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Inhibition by auranofin of the production of prostaglandin E_2 and nitric oxide in rat peritoneal macrophages

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Abstract

In rat peritoneal macrophages, 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (16.2 nM) stimulated production of both prostaglandin E_2 and nitric oxide. TPA also increased the levels of mRNA for cyclooxygenase-2 and inducible nitric oxide synthase, suggesting that the increase in the production of prostaglandin E_2 and nitric oxide is due to the increase in the levels of mRNA for cyclooxygenase-2 and inducible nitric oxide synthase, respectively. The TPA-induced increase in prostaglandin E_2 production was partially inhibited by the inhibitor of nitric oxide synthase L-N^G-monomethyl-L-arginine acetate (L-NMMA), and the TPA-induced increase in nitric oxide production was partially inhibited by the cyclooxygenase inhibitor indomethacin, suggesting that both the production of prostaglandin E_2 and nitric oxide in TPA-stimulated macrophages is influenced by each other. The orally active chrysotherapeutic agent auranofin, at 3 and 10 μM, inhibited the TPA-stimulated production of prostaglandin E_2 and nitric oxide, and suppressed the TPA-induced increase in the levels of mRNA for cyclooxygenase-2 and inducible nitric oxide synthase. These findings indicate that the inhibition by auranofin of the TPA-stimulated production of prostaglandin E_2 and nitric oxide is due to the decrease in the levels of mRNA for cyclooxygenase-2 and inducible nitric oxide synthase, respectively, and the interaction of the production between prostaglandin E_2 and nitric oxide may partly be involved in the mechanism for the inhibition by auranofin of the production of both prostaglandin E_2 and nitric oxide.

Keywords: TPA (12-*O*-tetradecanoylphorbol 13-acetate); Prostaglandin E_2; Nitric oxide (NO); Nitric oxide (NO) synthase inducible; Cyclooxygenase-2; Auranofin; Macrophage

1. Introduction

The levels of nitrate and nitrite, the stable end-products of nitric oxide (NO) produced by spontaneous oxidation, are increased in the synovial fluid of patients with rheumatoid arthritis (Farrell et al., 1992). In addition, the overproduction of NO via the expression of inducible NO synthase is suggested to reflect abnormalities of immunoregulation in the joints of patients with rheumatoid arthritis (Ueki et al., 1996), and treatment with the inhibitor of inducible NO synthase in a rat adjuvant arthritis model caused a significantly lower articular index, paw volume, and synovial fluid cell count (Connor et al., 1995; Santos et al., 1997). Furthermore, cyclooxygenase-2 protein is elevated in the synovial tissue of patients with rheumatoid arthritis (Sano et al., 1992; Crofford et al., 1994), resulting in an increase in prostaglandin E_2 production (Levi and Shaw-Smith, 1994).

In the lipopolysaccharide-stimulated murine macrophage cell line J774, treatment with indomethacin significantly reduced NO secretion (Milano et al., 1995). Therefore, it is suggested that prostaglandin E_2 may mediate an increase in NO production. On the other hand, cyclooxygenase inhibition abolishes the hypoxia-induced decrease in NO production in bovine aortic endothelium, indicating that prostanoids produced in response to hypoxia down-regulate NO production (Xu et al., 1995). The interactions between prostaglandin E_2 production and NO production thus appear to be complex, and remain to be clarified.

Auranofin ((2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)(triethylphosphine) gold) is an orally active chrysotherapeutic agent (Ward et al., 1983; Wenger et al., 1983). We have reported (Yamada et al., 1997; Yamashita et al., 1997b) that auranofin inhibits prostaglandin E_2 production in rat peritoneal macrophages stimulated by the

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protein kinase C activator 12-*O*-tetradecanoylphorbol 13-acetate (TPA) (Nishizuka, 1992), the endomembrane Ca^{2+}-ATPase inhibitor thapsigargin (Ali et al., 1985; Thastrup et al., 1987; Ohuchi et al., 1988; Garcia Rodriguez et al., 1993; Watanabe et al., 1995), or the Ca^{2+} ionophore A23187. In addition, we reported (Yamashita et al., 1997a) that auranofin inhibits production of NO in lipopolysaccharide-stimulated RAW 264.7 cells, an Abelson leukemia virus-transformed murine macrophage cell line (Raschke et al., 1978), by inhibiting the expression of inducible NO synthase.

In the present study, we attempted to determine whether auranofin inhibits production of both prostaglandin E_2 and NO in one type of inflammatory cell, rat peritoneal macrophages, because of the fact that RAW 264.7 cells produce little prostaglandin E_2 in response to several stimulators such as TPA and lipopolysaccharide (unpublished observations and (Reddy and Herschman, 1994; Guastadisegni et al., 1997).

First, we examined whether rat peritoneal macrophages produce NO in response to stimulation with TPA. Second, we examined the interaction between prostaglandin E_2 production and NO production in TPA-stimulated macrophages using the cyclooxygenase inhibitor indomethacin and the NO synthase inhibitor L-N^G-monomethyl-L-arginine acetate (L-NMMA). Third, we examined whether auranofin inhibits the TPA-induced increase in the levels of cyclooxygenase-2 and inducible NO synthase mRNAs, and the TPA-induced production of prostaglandin E_2 and NO in rat peritoneal macrophages.

2. Materials and methods

2.1. Preparation of rat peritoneal macrophages

A solution of soluble starch (Wako, Osaka, Japan) and bacto peptone (Difco Laboratory, Detroit, MI, USA), 5% each, was injected intraperitoneally into male Sprague–Dawley strain 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 sacrificed by cutting the carotid artery under diethylether anesthesia and peritoneal cells were harvested. The animal experiments were performed in accordance with the procedures approved by the Animal Ethics Committee of the Faculty of Pharmaceutical Sciences, Tohoku University, Japan.

The peritoneal cells were suspended in Eagle's minimal essential medium (EMEM, Nissui, Tokyo, Japan) containing 10% calf serum (Dainippon Pharmaceutical, Osaka, Japan), penicillin G potassium (18 μg/ml) and streptomycin sulfate (50 μg/ml) (Meiji Seika, Tokyo, Japan) at a density of 1.5×10^6 cells/ml. One milliliter of the cell suspension was poured into each well of a 12-well plastic tissue culture plate (Costar, Cambridge, MA, USA), and incubated for 2 h at 37°C. The wells were then washed three times with the medium to remove non-adherent cells, and the adherent cells were further cultured for 20 h. After three washes, the adherent cells were used for the subsequent experiments. More than 95% of the adherent cells were identified as macrophages (Ohuchi et al., 1985).

2.2. Determination of prostaglandin E_2 concentration

After incubation, the conditioned medium was collected, centrifuged at $1500 \times g$ and 4°C, and the prostaglandin E_2 concentration in the supernatant fraction was measured by radioimmunoassay (Ohuchi et al., 1985). Prostaglandin E_2 antiserum was purchased from PerSeptive Diagnostics (Cambridge, MA, USA).

2.3. Determination of NO production

NO production by macrophages was expressed as nitrite concentration in the conditioned medium, determined by using Griess reagent according to the method described by Green et al. (1982). Briefly, 100 μl aliquots of the conditioned medium were mixed with an equal volume of Griess reagent (0.1% (w/v) *N*-1-naphthylethylenediamine dihydrochloride, 1% (w/v) sulphanilamide in 2.5% (v/v) phosphoric acid). The absorbance at 540 nm was measured using a microplate reader (Model 450, Bio-Rad Laboratories, Hercules, CA, USA) and the nitrite concentration was determined by interpolation using a calibration curve of standard sodium nitrite concentrations vs. absorbance. All assays were performed on three to four replicates and repeated in at least three independent experiments.

2.4. Semiquantitation of the levels of mRNA for cyclooxygenase-2 and inducible NO synthase by reverse transcription-polymerase chain reaction (RT-PCR)

Rat peritoneal macrophages (1.5×10^7 cells) were incubated for 20 h at 37°C in 10 ml of medium containing TPA (16.2 nM) with or without auranofin. After incubation, cells were rinsed three times with ice-cold phosphate-buffered saline and scraped off the plate with a rubber policeman. Total RNA was then prepared by acid guanidinium thiocianate–phenol–chloroform extraction (Chomczynski and Sacchi, 1987), and the yield of RNA extracted was determined spectrophotometrically. One microgram of each sample was reverse transcribed at 37°C for 1 h in 20 μl of the buffer (50 mM Tris–HCl, pH 8.3, 75 mM KCl and 3 mM $MgCl_2$) containing 5 μM random hexamer oligonucleotides (Gibco BRL, Gaithersburg, MD, USA), 200 U of the reverse transcriptase from Moloney murine leukemia virus (Gibco BRL), each 2′-deoxynucleotide 5′-triphosphate (dNTP, Pharmacia Biotech, Uppsala, Sweden) at 500 μM and 10 mM dithiothreitol.

PCR primers for rat cyclooxygenase-2 were designed according to the nucleotide sequence of rat cyclooxy-

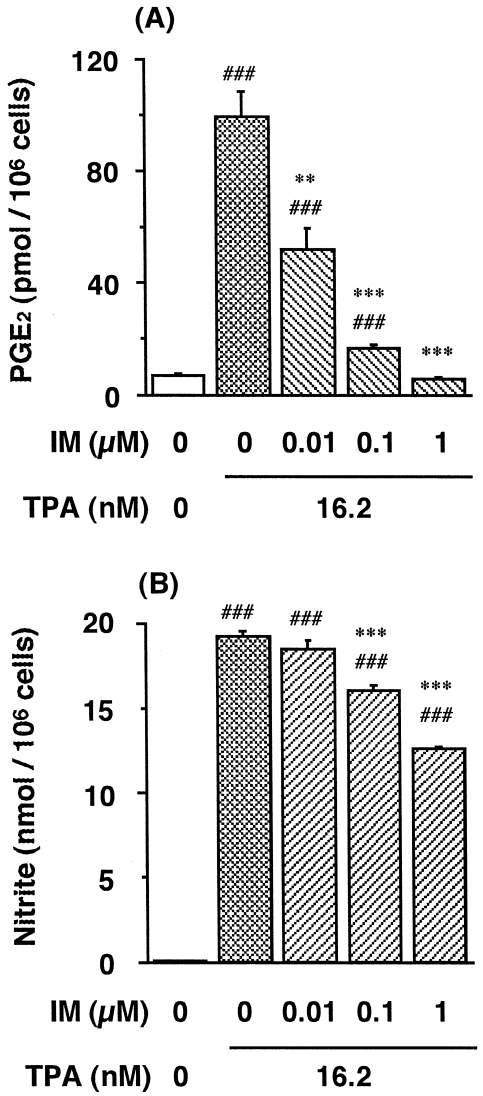

Fig. 1. Effects of indomethacin on the TPA-induced production of prostaglandin E_2 and NO. Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37°C for 20 h in 1 ml of the medium containing TPA (16.2 nM) and the indicated concentrations of indomethacin (IM). Total amounts of prostaglandin E_2 (PGE_2) (A) and nitrite (B) in the conditioned medium were determined. Values are the means ± S.E.M. of 4 samples. Statistical significance: $^{\#\#\#}P < 0.001$ vs. TPA control; $**P < 0.01$, $***P < 0.001$ vs. IM control.

genase-2 (Feng et al., 1993): (forward) 5′-ACTTGCTCACTTTGTTGAGTCATTC-3′, and (reverse) 5′-TTTGATTAGTACTGTAGGGTTAATG-3′, which amplify a 583-base pair cyclooxygenase-2 fragment. PCR was performed for 25 cycles, each cycle consisting of 30 s of denaturation at 94°C, 1 min of annealing at 54°C, and 2 min of extension at 72°C, using a thermal cycler (GeneAmp™ PCR System 2400, Perkin Elmer, Norwalk, CT, USA).

PCR primers for inducible NO synthase were designed according to Nadeau et al. (1995): (forward) 5′-GTGTTCCACCAGGAGATGTTG-3′, and (reverse) 5′-CTCCTGCCCACTGAGTTCGTC-3′, which amplify a 576-base pair inducible NO synthase fragment. PCR was performed for 27 cycles, each cycle consisting of 30 s of denaturation at 94°C, 1 min of annealing at 54°C, and 1 min of extension at 72°C.

The level of mRNA for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was determined as an internal control. PCR primers for rat GAPDH were: (forward) 5′-TGATGACATCAAGAAGGTGGTGGA-3′, and (reverse) 5′-TCCTTGGAGGCCATGTAGGCCAT-3′, which amplify a 249-base pair GAPDH fragment (Robbins and McKinney, 1992). PCR was performed for 18 cycles, each cycle consisting of 30 s of denaturation at 94°C, 1 min of annealing at 57°C, and 1 min of extension at 72°C. PCR

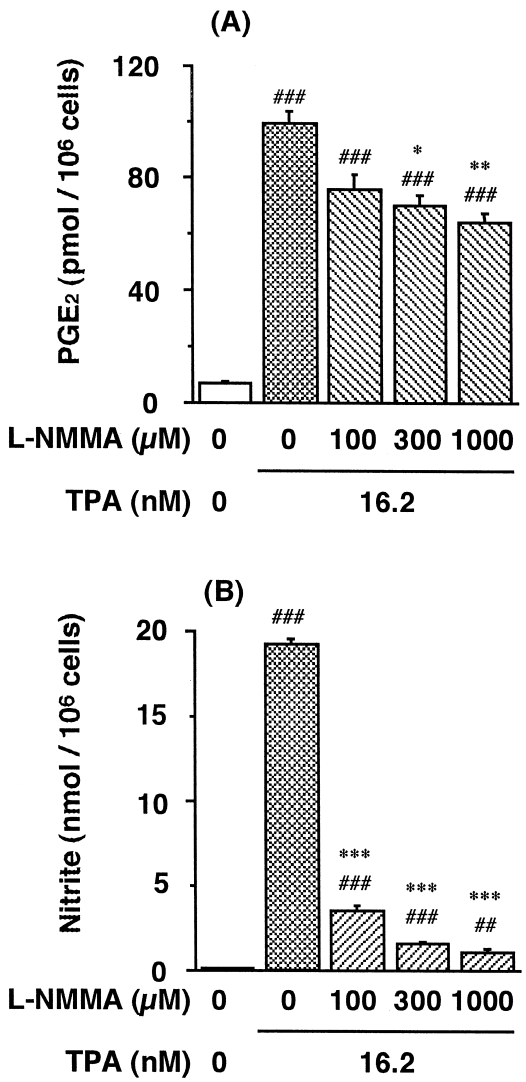

Fig. 2. Effects of L-NMMA on the TPA-induced production of prostaglandin E_2 and NO. Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37°C for 20 h in 1 ml of the medium containing TPA (16.2 nM) and the indicated concentrations of L-NMMA. Total amounts of prostaglandin E_2 (PGE_2) (A) and nitrite (B) in the conditioned medium were determined. Values are the means ± S.E.M. of 4 samples. Statistical significance: $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. TPA control; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. L-NMMA control.

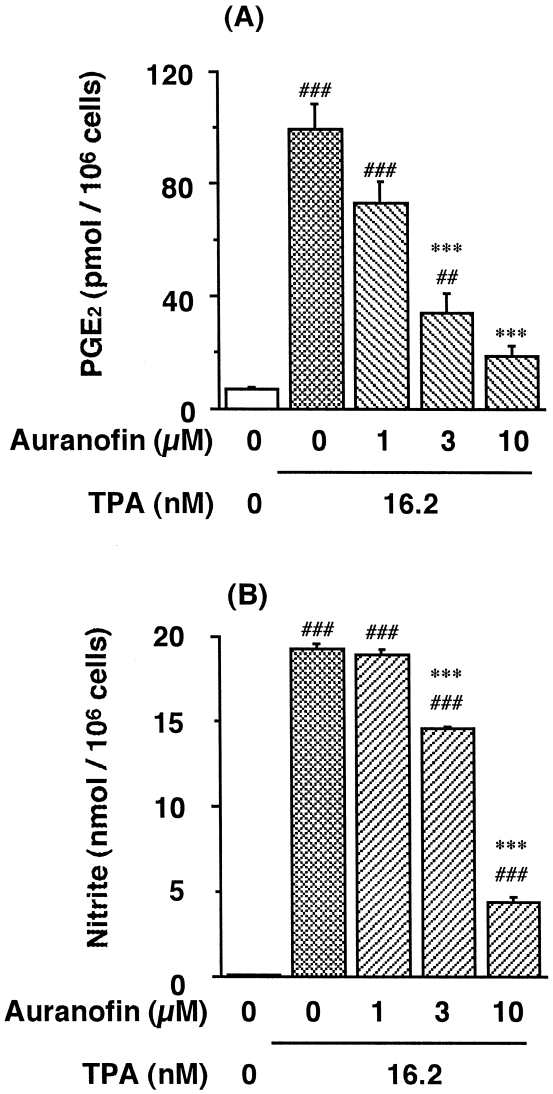

Fig. 3. Effects of auranofin on the TPA-induced production of prostaglandin E_2 and NO. Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37°C for 20 h in 1 ml of medium containing TPA (16.2 nM) and the indicated concentrations of auranofin. Total amounts of prostaglandin E_2 (PGE_2) (A) and nitrite (B) in the conditioned medium were determined. Values are the means ± S.E.M. of 4 samples. Statistical significance: $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. TPA control; $^{***}P < 0.001$ vs. auranofin control.

reactions contained 10 μl of the reverse-transcribed RNA solution, and 40 ml of PCR buffer (10 mM Tris–HCl, pH 8.3, 50 mM KCl, and 1.5 mM $MgCl_2$) containing each primer at 200 μM, 200 μM dNTP, and 1.25 U of Taq polymerase (Takara Shuzo, Shiga, Japan). After PCR, 10 μl of the PCR reaction mixture was electrophoresed on 1.6% agarose minigel, and the PCR products were visualized by ethidium bromide staining after electrophoresis. The levels of mRNA for cyclooxygenase-2, inducible NO synthase and GAPDH were quantified by scanning densitometry, and the ratios of the densities of cyclooxygenase-2 and inducible NO synthase mRNA signals to those of GAPDH were calculated.

2.5. Drugs

TPA (Sigma, St. Louis, MO, USA), auranofin (Sigma) and indomethacin (Sigma) were dissolved in ethanol and added to the medium. L-NMMA (Wako) was directly dissolved in the medium. The final concentration of ethanol in the drug-containing and control media was adjusted to 0.1%. After treatment with drugs the viability of the cells was determined as the ability to exclude trypan blue dye; no cytotoxic effects were observed.

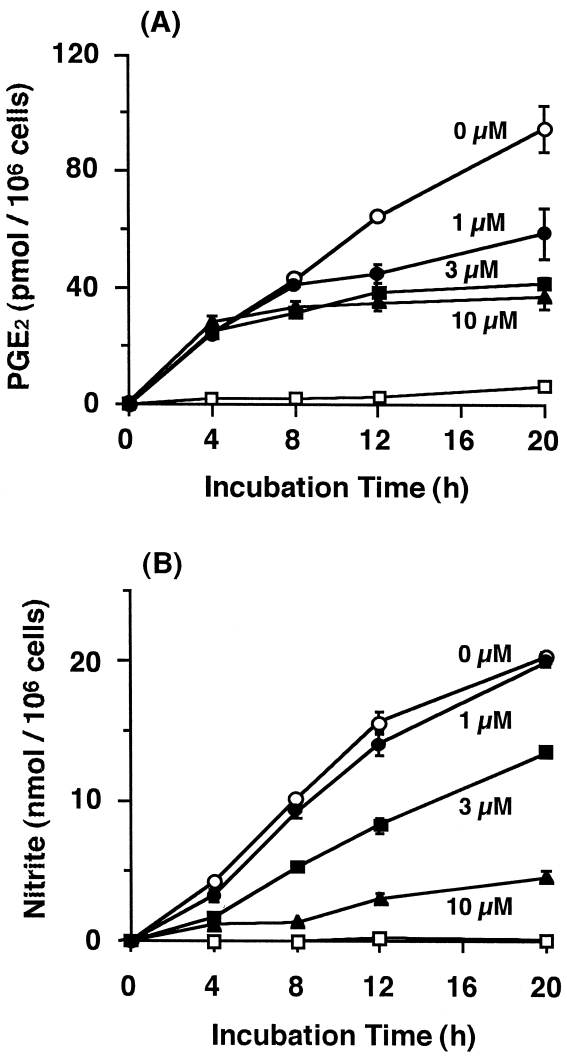

Fig. 4. Time-course of the effect of auranofin on the TPA-induced production of prostaglandin E_2 and NO. Rat peritoneal macrophages (1.5×10^6 cells) were incubated at 37°C for 4, 8, 12 and 20 h in 1 ml of the medium containing TPA (16.2 nM) and various concentrations of auranofin (○, 0 μM; ●, 1 μM; ■, 3 μM; ▲, 10 μM). Total amounts of prostaglandin E_2 (PGE_2) (A) and nitrite (B) in the conditioned medium were determined at the indicated times. Open squares represent the amount of prostaglandin E_2 (A) and nitrite (B) in macrophages incubated in the absence of TPA. Values are the means ± S.E.M. of 4 samples. Statistical significance: (A) At 8 h: auranofin at 3 or 10 μM vs. auranofin control, $P < 0.001$. At 12 and 20 h: auranofin at 1, 3 or 10 μM vs. auranofin control, $P < 0.001$. (B) At 4, 8, 12 and 20 h, auranofin at 3 or 10 μM vs. auranofin control, $P < 0.001$.

2.6. Statistical analysis

The results are expressed as means ± S.E.M. Comparisons were performed with the Student's unpaired *t*-test.

3. Results

3.1. Effects of TPA on the production of prostaglandin E_2 and NO

When rat peritoneal macrophages were incubated at 37°C for 20 h in medium containing various concentrations of TPA, the production of both prostaglandin E_2 and NO were increased in a concentration-dependent manner at TPA concentration from 4.86 to 48.6 nM (3 to 30 ng/ml) (data not shown). The effect of TPA at 16.2 nM (10 ng/ml) on the production of prostaglandin E_2 and NO was time-dependent when examined between 4 and 24 h (data not shown). Therefore, in subsequent experiments, the TPA concentration in the medium was set at 16.2 nM (10 ng/ml), and the cells were incubated for 20 h.

3.2. Effects of indomethacin and L-NMMA on the TPA-induced production of prostaglandin E_2 and NO

When macrophages were incubated in the medium containing TPA (16.2 nM) and various concentrations of indomethacin, the TPA-induced prostaglandin E_2 production at 20 h was decreased in a concentration-dependent manner at indomethacin concentrations from 0.01 to 1 μM; almost-complete inhibition was induced at 1 μM (Fig. 1A). Production of NO was also inhibited by indomethacin in a concentration-dependent manner at 0.1 to 1 μM; 35% inhibition was induced by 1 μM indomethacin (Fig. 1B). On the other hand, L-NMMA inhibited NO production in a concentration-dependent manner at 100 to 1000 μM, with 94% inhibition at 1000 μM (Fig. 2B). Prostaglandin E_2 production was also inhibited by L-NMMA, but significant inhibition was observed only at 300 and 1000 μM (32 and 37% inhibition, respectively) (Fig. 2A). These findings suggested an interaction between prostaglandin E_2 production and NO production in TPA-stimulated rat peritoneal macrophages.

3.3. Effects of auranofin on the TPA-induced production of prostaglandin E_2 and NO

When macrophages were incubated at 37°C for 20 h in medium containing TPA (16.2 nM) and various concentrations of auranofin, the TPA-induced production of prostaglandin E_2 was inhibited by auranofin in a concentration-dependent manner at auranofin concentrations of 1 to 10 μM (Fig. 3A). NO production was also inhibited by auranofin: significant inhibition was not observed at 1 μM, but was observed at 3 and 10 μM (Fig. 3B). Time-course

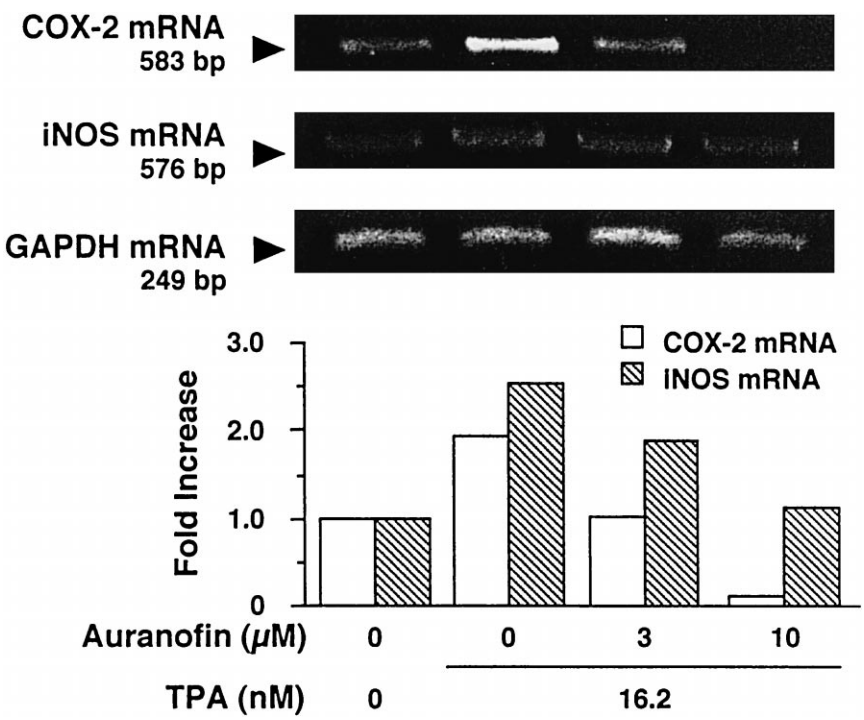

Fig. 5. Effects of auranofin on the TPA-induced increase in the levels of mRNAs for cyclooxygenase-2 and inducible NO synthase. Rat peritoneal macrophages (1.5×10^7 cells) were incubated at 37°C for 4 h in 10 ml of medium containing TPA (16.2 nM) and the indicated concentrations of auranofin. The levels of mRNAs for cyclooxygenase-2 (COX-2), inducible NO synthase (iNOS) and GAPDH were determined by RT-PCR. To facilitate comparison, the ratios of the densities of mRNAs for cyclooxygenase-2 or inducible NO synthase to that for GAPDH in macrophages incubated for 4 h without drugs were set as 1.0 for each mRNA.

experiments revealed significant inhibition of the TPA-stimulated prostaglandin E_2 production by auranofin at 3 and 10 μM at 8 h (Fig. 4A), and of the TPA-stimulated NO production at 4 h (Fig. 4B). Auranofin at 1 μM did not inhibit NO production (Fig. 4B), but inhibited prostaglandin E_2 production after more than 12 h incubation (Fig. 4A). These findings indicated that NO production is more susceptible to auranofin than is prostaglandin E_2 production in TPA-stimulated macrophages.

3.4. Effects of auranofin on the TPA-induced increase in the levels of mRNA for cyclooxygenase-2 and inducible NO synthase

Rat peritoneal macrophages were incubated at 37°C for 4 h in medium containing TPA (16.2 nM) and various concentrations of auranofin, and the levels of cyclooxygenase-2 and inducible NO synthase mRNAs were examined by RT-PCR. As shown in Fig. 5, TPA treatment increased the levels of both cyclooxygenase-2 and inducible NO synthase mRNAs, and auranofin at 3 and 10 μM inhibited the TPA-induced increase in the levels of mRNA for cyclooxygenase-2 and inducible NO synthase in a concentration-dependent manner. The levels of mRNA for GAPDH, a housekeeping gene product, did not change on treatment with TPA and auranofin (Fig. 5).

4. Discussion

The mechanisms of action of auranofin and other disease-modifying anti-rheumatic drugs were recently reviewed by Bondeson (1997). In that review, Bondeson concluded the anti-rheumatic efficacy of auranofin in rheumatoid arthritis can be explained by the potent inhibition of the induction of interleukin-1 and tumor necrosis factor-α. But as described above, the effects of auranofin on the levels of cyclooxygenase-2 and inducible NO synthase mRNAs may also play an important role in the therapeutic effect on rheumatoid arthritis. Non-steroidal anti-inflammatory drugs, which are known to inhibit cyclooxygenase, are also used for the treatment of rheumatoid arthritis (McKenna, 1998; Smyth, 1965). Therefore, a compound that inhibits both the production of NO and prostaglandin E_2 in the inflammatory cells by inhibiting the expression of inducible NO synthase protein and cyclooxygenase-2 protein could be a more potent agent than cyclooxygenase inhibitors. Patients suffered from rheumatoid arthritis are given auranofin orally. The present study showed that auranofin inhibits both the production of NO and prostaglandin E_2 in the cell culture system. Therefore, it might be worthwhile to examine the effect of auranofin when it is injected intra-articularly.

We reported earlier that treatment with TPA stimulates prostaglandin E_2 production (Ohuchi et al., 1985; Yamada et al., 1997; Yamashita et al., 1997b), and increases protein levels of cyclooxygenase-2 (Yamada et al., 1997; Yamashita et al., 1997b) in rat peritoneal macrophages. In the present study, we showed that treatment with TPA stimulates NO production as well as prostaglandin E_2 production in rat peritoneal macrophages. In addition, the levels of mRNAs for cyclooxygenase-2 and inducible NO synthase were increased by TPA treatment, suggesting that the TPA-induced increases in the production of prostaglandin E_2 and NO are due to increases in the levels of the mRNAs for cyclooxygenase-2 and inducible NO synthase, respectively. Elevation of the levels of inducible NO synthase mRNA and inducible NO synthase protein by TPA have also been reported in avian osteoclasts (Sunyer et al., 1997). In addition, overexpression of protein kinase C-ζ causes marked increases of the inducible NO synthase protein in rat mesangial cells (Miller et al., 1997). In contrast, Paul et al. (1997) reported that in RAW 264.7 murine macrophages and rat aortic smooth muscle cells, TPA alone did not result in inducible NO synthase protein expression. However, in the present investigation, we showed that the treatment with TPA alone elevated the mRNA level of inducible NO synthase and increased NO production in rat peritoneal macrophages.

As shown in this report, in rat peritoneal macrophages, the production of both prostaglandin E_2 and NO were increased by stimulation with TPA. As to the biological link between prostaglandin and NO biosynthesis, there are contradictory findings. For example, inhibition of NO synthesis enhances prostaglandin production in rat microglia cells, but does not affect it in RAW 264.7 cells (Guastadisegni et al., 1997). In contrast, in lipopolysaccharide-stimulated RAW 264.7 cells, treatment with the NO synthase inhibitor L-NMMA blocks production of both NO and prostaglandin E_2 (Salvemini et al., 1993). Inhibition of prostaglandin E_2 production by L-NMMA was also reported in isolated rat mesenteric arterial beds (Soma et al., 1996). In the present study, we showed that the inhibition of NO production by L-NMMA partially inhibited prostaglandin E_2 production in rat peritoneal macrophages (Fig. 2B). This suggests that there is an NO-mediated increase in the production of prostaglandin E_2. Moreover, inhibition of prostaglandin E_2 production by indomethacin partially inhibited NO production (Fig. 1B).

We previously reported that auranofin inhibits TPA-stimulated prostaglandin E_2 production in rat peritoneal macrophages by inhibiting the TPA-induced expression of cyclooxygenase-2 protein (Yamada et al., 1997; Yamashita et al., 1997b). In the present paper, we showed that auranofin abrogates the TPA-induced increase in the level of mRNA for cyclooxygenase-2 (Fig. 5). Therefore, it is possible that auranofin acts at the transcriptional level. However, it remains to be tested whether auranofin acts at the post-transcriptional level.

The TPA-induced NO production and the increase in the level of inducible NO synthase mRNA were also inhibited by auranofin (Fig. 3B, Fig. 4B, Fig. 5). There-

fore, the inhibition of NO production by auranofin may also be regulated at the transcriptional level. As for the induction of cyclooxygenase-2, nuclear factor interleukin-6, but not nuclear factor κB, is reported to play a key role in rat granulosa cells (Sirois and Richards, 1993). Yang et al. (1995) reported that the three gold compounds used for the treatment of chronic arthritis rank in potency for inhibiting binding of nuclear factor-κB to DNA as follows: aurothioglucose > aurothiomalate ≫ auranofin. However, we reported that, based on potency to inhibit the TPA-induced prostaglandin E_2 production and lipopolysaccharide-induced NO production, the compounds rank as follows: auranofin ≫ aurothioglucose > aurothiomalate (Yamashita et al., 1997a,b). Thus, the potency of the inhibition by auranofin of the binding of nuclear factor-κB to DNA does not correlate with the inhibition of prostaglandin E_2 production. In addition, the TPA-induced increase in NO production was inhibited by auranofin at 3 to 10 μM (Fig. 3), but not by aurothioglucose and aurothiomalate at these concentrations (data not shown). In mouse macrophages, it is reported that lipopolysaccharide increases inducible NO synthase transcription via its effect on nuclear factor-κB (Xie et al., 1994). Taken together, the previous and present data suggest that the transcription factors that mediate the TPA-induced increase in the levels of cyclooxygenase-2 and inducible NO synthase mRNAs might differ. Further investigation is necessary to clarify the precise mechanism of the transcriptional regulation by auranofin.

The time-course experiment revealed that the inhibition by auranofin of NO production at 3 and 10 μM was apparent at 4 h, while the inhibition of prostaglandin E_2 production was not observed at 4 h (Fig. 4A). Significant inhibition of prostaglandin E_2 production by auranofin was induced after 8 h incubation. The early increase in prostaglandin E_2 production induced by TPA might be partly due to constitutively expressed cyclooxygenase-1 via the following mechanism: TPA stimulates the release of arachidonic acid from membrane phospholipids of rat peritoneal macrophages (Ohuchi et al., 1988), and the released arachidonic acid is metabolized to prostaglandin E_2 by cyclooxygenase-1; thus, prostaglandin E_2 production is increased early on. We have previously reported that auranofin inhibits cyclooxygenase-2-dependent prostaglandin E_2 production, but stimulates cyclooxygenase-1-dependent prostaglandin E_2 production (Yamada et al., 1997). Therefore, at 4 h, it is possible that although cyclooxygenase-2-dependent prostaglandin E_2 production was inhibited by auranofin, cyclooxygenase-1-dependent prostaglandin E_2 production was increased, thus no inhibition by auranofin of TPA-induced prostaglandin E_2 production was observed.

In conclusion, we demonstrated that TPA stimulates production of both prostaglandin E_2 and NO in rat peritoneal macrophages by increasing the levels of mRNAs for cyclooxygenase-2 and inducible NO synthase, respectively, and that auranofin inhibits the TPA-induced increase in the production of prostaglandin E_2 and NO by lowering the levels of mRNAs for cyclooxygenase-2 and inducible NO synthase, respectively.

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