Prostaglandin E₂ Production Dependent upon Cyclooxygenase-1 and Cyclooxygenase-2 and Its Contradictory Modulation by Auranofin in Rat Peritoneal Macrophages

MASATERU YAMADA, HISAE NIKI, MASAMICHI YAMASHITA, SUETSUGU MUE and KAZUO OHUCHI

Department of Pathophysiological Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University, Sendai (M.Y., H.N., M.Y., K.O.) and Department of Health and Welfare Science, Faculty of Physical Education, Sendai College, Funaoka (S.M.), Miyagi, Japan

Accepted for publication January 21, 1997

ABSTRACT

Rat peritoneal macrophages were incubated in the presence of cycloheximide or dexamethasone to inhibit the induction of cyclooxygenase (COX)-2 protein synthesis. Thereafter, when the macrophages were incubated in the presence of arachidonic acid, PGE₂ production was increased. Western blot analysis demonstrated that COX-2 protein levels were low and were not affected by arachidonic acid treatment. COX-1 protein levels were not affected by arachidonic acid treatment either. The COX-2 inhibitors NS-398 and nimesulide only slightly inhibited PGE₂ production, whereas the COX-1/COX-2 inhibitors indo-0-tetradecanoylphorbol 13-acetate increased PGE₂ production. Furthermore, COX-2 protein levels were markedly increased by 12-0-tetradecanoylphorbol 13-acetate treatment, whereas COX-1 protein levels did not change. In this case, both the COX-2 and the COX-1/COX-2 inhibitors inhibited PGE₂ production. This suggests that under these conditions, PGE₂ production is dependent on COX-2. Effects of auranofin on COX-1-dependent and COX-2-dependent PGE₂ production were examined. We found that auranofin stimulated COX-1-dependent PGE₂ production but inhibited COX-2-dependent PGE₂ production in a concentration-dependent manner. The latter effect was found to be due to the inhibition of COX-2 protein induction. These findings might explain the mechanism of the antirheumatic and anti-inflammatory activities of auranofin.

COX, a prostaglandin endoperoxide synthase, is the key enzyme in the conversion of free arachidonic acids released from membrane phospholipids to prostaglandins and thromboxanes. It has two isoforms, COX-1 and COX-2 (Fletcher et al., 1992; O’Banion et al., 1992; Ryseck et al., 1992; Hsi et al., 1994). COX-1 is constitutively expressed in almost all types of cells (Funk et al., 1991; Simmons et al., 1991; O’Neil and Ford-Hutchinson, 1993; Smith et al., 1994) and is probably involved in cellular housekeeping (DeWitt and Smith, 1988; Merlie et al., 1988). In contrast, COX-2 is induced by several kinds of stimuli, including serum, proinflammatory cytokines (DuBois et al., 1994; Mitchell et al., 1994), bacterial lipopolysaccharide (O’Sullivan et al., 1992a; 1992b) and the tumor promoter TPA (Kubuji et al., 1991) in macrophages (Lee et al., 1992), fibroblasts (Evett et al., 1993) and inflamed tissues (Sano et al., 1992; Masferrer et al., 1994; Seibert et al., 1994; Appleton et al., 1995; Niki et al., 1997).

Increase of the COX-2 protein level is correlated with elevated synthesis of prostanoids, and the anti-inflammatory steroid dexamethasone inhibits the induction of COX-2 protein synthesis (Kubuji and Herschman, 1992; Masferrer et al., 1992; O’Banion et al., 1992; Niki et al., 1997). The non-steroidal anti-inflammatory drugs aspirin and indomethacin are widely used for the treatment of acute and chronic inflammatory disorders such as rheumatoid arthritis (Levi and Shaw-Smith, 1994; Simon, 1994), and they inhibit both COX-1 and COX-2 nonspecifically (Meade et al., 1993; Cromlish et al., 1994; O’Neil et al., 1994). Side effects caused by such nonselective COX-1/COX-2 inhibitors are observed in organs such as the stomach (Carson et al., 1987; Brooks and Day, 1991) and kidneys (Clive and Stoff, 1984; Black, 1986) and are thought to be due to the inhibition of COX-1, a housekeeping gene product. Recently, several kinds of selective COX-2 inhibitors, including NS-398 (Futaki et al., 1994), nimesulide (Barnett et al., 1994; Taniguchi et al., 1995), DuP

ABBREVIATIONS: COX, cyclooxygenase; NSAIDs, nonsteroidal anti-inflammatory drugs; TPA, 12-0-tetradecanoylphorbol 13-acetate; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide.
697 (Copeland et al., 1994), SC-58125 (Seibert et al., 1994) and meloxicam (Engelhardt et al., 1996a), have been developed. These inhibitors show fewer side effects than the non-selective COX-1/COX-2 inhibitors (Masferrer et al., 1994; Seibert et al., 1994; Engelhardt et al., 1996b).

Auranofin (2,3,4,6-tetra-O-acetyl-1-thio-β-D-glucopyranosato-S-triethylphosphine gold), an orally active chrysotherapeutic drug, is widely used for the treatment of rheumatoid arthritis (Ward et al., 1983; Wenger et al., 1983). Although it is reported that auranofin inhibits lysosomal enzyme release (DiMartino and Waltz, 1977; Finkestein 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, the precise mechanism of action of auranofin is still unclear. Reports vary as to the effect of auranofin on arachidonic acid metabolism. For example, Lewis et al. (1984) reported that auranofin inhibits zymosan-induced PGE2 production by peritoneal macrophages from collagen-arthritis rats. In contrast, in rat alveolar macrophages, auranofin stimulates arachidonic acid release and PGE2 production (Peters-Golden and Shelly, 1989). Recently, we found that the dual effect of auranofin on arachidonic acid metabolism is not due to the different cell types but rather to the conditions of cell culture (Yamashita et al., manuscript in preparation). The present study was intended to clarify the mechanism of action of auranofin by determining its effect on COX-1-dependent and COX-2-dependent PGE2 production, respectively.

**Materials and Methods**

**Preparation of rat peritoneal macrophages.** A solution of soluble starch (Wako Pure Chemical Ind., Osaka, Japan) and bacto peptone (Difco Laboratories, Detroit, MI), 5% each, that had been autoclaved at 120°C for 15 min was injected i.p. into male Sprague-Dawley rats (300–350 g, specific pathogen-free, Charles River Japan Inc., Kanagawa, Japan) at a dose of 5 ml per 100 g b.wt. Four days later, the rats were killed by cutting the carotid artery under diethylether anesthesia, and the peritoneal cells were harvested (Ohuchi et al., 1985). The rats were treated in accordance with procedures approved by the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Tohoku University, Japan.

**Macrophage culture for COX-1-dependent PGE2 production.** The peritoneal cells were suspended in Eagle’s minimal essential medium (Nissui Inc., Tokyo, Japan) containing 10% (v/v) calf serum, penicillin G potassium (100 U/ml) and streptomycin sulfate (50 μg/ml) at a density of 1.5 × 10^6 cells per milliliter. One milliliter of the cell suspension was poured into each well of a 12-well plastic tissue culture plate (Coster Co.), and the plates were incubated for 2 h at 37°C. The wells were then washed three times with medium to remove nonadherent cells (Ohuchi et al., 1985). The adherent cells were incubated for 4 h at 37°C in 1 ml of medium containing aspirin (Sigma Chemical Co.) (100 μM). After three washes to remove free aspirin, the cells were further incubated for 4 h at 37°C in 1 ml of medium containing TPA (Sigma Chemical Co.) (16.2 nM) and cycloheximide (1 μM) or dexamethasone (Sigma Chemical Co.) (10 μM) or dexamethasone (Sigma Chemical Co.) (10 μM) or dexamethasone (Sigma Chemical Co.) (10 μM) or dexamethasone (Sigma Chemical Co.) (10 μM). After incubation, the conditioned medium was collected to determine the PGE2 concentration.

**Macrophage culture for COX-2-dependent PGE2 production.** The peritoneal cells were suspended in Eagle’s minimal essential medium containing 10% (v/v) calf serum, penicillin G potassium (18 μg/ml) and streptomycin sulfate (50 μg/ml) at a density of 1.5 × 10^6 cells per milliliter. One milliliter of the cell suspension was poured into each well of a 12-well plastic tissue culture plate (Coster Co.), and the plates were incubated for 2 h at 37°C. The wells were then washed three times with medium to remove nonadherent cells (Ohuchi et al., 1985). The adherent cells were incubated for 4 h at 37°C in 1 ml of medium containing aspirin (Sigma Chemical Co.) (100 μM). After three washes to remove free aspirin, the cells were further incubated for 4 h at 37°C in 1 ml of medium containing TPA (Sigma Chemical Co.) (16.2 nM) in the absence of an arachidonic acid. After incubation, the conditioned medium was collected to determine the PGE2 concentration.

**Drug treatment.** The drugs used for preincubation of the cells were cycloheximide, dexamethasone and aspirin. They were dissolved in ethanol and added to the medium. To examine the effects on PGE2 production, we used the COX-1/COX-2 inhibitors indomethacin, piroxicam, and tenoxicam (Sigma Chemical Co.); the COX-2 inhibitors NS-398 and nimesulide (Funakoshi Co., Tokyo, Japan); and the orally active chrysotherapeutic drug auranofin (Funakoshi Co.). 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 the vehicle.

**Viability assay.** The viability of the cells was examined in each set of experiments by the MTT method (Mossman, 1983; Tada et al., 1986), which is based on the ability of mitochondrial succinate dehydrogenase to cleave MTT to the blue compound formazan. The cells were incubated for the indicated periods in 1 ml of medium containing 10% calf serum in the presence or absence of drugs. Then 100 μl of MTT solution in phosphate-buffered saline (PBS, pH 7.4) (5 mg/ml) was added to each well, and the cells were further incubated for 4 h at 37°C. After 1 ml of 0.04 N HCl solution in isopropanol was added, the cells were sonicated using a Handy Sonic Disruptor (UR-20P, Tomy, Tokyo, Japan) at 10% maximum power for 3 s, and the resultant colored product was read on a Microplate Reader (Bio-Rad, Richmond, CA) at 570 nm. Treatment with drugs showed no significant changes in viability of the cells.

**Measurement of PGE2 concentrations.** The conditioned medium was centrifuged at 1500 × g and 4°C for 5 min, and the PGE2 concentration in the supernatant fraction was radioimmunoassayed (Ohuchi et al., 1985). PGE2 antiserum was purchased from PerSeptive Diagnostics, Cambridge, MA.

**Western blot analysis of COX-1 and COX-2.** For the Western blot analysis, 1.5 × 10^5 peritoneal macrophages were incubated in 10 ml of medium under several conditions as described above. After incubation, the cells were washed three times with PBS, scraped off the plate by a rubber policeman, and centrifuged at 800 × g and 4°C for 5 min. The precipitate was sonicated five times (10 s each time) in 1 ml of ice-cold solubilization buffer (Tris, 50 mM; EDTA, 10 mM; Tween 20, 1% (v/v); N,N-dimethyldithiocarbamate, 1 mM; phenylmeth-ylsulphonyl fluoride, 1 mM; pepstatin A, 10 μM; leupeptin, 10 μM; pH 8.0) using a Handy Sonic Disruptor (UR-20P, Tomy) at 90% maximum power. The sonicates were then centrifuged at 100,000 × g and 4°C for 1 h. Protein concentrations in the supernatant fractions were determined according to the procedure described by Wang and Smith (1975). An aliquot of 30 μg protein was boiled for 3 min at a ratio of 1:1 (v/v) with 2 × gel loading buffer (Tris, 50 mM; SDS, 4% (v/v); glycerol, 10% (v/v); 2-mercaptoethanol, 4% (v/v); bromophenol blue, 0.05 mg/ml; pH 7.4). The samples were then loaded onto a gradient gel (4–10% Tris-glycine, pH 8.3) and subjected to electrophoresis (4 h at 15 mA). The separated proteins were transferred onto a nitrocellulose membrane (Bio-Rad) (2 h at 150 mA), and the blot was incubated in blocking solution (Block Ace, Dainippon Pharmaceutical Co., Osaka, Japan) for 1 h and then with primary antibodies for 2 h at room temperature. The primary antibodies used were a rabbit antibody to murine COX-2 (dilution 1:100, Oxford Biomedical Research, Inc., MI) and a goat antibody to sheep seminal COX-1 (dilution 1:25,000, Oxford Biomedical Research, Inc.). The blot was then incubated with secondary antibodies (dilution 1:2000), an anti-rabbit IgG for COX-2 and an anti-goat IgG for COX-1 (Vector...
Laboratories, Burlingame, CA), at 4°C for 3 h. Finally, the blot was incubated with VECTSTATIN ABC reagent (Vector Laboratories) at room temperature for 30 min. The blot was then incubated with the drugs for 3 min at 37°C, after which the amount of PGE2 was measured by radioimmunoassay. Individual band density values for each point were expressed as the relative density signal.

**Determination of inhibitory effects of drugs on 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 procedure described by Mancini et al. (1995). One unit of COX-1 (isolated from sheep seminal vesicle, Cayman Chemical Co., Ann Arbor, MI) or COX-2 (isolated from sheep placenta, purity 70%, Cayman Chemical Co.) was dissolved in 210 μl of Tris-HCl (100 mM, pH 7.4) containing 10 mM ethylenediamine tetraacetic acid, 1 mM reduced glutathione, 1 μM hematin and 0.5 mM phenol. The reaction mixture was preincubated with the drugs for 3 min at 37°C, after which arachidonic acid (20 μM) was added, and the mixture was incubated for 3 min at 37°C. 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 PGE2 was measured by radioimmunoassay.

**Statistical analysis.** The statistical significance of the results was analyzed by Dunnett’s test for multiple comparison and Student’s t test for unpaired observations.

**Results**

Protein levels of COX-1 and COX-2 in rat peritoneal macrophages under conditions for COX-1-dependent PGE2 production and COX-2-dependent PGE2 production. In the cycloheximide (1 μM)-pretreated macrophages, further incubation with arachidonic acid (10 μM) for 4 h in the presence of cycloheximide (1 μM) did not affect COX-2 protein levels (fig. 1, A and C). The COX-2 levels were also very low just before the incubation and 1 and 2 h after incubation in the presence of arachidonic acid (10 μM) and cycloheximide (1 μM) (data not shown). In contrast, COX-1 protein levels were high and remained so from 0 to 4 h after incubation in the presence of arachidonic acid (10 μM) and cycloheximide (1 μM) (fig. 1, A and C). The same results were obtained in macrophages that had been pretreated with dexamethasone (10 μM) for 4 h and further incubated for 4 h in medium containing arachidonic acid (10 μM) and dexamethasone (10 μM) (fig. 1, A and C).

COX-2 protein levels in the aspirin-pretreated macrophages were markedly increased by treatment with TPA (16.2 nM) for 4 h (fig. 1, B and C). However, COX-1 protein levels in the aspirin-pretreated macrophages did not change upon treatment with TPA for 4 h (fig. 1, B and C).

**Effects of various NSAIDs on COX-1-dependent PGE2 production.** In the cycloheximide (1 μM)-pretreated macrophages, PGE2 production at 4 h in the presence of cycloheximide (1 μM) was significantly increased by the addition of arachidonic acid (10 μM) (fig. 2A). The arachidonic acid-induced PGE2 production was inhibited by the COX-1/COX-2 inhibitors indomethacin, piroxicam and tenoxicam in a concentration-dependent manner (fig. 2A). In contrast, only a slight inhibition was induced by the COX-2 inhibitors NS-398 and nimesulide at 1 μM (fig. 2A).

In the dexamethasone (10 μM)-pretreated macrophages, exogenous arachidonic acid (10 μM) increased PGE2 production at 4 h in the presence of dexamethasone (10 μM), and the COX-1/COX-2 inhibitors indomethacin, piroxicam and tenoxicam inhibited the arachidonic acid-induced PGE2 production in a concentration-dependent manner (fig. 2B). Again, only a slight inhibition was induced by NS-398 and nimesulide at 1 μM.
For the control group, the PGE$_2$ concentration at 4 h was 0.1 ng/ml and the nimesulide-treated group were 0.29 ± 0.01 ng/ml containing CHI (1 μM). The cells were further incubated for 4 h at 37°C in 1 ml of medium containing arachidonic acid (10 μM). Tenoxicam (TXC) in the presence of arachidonic acid (AA, 10 μM) and tenoxicam also inhibited the TPA-induced PGE$_2$ production (fig. 3). Indomethacin showed almost the same potency as NS-398 and nimesulide at a concentration of 1 μM (fig. 2B). At 10 μM, NS-398 and nimesulide showed strong inhibition of PGE$_2$ production, but the inhibitory effects by such COX-2 inhibitors were weaker than those by the COX-1/COX-2 inhibitors; the PGE$_2$ concentrations at 4 h for the NS-398-treated group and the nimesulide-treated group were 0.29 ± 0.01 and 0.34 ± 0.02 ng/ml, respectively, whereas those for the indomethacin-, piroxicam- and tenoxicam-treated groups were 0.10 ± 0.01, 0.09 ± 0.02, and 0.05 ± 0.01 ng/ml, respectively. For the control group, the PGE$_2$ concentration at 4 h was 0.82 ± 0.02 ng/ml (means ± S.E.M. from four samples).

**Effects of various NSAIDs on COX-1-dependent PGE$_2$ production.** In the aspirin-pretreated macrophages, stimulation by TPA (16.2 nM) for 4 h markedly increased PGE$_2$ production (fig. 3). In the presence of the COX-1 inhibitor indomethacin, piroxicam and tenoxicam also inhibited the TPA-induced PGE$_2$ production in a concentration-dependent manner (fig. 3). Indomethacin showed almost the same potency as NS-398 and nimesulide, but the effects of piroxicam and tenoxicam were much weaker.

**Effects of various NSAIDs on COX-2-dependent PGE$_2$ production and COX-2-dependent PGE$_2$ production.** The arachidonic acid (10 μM)-induced PGE$_2$ production at 4 h in the cycloheximide (1 μM)-pretreated macrophages was further enhanced by the addition of auranofin in a concentration-dependent manner at 1 to 10 μM (fig. 4A). Also in the dexamethasone (10 μM)-pretreated macrophages, auranofin at concentrations of 1 to 10 μM enhanced the arachidonic acid (10 μM)-induced PGE$_2$ production (data not shown).

In contrast, the TPA (16.2 nM)-induced PGE$_2$ production at 4 h in the aspirin (100 μM)-pretreated macrophages was suppressed by auranofin at 3 and 10 μM (fig. 4B). These findings strongly suggest that auranofin enhances COX-1-dependent PGE$_2$ production and suppresses COX-2-dependent PGE$_2$ production.

**Effects of auranofin on the enzyme activities of COX-1 and COX-2.** The direct effects of auranofin on isolated COX-1 and COX-2 were examined. As shown in figure 5A, the COX-1/COX-2 inhibitor indomethacin inhibited COX-1 activity in a concentration-dependent manner, whereas auranofin and the COX-2 inhibitor NS-398 had no inhibitory effect on COX-1. COX-2 activity was not inhibited by auranofin either, but it was inhibited by the COX-1/COX-2 inhibitor indomethacin and the COX-2 inhibitor NS-398 (fig. 5B). These findings indicate that the inhibition by auranofin of the TPA-induced PGE$_2$ production (COX-2-dependent PGE$_2$ production) is not due to the inhibition of COX-2 activity.

**Effects of auranofin on the protein levels of COX-1 and COX-2.** In the cycloheximide (1 μM)-pretreated macrophages, COX-1 protein levels 4 h after incubation in the presence of arachidonic acid (10 μM) and cycloheximide (1 μM) did not change upon auranofin (10 μM) treatment (fig. 6). Also in the dexamethasone (10 μM)-pretreated macrophages, COX-1 protein levels 4 h after incubation in the presence of arachidonic acid (10 μM) and dexamethasone (10 μM) were not changed by auranofin (10 μM) treatment (data...
not shown). In these cells, COX-2 protein levels were not affected by auranofin (10 μM)-treatment (fig. 6).

In contrast, the TPA (16.2 nM)-induced increase of COX-2 protein levels in the aspirin (100 μM)-pretreated macrophages was markedly suppressed by auranofin (10 μM) treatment at 4 h (fig. 6). Again, auranofin did not affect COX-1 protein levels in these cells (fig. 6).

**Discussion**

The results of studies of the effect of auranofin on the metabolism of arachidonic acid vary. For example, in rat alveolar macrophages, auranofin stimulated the release of arachidonic acid and the production of PGE₂ and thromboxane A₂ (Peters-Golden and Shelly, 1989), whereas in peritoneal macrophages from collagen arthritic rats, zymosan-induced PGE₂ production was suppressed by auranofin (Lewis et al., 1984). These contradictory results might be due to the different cell types used in the experiments. However, we recently found that the effect of auranofin varied within the same type of cell, rat peritoneal macrophages (Yamashita et al., manuscript in preparation). Specifically, PGE₂ production stimulated by the protein kinase C activator TPA (Nishizuka, 1992) or the endomembrane Ca²⁺-ATPase inhibitor thapsigargin (Ali et al., 1985; Thastrup et al., 1987; Ohuchi et al., 1988; Rodriguez et al., 1993; Watanabe et al., 1995) was inhibited by auranofin, but the spontaneous PGE₂ production was enhanced by auranofin. The effects of auranofin seemed to vary dependent on cell culture conditions. Therefore, in the present study we attempted to clarify the mechanism of the action of auranofin on the metabolism of arachidonic acid.
Because COX-2 protein is induced by several stimuli (Rosen et al., 1989; Kubju et al., 1991; O’Banion et al., 1991; Xie et al., 1991; Hla and Neilson, 1992; Lee et al., 1992; O’Sullivan et al., 1992a; 1992b; Ryseck et al., 1992), we hypothesized that auranofin inhibits the induction of COX-2 and inhibits PGE2 production under stimulated conditions. To prove this, we examined the effect of auranofin on COX-2-dependent PGE2 production. COX-2-dependent PGE2 production was induced by stimulation with TPA in rat peritoneal macrophages that had been pretreated with aspirin to inactivate the preexisting COX (Rome et al., 1989; O’Banion et al., 1991; Roth et al., 1983). Under these conditions, the protein level of COX-2 in the cells was increased, and PGE2 production was inhibited by the specific COX-2 inhibitors NS-398 and nimesulide. Furthermore, auranofin inhibited the induction of COX-2 protein and PGE2 production. These findings strongly support our notion that auranofin inhibits PGE2 production by inhibiting the induction of COX-2. It should be noted that the induction of interleukin 1ß and tumor necrosis factor ß mRNA was also inhibited by auranofin in mouse macrophages stimulated by zymosan, by lipopolysaccharide or by various bacteria (Bondeson and Sundler, 1995). The mechanism of the suppression of COX-2 protein by auranofin remains to be elucidated.

In nonstimulated rat peritoneal macrophage culture, we observed that auranofin stimulated PGE2 production (Yamashita et al., manuscript in preparation). To confirm this observation, in the present study, we examined the effect of auranofin on COX-1 dependent PGE2 production. COX-1-dependent PGE2 production was induced by the addition of arachidonic acid to medium of the cells that had been pretreated with cycloheximide, a protein synthesis inhibitor, or with dexamethasone, a glucocorticoid that suppresses COX-2 induction (O’Banion et al., 1992; Dubois et al., 1994; Mitchell et al., 1994; Niki et al., in press). Under such culture conditions, PGE2 production was only slightly inhibited by the specific COX-2 inhibitor NS-398 and nimesulide but was strongly inhibited by the COX-1/COX-2 inhibitor indomethacin, the level of COX-2 protein in the cells was low and did not change upon the addition of arachidonic acid and the level of COX-1 protein was more clearly detectable than that of COX-2. Therefore, we suggest that PGE2 production under these culture conditions is dependent on COX-1. Under these conditions, auranofin stimulated PGE2 production in a concentration-dependent manner. Previously, we observed that the same concentrations of auranofin stimulated arachidonic acid release from cells under nonstimulated conditions (Yamashita et al., manuscript in preparation). The stimulation by auranofin of the release of arachidonic acid from rat alveolar macrophages was also observed by Peters-Golden and Shelly (1989). In the culture for COX-1-dependent PGE2 production, arachidonic acid was exogenously added, but auranofin stimulated PGE2 production. Therefore, the stimulation of PGE2 production by auranofin in this culture is not due to the stimulation of the release of arachidonic acid. Further investigation is necessary to clarify the mechanism of the stimulation of PGE2 production under these culture conditions.

In conclusion, auranofin has a dual effect on PGE2 production. Under stimulated conditions where COX-2 is induced, auranofin inhibits PGE2 production by suppressing the induction of COX-2 protein. Under nonstimulated conditions, auranofin enhances COX-1-dependent PGE2 production. These findings might partially explain the mechanism of the effect of auranofin on chronic rheumatoid arthritis. Finally, the cell culture conditions described here are useful for estimation of the specific inhibitors of COX-1 and COX-2.

References


Send reprint requests to: Kazuo Ohuchi, Ph.D., Prof., Department of Pathophysiological Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University, Aoba Aramaki, Aoba-ku, Sendai, Miyagi 980-77, Japan.