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Dual effects of auranofin on prostaglandin E_2 production by rat peritoneal macrophages

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Abstract

Incubation of rat peritoneal macrophages in medium containing various concentrations of auranofin (1, 3 and 10 μM) increased prostaglandin E_2 production at 4 h in a concentration-dependent manner, in accordance with the increase in the release of [^{3}H]arachidonic acid from membrane phospholipids. However, at 20 h, no stimulation of prostaglandin E_2 production by auranofin was observed. When the peritoneal macrophages were incubated in the presence of 12-*O*-tetradecanoylphorbol 13-acetate (TPA), thapsigargin or A23187, prostaglandin E_2 production at 4 and 20 h was enhanced. The stimulator-induced prostaglandin E_2 production at 20 h was suppressed by 10 μM of auranofin. Western blot analysis demonstrated that auranofin inhibited the induction of cyclooxygenase 2 by TPA, thapsigargin or A23187 at 4 and 20 h. The level of cyclooxygenase 1 did not change by treatment with these stimulators in the presence or absence of auranofin. These findings suggest that auranofin has dual effects on prostaglandin E_2 production: without stimulation, auranofin increases prostaglandin E_2 production at 4 h due to the increased release of arachidonic acid which is converted to prostaglandin E_2 mainly by cyclooxygenase 1, but inhibits the stimulator-induced late-phase prostaglandin E_2 production by inhibiting the induction of cyclooxygenase 2. © 1997 Elsevier Science B.V.

Keywords: Auranofin; Prostaglandin E_2; 12-*O*-Tetradecanoylphorbol 13-acetate; Thapsigargin; A23187; Cyclooxygenase 2; Cyclooxygenase 1; Macrophage

1. Introduction

Auranofin (2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*) (triethylphosphine) gold, an orally active chrysotherapeutic agent, is used for the treatment of inflammatory joint diseases such as rheumatoid arthritis (Ward et al., 1983; Wenger et al., 1983) and psoriatic arthritis (Carette et al., 1989). It inhibits lysosomal enzyme release (DiMartino and Waltz, 1977; Finkelstein et al., 1977), chemotaxis (Scheinberg et al., 1982; Hafstrom et al., 1983), phagocytosis (Hafstrom et al., 1983) and superoxide generation (Davis et al., 1983) in leukocytes. As to the effect of auranofin on the production of arachidonate metabolites, Parente et al. (1986) reported that auranofin enhances production of leukotriene B_4 and C_4 but does not affect prostaglandin E_2 production in human peripheral polymorphonuclear leukocytes stimulated with formyl-methionyl-leucyl-phenylalanine. However, Honda et al. (1987) and Elmgreen et al. (1989) reported that auranofin inhibits leukotriene B_4 production in human peripheral polymorphonuclear leukocytes stimulated with A23187. In rat alveolar macrophages, auranofin stimulates arachidonic acid release and production of prostaglandin E_2 and thromboxane A_2 (Peters-Golden and Shelly, 1988), and inhibits 5-lipoxygenase metabolism (Peters-Golden and Shelly, 1989). In contrast, Lewis et al. (1984) reported that auranofin inhibits the zymosan-induced prostaglandin E_2 production by peritoneal macrophages in collagen arthritic rats. Therefore, the effects of auranofin on arachidonate metabolism are conflicting. In the present investigation, we examined the effects of auranofin on prostaglandin E_2 production in rat peritoneal macrophages under several incubation conditions to analyze the mechanism of action of auranofin.

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2. Materials and methods

2.1. Preparation of rat peritoneal macrophages

A solution of soluble starch (Wako, Osaka, Japan) and bacto peptone (Difco, Detroit, MI, USA), 5% each, was injected intraperitoneally into male Sprague-Dawley rats (300–350 g, specific pathogen-free, Charles River Japan, Kanagawa, Japan) at a dose of 5 ml per 100 g body weight. Four days later, the rats were killed by cutting the carotid artery under diethylether anesthesia and peritoneal cells were harvested (Ohuchi et al., 1985). The animal experiments were done in accordance with the procedure approved by the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Tohoku University, Japan.

2.2. Macrophage culture

The peritoneal cells were suspended in Eagle's minimal essential medium (EMEM, Nissui, Tokyo, Japan) containing 10% calf serum (Flow Laboratories, North Rydge, Australia), penicillin G potassium (18 μg/ml) and streptomycin sulphate (50 μg/ml) (Meiji Seika, Tokyo, Japan), then seeded at a density of 6×10^6 cells per plastic tissue culture dish (50 mm in diameter, Corning Glass Works, Corning, NY, USA) in 4 ml of medium, and incubated for 2 h at 37°C. The dishes were then washed three times with medium to remove non-adherent cells, and the adherent cells were further incubated for 20 h. After three washes, the adherent cells were used for the following experiments. More than 95% of the adherent cells were identified as macrophages (Ohuchi et al., 1981).

2.3. Incubation of macrophages with drugs

The adherent cells were incubated for 4 or 20 h at 37°C in 4 ml of medium containing 10% calf serum and various concentrations of auranofin (Sigma, St. Louis, MO, USA) in the presence or absence of drugs. The drugs used were the protein kinase C activator, 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (Nishizuka, 1992), the endomembrane Ca^{2+}-ATPase inhibitor, thapsigargin (Thastrup et al., 1987; Ohuchi et al., 1988; Watanabe et al., 1995), and the Ca^{2+} ionophore, A23187 (Sigma). The drugs were dissolved in ethanol and added to the medium. The final concentration of ethanol was adjusted to 0.1% (v/v). The control medium contained the same amount of ethanol.

2.4. Measurement of prostaglandin E_2 concentrations

4 or 20 h after incubation, the conditioned medium was obtained, centrifuged at $1500 \times g$ and 4°C for 5 min, and prostaglandin E_2 concentrations in the supernatant fraction were radioimmunoassayed (Ohuchi et al., 1985). Prostaglandin E_2 antiserum was purchased from PerSeptive Diagnostics (Cambridge, MA, USA).

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

The peritoneal cells were collected and 6×10^6 cells were incubated for 2 h, washed three times to remove non-adherent cells, and further incubated for 18 h in medium containing 10% calf serum. The cells were then washed three times and incubated for 2 h at 37°C in 4 ml of medium containing 10% calf serum and 37 kBq of [^{3}H]arachidonic acid (2.26 TBq/mmol, New England Nuclear, Boston, MA, USA). The adherent cells were washed three times with medium to remove free [^{3}H]arachidonic acid, and incubated in 4 ml of medium containing 10% calf serum and various concentrations of auranofin. The conditioned medium was withdrawn at 4 h, centrifuged at $1500 \times g$ and 4°C for 15 min, and the radioactivity of the supernatant fraction was determined (Ohuchi et al., 1988).

2.6. Western blot analysis of cyclooxygenase 1 and cyclooxygenase 2

4 or 20 h after incubation in 4 ml of medium in the presence or absence of each stimulator and 10 μM of auranofin, the cells were washed three times with phosphate-buffered saline (PBS, pH 7.4), scraped off the plate, and centrifuged at $800 \times g$ for 5 min at 4°C. The precipitate was sonicated for five times using a Handy Sonic Disrupter (UR-20P, TOMY, Tokyo, Japan) at 90% maximum power for 10 s at a time in 1 ml of the ice-cold solubilization buffer (Tris, 50 mM; EDTA, 10 mM; Tween 20, 1% (v/v); *N,N*-dimethyldithiocarbamate, 1 mM; phenylmethylsulphonyl fluoride, 1 mM; pepstatin A, 10 μM; and leupeptin, 10 μM; pH 8.0). The sonicates were centrifuged at $100\,000 \times g$ and 4°C for 1 h, and the supernatant fractions were obtained. Protein concentrations in the supernatant fractions were determined (Wang and Smith, 1975), and an aliquot of 30 μg protein was then boiled for 3 min at a ratio of 1:1 (v/v) with 2 × gel loading buffer (Tris, 50 mM; SDS, 4% (w/v); glycerol, 10% (v/v); 2-mercaptoethanol, 4% (v/v); and bromophenol blue, 0.05 mg/ml; pH 7.4). Samples were then loaded onto gradient gels (4–10% Tris-glycine, pH 8.3) and subjected to electrophoresis (4 h at 15 mA). The separated proteins were transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA, USA; 2 h at 150 mA). After transfer to the nitrocellulose membrane, the blot was incubated in the blocking solution (Block Ace, Dainippon, Osaka, Japan) at room temperature for 1 h and then incubated with primary antibodies at 23°C for 2 h. The antibodies used were a rabbit antibody to murine cyclooxygenase 2 (dilution 1:100, Oxford Biomedical Research, Ann Arbor, MI, USA) and a goat antibody to sheep seminal cyclooxygenase 1 (dilution 1:25 000, Oxford Biomedical Research). The blot was then incubated with secondary antibodies (dilution 1:2000), which were an anti-rabbit immunoglobulin G (Vector Laboratories,

Burlingame, CA, USA) for cyclooxygenase 2, and an anti-goat immunoglobulin G (Vector Laboratories) for cyclooxygenase 1, at 4°C for 3 h. Finally, the blot was incubated with Vectastain ABC reagent (Vector Laboratories) at room temperature for 30 min. The blot was then incubated with ECL detection reagent (Amersham International, Amersham, UK) at room temperature for 2 min, exposed to Kodak X-OMAT AR film at room temperature for 50 s, and photographed. The cyclooxygenase 1 and cyclooxygenase 2 levels were quantified by scanning densitometry, and individual band density values for each point were expressed as the relative density signal.

2.7. Statistical analysis

Results were analyzed for statistical significance by Dunnett's test for multiple comparison and Student's *t*-test for unpaired observations.

3. Results

3.1. Effects of auranofin on the spontaneous prostaglandin E_2 production by macrophages

When the peritoneal macrophages were incubated in medium containing 10% calf serum and various concentrations of auranofin, the prostaglandin E_2 concentrations in the conditioned medium at 4 h were increased significantly by auranofin at concentrations of 1, 3 and 10 μM in a concentration-dependent manner (Fig. 1). However, the stimulative effects of auranofin were not observed when examined at 20 h (Fig. 1). Time-course experiments revealed that at 8 h, 3 and 10 μM of auranofin significantly increased prostaglandin E_2 production ($P < 0.01$); at 12 h, only 10 μM of auranofin enhanced prostaglandin E_2 production ($P < 0.01$); and at 20 h, no significant stimulation was induced at any concentrations of auranofin.

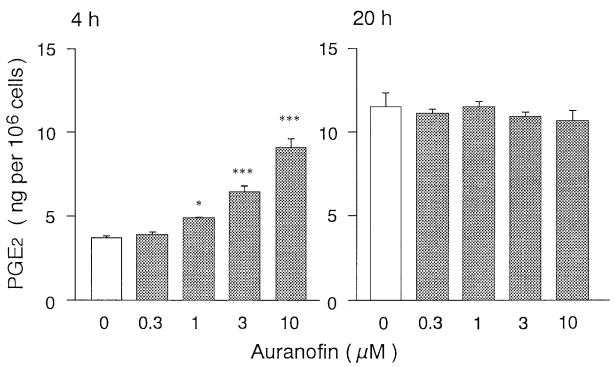

Fig. 1. Effects of various concentrations of auranofin on spontaneous prostaglandin E_2 production in rat peritoneal macrophages. Rat peritoneal macrophages (6×10^6 cells) were incubated at 37°C for 4 or 20 h in 4 ml of medium containing 10% calf serum and indicated concentrations of auranofin. Prostaglandin E_2 (PGE_2) concentrations in the conditioned medium were radioimmunoassayed. Values are the means from four samples with S.E.M. shown by vertical bars. Similar results were obtained in three additional experiments. Statistical significance: $^{*}P < 0.05$, $^{***}P < 0.001$ vs. the control.

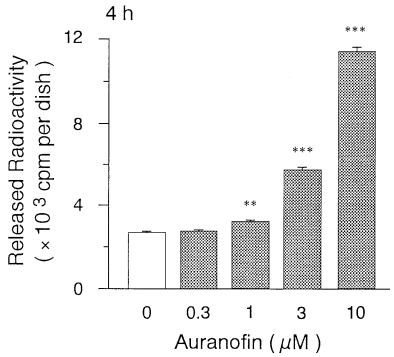

Fig. 2. Effects of auranofin on the release of radioactivity from [^{3}H]arachidonic acid-labeled macrophages. Rat peritoneal macrophages (6×10^6 cells) were incubated at 37°C for 2 h in medium containing 10% calf serum and 37 kBq of [^{3}H]arachidonic acid. After three washes, the cells were incubated at 37°C for 4 h in 4 ml of medium containing various concentrations of auranofin, and the radioactivity released into the medium was determined. Values are the means from four samples with S.E.M. shown by vertical bars. Similar results were obtained in three additional experiments. Statistical significance: $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the control.

3.2. Effects of auranofin on the release of radioactivity from [^{3}H]arachidonic acid-labeled macrophages

Auranofin at concentrations of 1, 3 and 10 μM increased the release of radioactivity from [^{3}H]arachidonic acid-labeled macrophages in a concentration-dependent manner when determined at 4 h (Fig. 2), in accordance with the increase in prostaglandin E_2 production as shown in Fig. 1.

3.3. Effects of auranofin on prostaglandin E_2 production in TPA-stimulated macrophages

The peritoneal macrophages were incubated in medium containing TPA (16.2 nM, 10 ng/ml) with or without 10 μM auranofin. TPA treatment stimulated prostaglandin E_2 production time-dependently, but in the presence of 10 μM of auranofin, TPA-induced prostaglandin E_2 production at 8, 12, and 20 h was inhibited. The concentrations of prostaglandin E_2 in the conditioned medium of the group treated with TPA and auranofin were almost the same as that of the group treated with auranofin alone (Fig. 3). At 4 h, TPA-induced prostaglandin E_2 production was not affected by auranofin. Without TPA, auranofin enhanced prostaglandin E_2 production at 4 h, and thereafter prostaglandin E_2 production increased gradually. At 20 h, there was no significant difference in prostaglandin E_2 levels between the control and auranofin-treated groups. TPA-induced prostaglandin E_2 production at 20 h was not inhibited by 1 μM of auranofin, but was significantly ($P <$

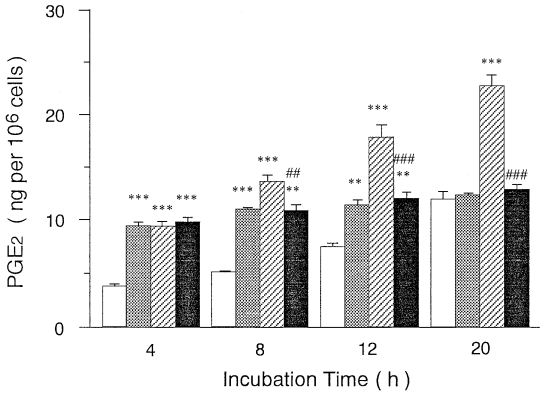

Fig. 3. Time course of the effects of auranofin on TPA-induced prostaglandin E_2 production in rat peritoneal macrophages. Rat peritoneal macrophages (6×10^6 cells) were incubated at 37°C for various periods in 4 ml of medium containing 10% calf serum in the presence or absence of TPA (16.2 nM, 10 ng/ml) and 10 μM of auranofin. Prostaglandin E_2 (PGE_2) concentrations in the conditioned medium were radioimmunoassayed. Control (blank columns), auranofin 10 μM (shadowed columns), TPA 16.2 nM (hatched columns), and auranofin 10 μM with TPA 16.2 nM (closed columns) are shown. Values are the means from four samples with S.E.M. shown by vertical bars. Statistical significance: $^{**}P<0.01$, $^{***}P<0.001$ vs. the corresponding control, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. their corresponding TPA control.

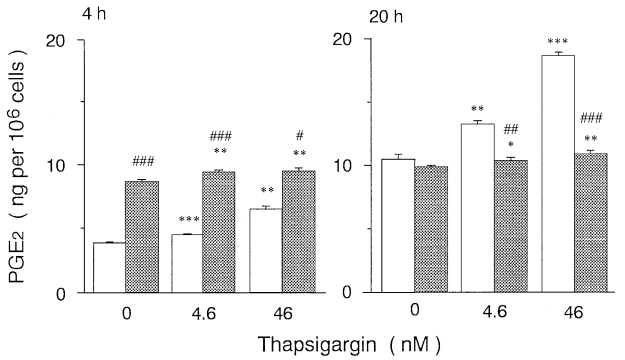

Fig. 4. Effects of auranofin on prostaglandin E_2 production in thapsigargin-stimulated macrophages. Rat peritoneal macrophages (6×10^6 cells) were incubated at 37°C for 4 and 20 h in 4 ml of medium containing 10% calf serum in the presence or absence of thapsigargin (4.6 and 46 nM, 3 and 30 ng/ml, respectively) and 10 μM of auranofin. Prostaglandin E_2 (PGE_2) concentrations in the conditioned medium were radioimmunoassayed. Without auranofin, blank columns; and with auranofin, shadowed columns. Values are the means from four samples with S.E.M. shown by vertical bars. Similar results were obtained in three additional experiments. Statistical significance: $^{**}P<0.01$, $^{***}P<0.001$ vs. the control. $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. the corresponding auranofin-free control.

0.001) inhibited by 3 μM of auranofin (prostaglandin E_2 (ng per 10^6 cells): TPA, 22.4 ± 1.0; TPA + auranofin 3 μM, 18.6 ± 0.5; TPA + auranofin 10 μM, 12.3 ± 0.6; means ± S.E.M. from 4 samples).

3.4. Effects of auranofin on prostaglandin E_2 production in thapsigargin-stimulated macrophages

The peritoneal macrophages were incubated in medium containing various concentrations of thapsigargin with or without 10 μM of auranofin. Thapsigargin (4.6 and 46 nM, 3 and 30 ng/ml, respectively) stimulated prostaglandin E_2 production in a concentration-dependent manner at 4 and 20 h (Fig. 4). At 4 h, auranofin enhanced prostaglandin E_2 production in the presence or absence of thapsigargin. However, when determined at 20 h, 10 μM of auranofin inhibited thapsigargin-induced prostaglandin E_2 production, but not the spontaneous prostaglandin E_2 production (Fig. 4). At 20 h, 3 μM of auranofin significantly ($P<0.001$) inhibited thapsigargin-induced prostaglandin E_2 production, and 1 μM of auranofin showed no inhibitory effect (data not shown).

3.5. Effects of auranofin on prostaglandin E_2 production in A23187-stimulated macrophages

The peritoneal macrophages were incubated in medium containing various concentrations of A23187 with or without 10 μM of auranofin. A23187 at 0.3 and 1 μM (0.18 and 0.52 μg/ml, respectively) stimulated prostaglandin E_2 production in a concentration-dependent manner at 4 and 20 h (Fig. 5). At 4 h, auranofin enhanced prostaglandin E_2 production in the presence or absence of A23187. However, when determined at 20 h, auranofin inhibited A23187-induced prostaglandin E_2 production, but not the spontaneous prostaglandin E_2 production (Fig. 5). At 20 h, 3 μM of auranofin significantly ($P<0.001$) inhibited A23187-induced prostaglandin E_2 production, and 1 μM of auranofin showed no inhibitory effect (data not shown). Inhibition by auranofin of prostaglandin E_2 production

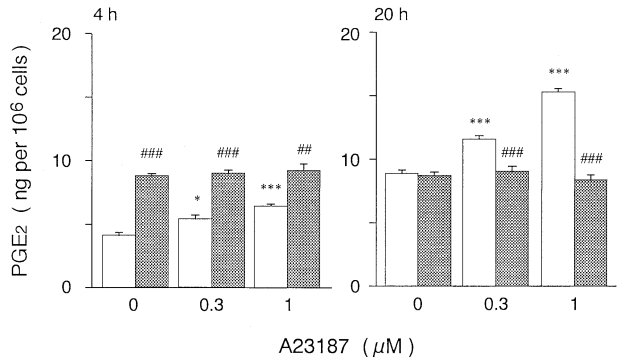

Fig. 5. Effects of auranofin on prostaglandin E_2 production in A23187-stimulated macrophages. Rat peritoneal macrophages (6×10^6 cells) were incubated at 37°C for 4 and 20 h in 4 ml of medium containing 10% calf serum in the presence or absence of A23187 (0.3 and 1 μM) and 10 μM of auranofin. Prostaglandin E_2 (PGE_2) concentrations in the conditioned medium were radioimmunoassayed. Without auranofin, blank columns; and with auranofin, shadowed columns. Values are the means from four samples with S.E.M. shown by vertical bars. Similar results were obtained in three additional experiments. Statistical significance: $^{*}P<0.05$, $^{***}P<0.001$ vs. the control. $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. the corresponding auranofin-free control.

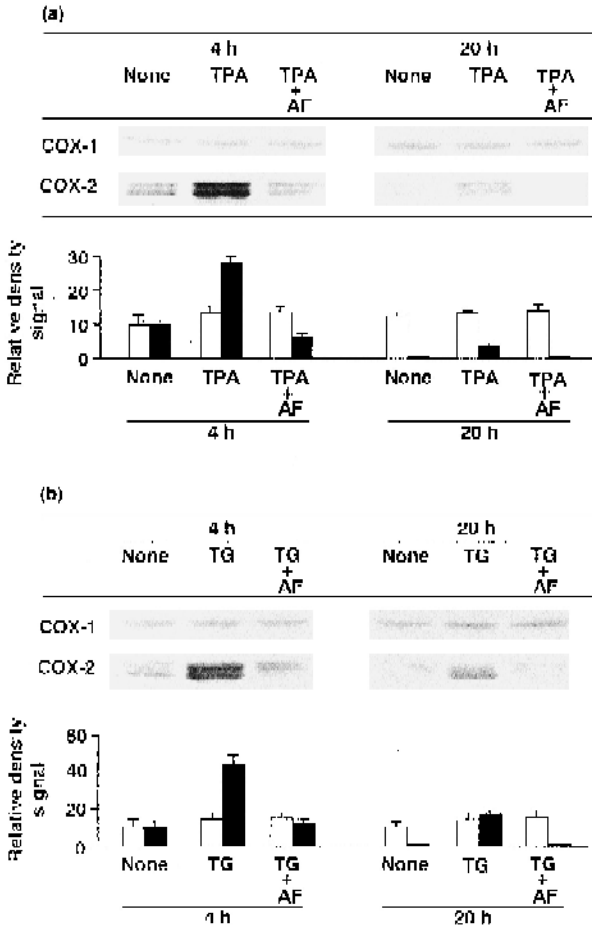

Fig. 6. Effects of auranofin on the levels of cyclooxygenase 1 and cyclooxygenase 2 in TPA- and thapsigargin-stimulated macrophages. Rat peritoneal macrophages (6×10^6 cells) were incubated at 37°C for 4 and 20 h in 4 ml of medium containing 10% calf serum in the presence or absence of TPA (16.2 nM, 10 ng/ml) and auranofin (AF, 10 μM) (a), and thapsigargin (TG, 46.1 nM, 30 ng/ml) and auranofin (AF, 10 μM) (b). The protein levels of cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) were determined by Western blot analysis. To facilitate comparisons, the relative optical density signal of each band is shown as a histogram. Relative cyclooxygenase 1 and cyclooxygenase 2 levels in the cells incubated for 4 h in medium containing no drugs were set to ten. Histograms are the means ± S.E.M. shown by vertical bars from three independent experiments. Open columns represent cyclooxygenase 1, and closed columns represent cyclooxygenase 2. Statistical significance: (a) TPA vs. None or TPA + AF, $P < 0.001$, at 4 and 20 h. (b) TG vs. None or TG + AF, $P < 0.001$, at 4 and 20 h.

was not due to cytotoxicity as assessed by trypan blue exclusion.

3.6. Effects of auranofin on the levels of cyclooxygenase 1 and cyclooxygenase 2 in macrophages

Western blot analysis revealed that cyclooxygenase 1 levels in macrophages did not change by treatment with TPA (16.2 nM, 10 ng/ml) or thapsigargin (46.1 nM, 30 ng/ml) when determined at 4 and 20 h (Fig. 6). Treatment with 10 μM of auranofin in the presence of TPA or thapsigargin also did not affect the cyclooxygenase 1 levels in macrophages at 4 and 20 h (Fig. 6). In contrast, cyclooxygenase 2 levels at 4 and 20 h were increased by treatment with TPA or thapsigargin. In both cases, cyclooxygenase 2 levels at 4 h were higher than that at 20 h. The induction of cyclooxygenase 2 caused by TPA or thapsigargin was inhibited by 10 μM of auranofin at 4 and 20 h (Fig. 6). Auranofin at 3 μM partially inhibited the induction of cyclooxygenase 2 caused by TPA or thapsigargin, and 1 μM of auranofin showed no effect (data not shown). Treatment with A23187 (1 μM, 0.524 μg/ml) also increased cyclooxygenase 2 levels at 4 and 20 h, and the induction of cyclooxygenase 2 caused by A23187 was also inhibited by 10 μM of auranofin (data not shown).

4. Discussion

In mouse peritoneal macrophages, auranofin (1–20 μM) stimulated the mobilization of arachidonate at 30 min in the absence of stimulators (Bondeson and Sundler, 1993). We confirmed these findings by showing that the release of arachidonic acid and prostaglandin E_2 production in rat peritoneal macrophages were increased by auranofin at 4 h. Because the rat peritoneal macrophages produced arachidonate metabolites in the following order: prostaglandin E_2 > 6-keto-prostaglandin $F_{1\alpha}$ > prostaglandin $F_{2\alpha}$ ≫ thromboxane B_2 (data not shown), we determined prostaglandin E_2 production as an index of the metabolism of arachidonic acid. The increased production of prostaglandin E_2 by auranofin is transient, because the stimulation of prostaglandin E_2 production by auranofin did not continue for a long period (20 h).

In the presence of stimulators, auranofin did not inhibit the stimulator-induced prostaglandin E_2 production at 4 h. However, when the effect of auranofin was determined at 20 h, it was demonstrated that auranofin suppressed the stimulator-induced prostaglandin E_2 production. Western blot analysis revealed that auranofin inhibited the induction of cyclooxygenase 2 caused by TPA or thapsigargin (Fig. 6). Therefore, the inhibition of prostaglandin E_2 production by auranofin at 20 h in the presence of these stimulators might be due to the inhibition of cyclooxygenase 2 induction. During the short time period of incubation (4 h), auranofin suppressed the cyclooxygenase 2 induction caused by TPA or thapsigargin (Fig. 6), but had no inhibitory effects on prostaglandin E_2 production (Figs. 3–5). This might be explained as follows: at the early phase, auranofin stimulates the release of arachidonic acid, which is metabolized into prostaglandin E_2 mainly by pre-existing cyclooxygenase 1 although the induction of cyclooxygenase 2 is inhibited by auranofin. The mechanism of the inhibition caused by auranofin of cyclooxygenase 2 induction remains to be elucidated. Gold compounds are reported to inhibit the binding of nuclear factor kappa B (NF-κB) to DNA (Yang et al., 1995), and NF-κB controls the production of interferon-β, tumor necrosis factor-α, interleukin-2, -6 and -8, granulocyte-macrophage

colony stimulating factor and the intercellular adhesion molecule-1 (Degitz et al., 1991; Neish et al., 1992). Bondeson and Sundler (1995) reported that auranofin inhibits the induction of interleukin-1β and tumor necrosis factor-α messenger RNA in mouse macrophages. However, as to the induction of cyclooxygenase 2, nuclear factor interleukin-6 but not NF-κB is reported to play a key role in rat granulosa cells (Sirois and Richards, 1993). In addition, Yang et al. (1995) reported that the potency of the gold compounds to inhibit the binding of NF-κB to DNA was as follows: aurothioglucose > aurothiomalate ≫ auranofin. Our preliminary experiments revealed that the order to inhibit TPA (16.2 nM, 10 ng/ml)-induced prostaglandin E_2 production at 20 h was as follows: auranofin ≫ aurothioglucose > aurothiomalate (prostaglandin E_2 (ng per 10^6 cells): None, 11.7 ± 0.6; TPA, 25.4 ± 1.4; TPA + auranofin (10 μM), 12.5 ± 0.7 ($P < 0.001$ vs. TPA); TPA + aurothioglucose 100 μM, 19.8 ± 1.0 ($P < 0.05$ vs. TPA); TPA + sodium aurothiomalate 100 μM, 26.1 ± 0.9; the means ± S.E.M. from 4 samples). These results indicate that the inhibition of the binding of NF-κB to DNA does not correlate with the inhibition of prostaglandin E_2 production. Therefore, it is possible that auranofin inhibits the binding of nuclear factor interleukin-6 to DNA in rat peritoneal macrophages. In contrast, auranofin enhances the synthesis of heme oxygenase and 23-kDa stress protein in mouse peritoneal macrophages at doses above 12.5 μM (Sato et al., 1995).

It has been reported that cyclooxygenase 2 is induced in the synovial tissues of patients with rheumatoid arthritis (Sano et al., 1992; Crofford et al., 1994). The present investigation suggests that treatment with auranofin would suppress the induction of cyclooxygenase 2 in the synovial tissue and inhibit cyclooxygenase 2-mediated prostaglandin E_2 production. The initial rise in prostaglandin E_2 production caused by auranofin is due to the mobilization of arachidonic acid that is probably metabolized by the pre-existing cyclooxygenase 1 and cyclooxygenase 2. Prostaglandin E_2 produced by cyclooxygenase 1 would be important for the cells to express their fundamental functions, because cyclooxygenase 1 is a housekeeping gene product (Lee et al., 1992). The initial rise in prostaglandin E_2 production through cyclooxygenase 1 and cyclooxygenase 2, and the late-phase suppression of prostaglandin E_2 production by the inhibition of the induction of cyclooxygenase 2 might be one of the mechanism of auranofin's therapeutic action on rheumatoid arthritis. Concentrations of auranofin employed in the present study are close to the therapeutic concentrations (1.5–5.1 μM in whole blood) as reported by Gottlieb (1982).

References

Bondeson, J., Sundler, R., 1993. Antirheumatic gold compounds and penicillamine enhance protein kinase C-mediated activation of the arachidonate-mobilizing phospholipase A_2 in mouse macrophages. J. Leukocyte Biol. 54, 329–335.

Bondeson, J., Sundler, R., 1995. Auranofin inhibits the induction of interleukin 1β and tumor necrosis factor α mRNA in macrophages. Biochem. Pharmacol. 50, 1753–1759.

Carette, S., Calin, A., McCafferty, J.P., Wallin, B.A., 1989. A double-blind placebo-controlled study of auranofin in patients with psoriatic arthritis. Arthritis Rheum. 32, 158–165.

Crofford, L.J., Wilder, R.L., Ristimaki, A.P., Sano, H., Remmers, E.F., Epps, H.R., Hla, T., 1994. Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. Effects of interleukin-1β, phorbol ester, and corticosteroids. J. Clin. Invest. 93, 1095–1101.

Davis, P., Johnston, C., Miller, C.L., Wong, K., 1983. Effects of gold compounds on the function of phagocytic cells. II. Inhibition of superoxide radical generation by tripeptide-activated polymorphonuclear leukocytes. Arthritis Rheum. 26, 82–86.

Degitz, K., Li, L.J., Caughman, S.W., 1991. Cloning and characterization of the 5′-transcriptional regulatory region of the human intercellular adhesion molecule 1 gene. J. Biol. Chem. 266, 14024–14030.

DiMartino, M.J., Waltz, D.T., 1977. Inhibition of lysosomal enzyme release from rat leukocytes by auranofin. Inflammation 2, 131–142.

Elmgreen, J., Ahnfelt-Ronne, I., Nielsen, O.H., 1989. Inhibition of human neutrophils by auranofin: chemotaxis and metabolism of arachidonate via the 5-lipoxygenase pathway. Ann. Rheum. Dis. 48, 134–138.

Finkelstein, A.E., Roisman, F.R., Waltz, D.T., 1977. Effect of auranofin, a new antiarthritic agent, on immune complex-induced release of lysosomal enzymes from human leukocytes. Inflammation 2, 143–150.

Gottlieb, N.L., 1982. Comparative pharmacokinetics of parenteral and oral gold compounds. J. Rheumatol. 9 (Suppl. 8), 99–109.

Hafstrom, I., Uden, A.M., Palmblad, J., 1983. Modulation of neutrophil functions by auranofin. Studies on effects in vitro and in rheumatoid arthritis patients. Scand. J. Rheumatol. 12, 97–105.

Honda, Z., Iizasa, T., Morita, Y., Matsuta, K., Nishida, Y., Miyamoto, T., 1987. Differential inhibitory effects of auranofin on leukotriene B_4 and leukotriene C_4 formation by human polymorphonuclear leukocytes. Biochem. Pharmacol. 36, 1475–1481.

Lee, S.H., Soyoola, E., Chanmugam, P., Hart, S., Sun, W., Zhong, H., Liou, S., Simmons, D., Hwang, D., 1992. Selective expression of mitogen-inducible cyclooxygenase in macrophages stimulated with lipopolysaccharide. J. Biol. Chem. 267, 25934–25938.

Lewis, A.J., Carlson, R.P., Chang, J., Delustro, F., 1984. Effect of gold salts, D-penicillamine and benoxaprofen on type II collagen-induced arthritis in rats. Agents Actions 14, 707–714.

Neish, A.S., Williams, A.J., Palmer, H.J., Whitley, M.Z., Collins, T., 1992. Functional analysis of the human vascular cell adhesion molecule 1 promoter. J. Exp. Med. 176, 1583–1593.

Nishizuka, Y., 1992. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 258, 607–614.

Ohuchi, K., Kamada, Y., Levine, L., Tsurufuji, S., 1981. Glycyrrhizin inhibits prostaglandin E_2 production by activated peritoneal macrophages from rats. Prostaglandins Med. 7, 457–463.

Ohuchi, K., Watanabe, M., Yoshizawa, K., Tsurufuji, S., Fujiki, H., Suganuma, M., Sugimura, T., Levine, L., 1985. Stimulation of prostaglandin E_2 production by 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-type and non-TPA-type tumor promoters in macrophages and its inhibition by cycloheximide. Biochim. Biophys. Acta 834, 42–47.

Ohuchi, K., Sugawara, T., Watanabe, M., Hirasawa, N., Tsurufuji, S., Fujiki, H., Christensen, S.B., Sugimura, T., 1988. Analysis of the stimulative effect of thapsigargin, a non-TPA-type tumour promoter, on arachidonic acid metabolism in rat peritoneal macrophages. Br. J. Pharmacol. 94, 917–923.

Parente, J.E., Wong, K., Davis, P., Burka J.F., Percy, J.S., 1986. Effects of gold compounds on leukotriene B_4, leukotriene C_4 and prostaglandin E_2 production by polymorphonuclear leukocytes. J. Rheumatol. 13, 47–51.

Peters-Golden, M., Shelly, C., 1988. The oral gold compound auranofin

triggers arachidonate release and cyclooxygenase metabolism in the alveolar macrophage. Prostaglandins 36, 773–786.

Peters-Golden, M., Shelly, C., 1989. Inhibition of alveolar macrophage 5-lipoxygenase metabolism by auranofin. Biochem. Pharmacol. 38, 1589–1595.

Sano, H., Hla, T., Maier, J.A.M., Crofford, L.J., Case, J.P., Maciag, T., Wilder, R.L., 1992. In vivo cyclooxygenase expression in synovial tissues of patients with rheumatoid arthritis and osteoarthritis and rats with adjuvant and streptococcal cell wall arthritis. J. Clin. Invest. 89, 97–108.

Sato, H., Yamaguchi, M., Shibasaki, T., Ishii, T., Bannai, S., 1995. Induction of stress proteins in mouse peritoneal macrophages by the anti-rheumatic agents gold sodium thiomalate and auranofin. Biochem. Pharmacol. 49, 1453–1457.

Scheinberg, M.A., Santos, L.M.B., Finkelstein, A.E., 1982. The effect of auranofin and sodium aurothiomalate on peripheral blood monocytes. J. Rheumatol. 9, 366–369.

Sirois, J., Richards, J.S., 1993. Transcriptional regulation of the rat prostaglandin endoperoxide synthase 2 gene in granulosa cells. Evidence for the role of a *cis*-acting C/EBP β promoter element. J. Biol. Chem. 268, 21931–21938.

Thastrup, O., Foder, B., Scharff, O., 1987. The calcium mobilizing and tumor promoting agent, thapsigargin elevates the platelet cytoplasmic free calcium concentration to a higher steady state level. A possible mechanism of action for the tumor promotion. Biochem. Biophys. Res. Commun. 142, 654–660.

Wang, C., Smith, R.L., 1975. Lowry determination of protein in the presence of Triton X-100. Anal. Biochem. 63, 414–417.

Ward, J.R., Williams, H.J., Egger, M.J., Reading, J.C., Boyce, E., Altz-Smith, M., Samuelson Jr., C.O., Willkens, R.F., Solsky, M.A., Hayes, S.P., Blocka, K.L., Weinstein, A., Meenan, R.F., Guttadauria, M., Kaplan, S.B., Klippel, J., 1983. Comparison of auranofin, gold sodium thiomalate, and placebo in the treatment of rheumatoid arthritis: a controlled clinical trial. Arthritis Rheum. 26, 1303–1315.

Watanabe, M., Yamada, M., Mue, S., Ohuchi, K., 1995, Enhancement by cyclo-oxygenase inhibitors of platelet-activating factor production in thapsigargin-stimulated macrophages. Br. J. Pharmacol. 116, 2141–2147.

Wenger, M.E., Alexander, S., Bland, J.H., Blechman, W.J., 1983. Auranofin versus placebo in the treatment of rheumatoid arthritis. Am. J. Med. 75, 123–127.

Yang, J.P., Merin, J.P., Nakano, T., Kato, T., Kitade, Y., Okamoto, T., 1995. Inhibition of the DNA-binding activity of NF-κB by gold compounds in vitro. FEBS Lett. 361, 89–96.