7 cells were stimulated with interferon-γ (10 IU/ml) and lipopolysaccharide (2 μg/ml) for 16 h. Cells were washed twice with fresh culture medium, and then incubated with arachidonic acid (30 μM) in the presence or absence of various concentrations of tryptanthrin for a further 40 min. The levels of prostaglandin E_2 in the supernatants were determined by enzyme immunoassay. The data represent the mean ± SD of three independent experiments. The P values represent comparisons with interferon-γ/lipopolysaccharide-pretreated control cells.

[a] $P < 0.05$.

[b] $P < 0.005$.

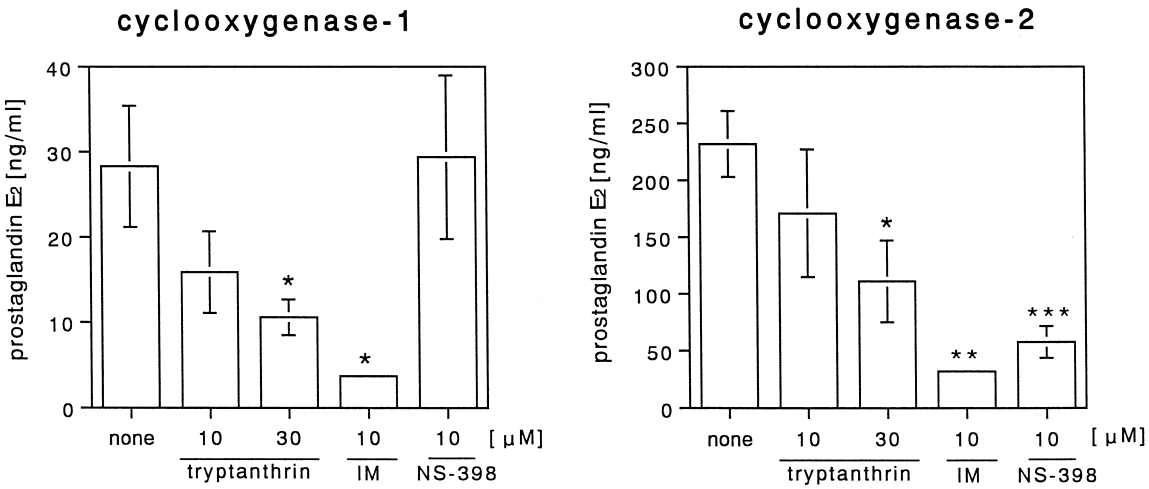

Fig. 6. Effect of tryptanthrin, indomethacin (IM) and NS-398 on cyclooxygenase enzyme activity. Cyclooxygenase enzyme activities were determined by cyclooxygenase inhibitor screening assay kit (Cayman Chemical) as described in Materials and methods. The activities are shown as the concentrations of prostaglandin E_2 produced in the reaction. The data represent the mean ± SD of three independent experiments. The P values represent comparisons with control: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.

most the same, indicating that tryptanthrin does not show selectivity towards either cyclooxygenase isoform. In contrast, the highly selective cyclooxygenase-2 inhibitor, NS-398, only inhibited cyclooxygenase-2 activity without affecting cyclooxygenase-1 activity.

4. Discussion

Tryptanthrin, one of the major compounds extracted from the medical plant *P. tinctorium*, has been known for its antimicrobial and anticarcinogenic effects. In our search for new anti-inflammatory drugs, we identified novel activities of tryptanthrin, namely, to suppress both NO and prostaglandin E_2 production by activated macrophages, and have revealed the molecular mechanisms of its actions.

Tryptanthrin did not inhibit NO production by activated macrophages when inducible NO synthase expression had already been induced. Although we did not measure NO synthase activity directly, this result suggests that tryptanthrin has no significant effect on NO synthase enzyme activity. Western blotting showed that inducible NO synthase protein levels were decreased after treatment with tryptanthrin, and this decrease correlated with the accumulation of nitrite. All these results indicate that tryptanthrin inhibits NO synthesis through a reduction in inducible NO synthase expression. Tryptanthrin may block de novo synthesis of inducible NO synthase or render inducible NO synthase more susceptible to degradation at the mRNA and/or protein levels.

In contrast to its action of suppressing inducible NO synthase expression, tryptanthrin did not change the levels of cyclooxygenase-2 protein. Inducible NO synthase and cyclooxygenase-2 share common mechanisms that regulate their gene expressions. Pro-inflammatory stimuli such as lipopolysaccharide, interferon-γ, interleukin-1β, and tumor necrosis factor-α are known to induce transcription of both inducible NO synthase and cyclooxygenase-2 genes through common transcription factors like NF-κB (Barnes and Karin, 1997). However, from our result that tryptanthrin inhibited inducible NO synthase expression without affecting cyclooxygenase-2 expression, it is unlikely that the target of the action of tryptanthrin is a common component involved in the signaling pathways leading to inducible NO synthase and cyclooxygenase-2 expression.

We found that the inhibitory effect of tryptanthrin on prostaglandin E_2 production was mediated by direct inhibition of cyclooxygenase enzyme activity. Of the two cyclooxygenase enzyme isoforms, cyclooxygenase-2 is thought to be the isoform predominantly involved in inflammatory responses. Therapeutic benefits of NSAIDs may derive from the inhibition of the cyclooxygenase-2 enzyme, whereas inhibition of the cyclooxygenase-1 enzyme may account for some of their adverse-effects (van Ryn and Pairet, 1999). Therefore, selective cyclooxygenase-2 inhibitors are expected to bypass the adverse-effects associated with cyclooxygenase-1 inhibition, although most NSAIDs used clinically inhibit both isoforms of cyclooxygenase. Unfortunately, tryptanthrin did not show selectivity towards the cyclooxygenase-2 isoform. However, it is known that selective cyclooxygenase-2 inhibitors only partially reduce the levels of prostaglandins at the sites of either acute or chronic inflammation, in comparison to NSAIDs that suppress both cyclooxygenase isoforms (Gilroy et al., 1998a,b; Wallace et al., 1998, 1999). Therefore, cyclooxygenase-1 may contribute, in part, to some inflammatory responses and inhibition of cyclooxygenase-1 as well as cyclooxygenase-2 may be necessary to achieve the desired anti-inflammatory effect.

Recent reports have shown that NO can positively or negatively affect cyclooxygenase activity or expression depending on the cell type (Corbett et al., 1993; Salvemini et al., 1996; Habib et al., 1997). For instance, NO stimulates cyclooxygenase activity in lipopolysaccharide-stimulated RAW 264.7 cells (Salvemimi et al., 1993), although contradictory results have also been reported (Patel et al., 1999). Therefore, it is possible that the effect of tryptanthrin at inhibiting prostaglandin E_2 production may be the result of inhibiting NO formation. However, tryptanthrin suppresses prostaglandin E_2 production at concentrations that have no influence on NO formation. Furthermore, tryptanthrin directly inhibited the activity of purified cyclooxygenase enzyme. These results suggest that the inhibitory effect of tryptanthrin on prostaglandin E_2 production is not a result of inhibition of NO formation. On the other hand, it has been reported that inhibition of prostaglandin E_2 production by some NSAIDs such as indomethacin causes a reduction in NO formation in the murine macrophage cell line, J774 (Lin et al., 1999). However, in our experiments using RAW 264.7, indomethacin inhibited prostaglandin E_2 production without affecting NO synthesis (data not shown). Therefore, prostaglandin E_2 is unlikely to be a regulator of NO synthesis at least in RAW 264.7 cells. Taken together, we conclude that the inhibitory effects of tryptanthrin on NO and prostaglandin E_2 production are independent of each other.

In summary, our data demonstrate that tryptanthrin inhibits both NO and prostaglandin E_2 production by separate mechanisms, suppression of inducible NO synthase expression and inhibition of cyclooxygenase activity, respectively. These results suggest that tryptanthrin exhibits anti-inflammatory properties and further imply that tryptanthrin may be therapeutically useful in the treatment of inflammatory diseases.

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