Involvement of Reactive Oxygen Intermediates in Cyclooxygenase-2 Expression Induced by Interleukin-1, Tumor Necrosis Factor-α, and Lipopolysaccharide

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Abstract

Reactive oxygen intermediates (ROIs) play an important role in inflammatory processes as mediators of injury and potentially in signal transduction leading to gene expression. Cyclooxygenase (COX) is a rate-limiting enzyme in prostanoid biosynthesis, and its recently cloned inducible form, COX-2, is induced by proinflammatory cytokines. This study linked ROIs to the signaling pathways that induce COX-2 expression. The hydroxyl radical scavengers DMSO (1%), as well as di- and tetramethylthiourea, inhibited IL-1-, TNFα-, and LPS-induced COX-2 expression in rat mesangial cells. The suppression of COX-2 mRNA expression correlated with the COX-2 protein level. In comparison with the prolonged induction of the inducible gene encoding protein-tyrosine phosphatase by hydrogen peroxide, the COX-2 gene was only transiently induced. Protein-tyrosine phosphatase is also induced by heat shock and chemical stress, whereas COX-2 is not. Superoxide was a more potent inducer for COX-2 than hydrogen peroxide. In addition, NADPH stimulated COX-2 expression, and an inhibitor of NADPH oxidase blocked COX-2 expression induced by TNFα. COX-2 and KC gene expression costimulated by IL-1 were inhibited differentially by the scavengers. These studies demonstrate that oxidant stress is a specific and important inducer of COX-2 gene expression. This induction may contribute to the deleterious amplification of prostanoids in inflammation and compound the direct effects of ROI production. (*J. Clin. Invest.* 1995. 95:1669–1675.) Key words: reactive oxygen intermediates • NADPH • mesangial cell • PTPase • KC

Introduction

Cyclooxygenase (COX)[1] is a rate-limiting enzyme in prostanoid synthesis. COX-2 is a recently cloned form of COX that is induced in a number of cells by proinflammatory stimuli (1–5). Exposure of a variety of cells or tissues, including rat mesangial cells, to IL-1, TNFα, or LPS results in increased levels of mRNA and peptide for COX-2, but not for COX-1 (6–9). We have recently cloned rat COX-1 and COX-2 and studied the regulation of both genes (10); however, the second messengers involved in the signal transduction pathways leading to induction of COX-2 remain incompletely defined. Hydrogen peroxide can stimulate prostaglandin production in vivo (11). Inhibitors of reactive oxygen formation and the scavengers of reactive oxygen species have been reported to block prostanoid production by quenching the generation of hydroperoxides, which are activators of COX (12). Reactive oxygen intermediates (ROIs) are agents commonly produced by inflammatory cells during inflammatory processes (13), including those occurring in arthritis and glomerulonephritis. In the latter, glomerular cells, particularly mesangial cells, can generate ROIs independently of infiltrating cells, which may serve an autacoid role in glomerular injury (14). IL-1 and TNFα induce both superoxide and hydrogen peroxide production in human mesangial cells, with production increasing during a 90-min exposure (15).

To determine whether ROIs are involved in the expression of COX-2 induced by IL-1, TNFα, and LPS, the effects of the radical scavengers DMSO and di- and tetramethylthiourea (DMTU and TMTU) and of other antioxidants on the expression of COX-2 were compared with those on COX-1 and the rat homolog of a known oxidant-inducible protein, tyrosine phosphatase (PTPase, CL100/3CH134) (16, 17). To show the specificity of ROIs in COX-2 expression, the effects of the radical scavengers on the expression of the rat KC gene, a chemokine also induced by IL-1 (18), were compared with their effects on COX-2. Membrane-bound NADPH-dependent oxidase components have been identified on human mesangial cells and have been suggested as a source for generation of ROIs by mesangial cells (19, 20). To study the role of NADPH, both NADPH stimulation and inhibition were used.

Methods

Culture of rat mesangial cells. Rat mesangial cells were a gift from Dr. Jeffrey I. Kreisberg (Department of Pathology, University of Texas Health Science Center at San Antonio, San Antonio, TX). Mesangial cells were identified by ultrastructural, biochemical, and immunofluorescence techniques (21). Cells were cultured in RPMI 1640 tissue culture medium containing 20% FCS with antibiotics and an antimycotic. All experiments were performed on 80% confluent cells between the 8th and 12th passages.

Reverse transcriptase PCR cloning of rat PTPase (CL100/3CH134) and riboprobe generation. CL100/3CH134 sense and antisense primers were designed by alignment of human (CL100) and murine (3CH134) (16, 17), 5′-GGATCCGAAGCTGTTTTCGGCTTCCTGC-3′ with BamHI site at the 5′ end; and 5′-CTTGTACTGGTAGTGACCCTC-3′

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1. *Abbreviations used in this paper:* CHX, cycloheximide; COX, cyclooxygenase; DMTU, dimethylthiourea; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMAP, 4′-hydroxy-3′ methyl-acetophenone; PTPase, protein-tyrosine phosphatase; ROI, reactive oxygen intermediate; TMTU, tetramethylthiourea.

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for antisense. In vivo excised DNA from an LPS-stimulated rat peritoneal macrophage cDNA library constructed in λZapII (a gift of Dr. Wolfgang Northemann, The Scripps Research Institute) was used as a template to clone a PTPase 298-bp cDNA fragment (from 376 to 664 bp of the mouse homolog). PCR was performed for 30 cycles with denaturing at 93°C for 30 s, annealing at 60°C for 40 s, and elongating at 72°C for 30 s. The PCR products were digested with BamHI and subcloned in pBluescript (Stratagene, La Jolla, CA) digested with BamHI and EcoRV. This probe was used to screen the rat peritoneal macrophage cDNA library. Several full-length clones were obtained (Feng, L., Y. Xia, D. Seiffert, and C. B. Wilson, manuscript in preparation). The cDNAs were sequenced with an automated DNA sequencer (ABI 373A; Applied Biosystems, Foster City, CA) using appropriate primers. A 177-bp NcoI fragment of rat PTPase (114–291) was subcloned into pGEM5Z (Promega Corp., Madison, WI), and the clone with the 3′ end toward the T_7 promoter was selected. The recombinant plasmid was linearized with EcoRV and transcribed using T_7 polymerase. A 132-bp rat KC fragment was subcloned from rat KC by digesting cDNA with PstI and NcoI and was then ligated into pGEM5Z. The recombinant plasmid was linearized with SalI and transcribed with T_7 RNA polymerase. The 340-bp rat COX-1, 242-bp rat COX-2, and 114-bp rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH), termed GAP 114, probes have been described previously (10, 22).

Total RNA extract and RNase protection assay. The total RNA was prepared by a single-step method (23), quantitated by its absorption at 260 nm, and frozen at −70°C. 1 μg of total RNA was hybridized with 10^5 cpm of each ^{32}P-labeled rat antisense riboprobe. The RNase protection assay was performed as described previously, and the radioactivity in the protected bands was quantitated by the Radioanalytic Imaging System (AMBIS Systems, San Diego, CA) (10, 22).

Western blot determination of total enzyme protein levels. The level of COX-2 was analyzed by Western blot analysis using an anti–COX-2 antibody as described previously (10). Briefly, cells were sonicated in PBS, pH 7.4, containing 10 mM EDTA, 5 mM EGTA, 1 mM PMSF, and 10 μM leupeptin for 30 s. Microsomes from the lysed cells were electrophoresed on an 8% SDS–polyacrylamide gel and were transblotted onto a nitrocellulose filter. The blot was blocked with 5% nonfat milk, incubated in the antibody solution (diluted 1:250) overnight, and treated with goat anti–rabbit IgG conjugated with alkaline phosphatase (diluted 1:1,500). Color development was done with alkaline phosphatase color reagents (Boehringer Mannheim, Indianapolis, IN).

Nuclear run-off transcription assay. Nuclear run-off analysis was performed as described previously (18). Briefly, nuclei were prepared by lysing 10^8 cells in a buffer (10 mM Tris-HCl, pH 7.4, 3 mM $CaCl_2$, 2 mM $MgCl_2$) containing 0.6% NP-40. Nuclei were collected by centrifugation, placed in a storage buffer (50 mM Tris-HCl, pH 8.3, 40% glycerol, 5 mM $MgCl_2$, 0.1 mM EDTA), and held at −80°C. Upon thawing, nuclei were resuspended and incubated with an equal volume of labeling buffer (300 mM KCl, 0.5 mM each ATP, GTP, and CTP), containing 200 μCi of [α-^{32}P]UTP (DuPont-NEN, Boston, MA) at 30°C for 1 h. After pulse labeling, radiolabeled RNA was prepared by sequential DNase I digestion, organic extraction, ethanol precipitation, and TCA precipitation. For probing, plasmids containing full-length COX-1, COX-2, PTPase, or GAP 114 cDNA (10 μg per dot) were blotted onto membranes using a slot–blot apparatus (Schleicher & Schuell, Keene, NH) and hybridized for 48 h at 65°C with pulse-labeled RNA. After washing at high stringency, the membranes were exposed to x-ray film at −70°C for 5 d. After autoradiography, the quantities of the transcript were determined by scanning the x-ray film with a densitometer, and the final values were factored by the GAP 114 mRNA.

Prostaglandin E_2 assay. To determine COX activity, cell supernatants were assayed for prostaglandin E_2 (PGE_2) using previously published methods (10).

Results

Radical scavengers suppress COX-2 expression induced by IL-1, TNFα, and LPS. Rat mesangial cells stimulated by proinflammatory cytokines and LPS express both COX-2 and PTPase, but not COX-1 (Fig. 1 *A*). All three of the hydroxyl radical scavengers, DMSO, DMTU, and TMTU, reduced the expression of COX-2 and PTPase in cytokine-stimulated mesangial cells at 4 h without affecting levels of COX-1 or the housekeeping gene GAPDH (Fig. 1 *A*). DMTU and TMTU, at a dose of 10 mM, almost completely blocked steady-state COX-2 mRNA induced at 4 h by all three stimuli (IL-1, TNFα, and LPS), whereas equimolar urea as a control had no effect on the expression of COX-2 and PTPase. In contrast to DMTU and TMTU, DMSO, which inhibited TNFα-induced COX-2 expression, only partially inhibited IL-1–induced COX-2 expression (55%) and had no effect on LPS-induced COX-2 expression. DMSO also was relatively ineffective in reducing the induced PTPase mRNA levels. The decrease in COX-2 steady-state mRNA levels was mirrored by a decrease in COX activity, as assessed by PGE_2 production (Fig. 1 *B*).

To examine the possible role of protein synthesis in the DMTU inhibition of IL-1–induced COX-2 expression, a study was done using cycloheximide (CHX). IL-1 with or without DMTU was used to stimulate mesangial cells in the presence or absence of 25 μg/ml CHX. Using the ratios of (COX-2/GAPDH counts per minute) × 10 as determined by the AMBIS system, readout values of 0.06, 0.92, 0.62, 1.16, 0.10, and 0.12 were found for control, IL-1 alone, CHX alone, IL-1 + CHX, IL-1 + DMTU, and IL-1 + DMTU + CHX, respectively. This indicated that COX-2 expression was superinduced by IL-1 in the presence of CHX. It also showed that the DMTU inhibition of IL-1–induced COX-2 expression was not changed by CHX, indicating that protein synthesis was not required for the inhibitory effect.

DMTU suppresses COX-2 induction by IL-1 in a dose- and time-dependent manner. As shown in Fig. 2 *A*, the addition of DMTU suppressed the COX-2 expression in a dose-dependent fashion at 4 h. DMTU at 1 mM inhibited COX-2 expression to 40%, and nearly complete suppression was observed at 10 mM. In the time course study, when DMTU (10 mM) was incubated concurrently with IL-1 a mean suppression (n = 3 studies) of COX-2 steady-state mRNA levels of 20, 56, 93, and 83% was found at 1, 2, 4, and 8 h, respectively. The COX-2 protein level (Fig. 2 *B*) correlated well with the COX-2 mRNA level.

Effects of DMTU and TMTU on mRNA levels for COX-2 and PTPase induced by serum or PMA. In addition to proinflammatory cytokines, serum and PMA are strong inducers of COX-2. As previously described, the later stage (2–4 h) induction of COX-2 gene expression by LPS, TNFα, and IL-1α is oxidant dependent. We examined whether ROIs might be involved in the intracellular signaling after serum induction of COX-2. As shown in Fig. 3, DMTU and TMTU had no effect on serum-induced COX-2 expression and incompletely suppressed PMA-induced COX-2 expression, suggesting that the signaling involved in serum induction is different from that of the proinflammatory cytokines. The scavengers had no effect on the PTPase mRNA induced by PMA, again suggesting different regulatory mechanisms for COX-2 and PTPase.

Selectivity of scavenger effects using KC expression induced by IL-1. To verify further the specificity of the scavenger effect on oxidant stress genes, the effect of the scavengers on another IL-1–inducible gene, KC, was studied. As shown in Fig. 4, KC was strongly induced by IL-1; however, the scavengers had no effect on KC expression. Both COX-2 and PTPase expression were dramatically inhibited, as expected.

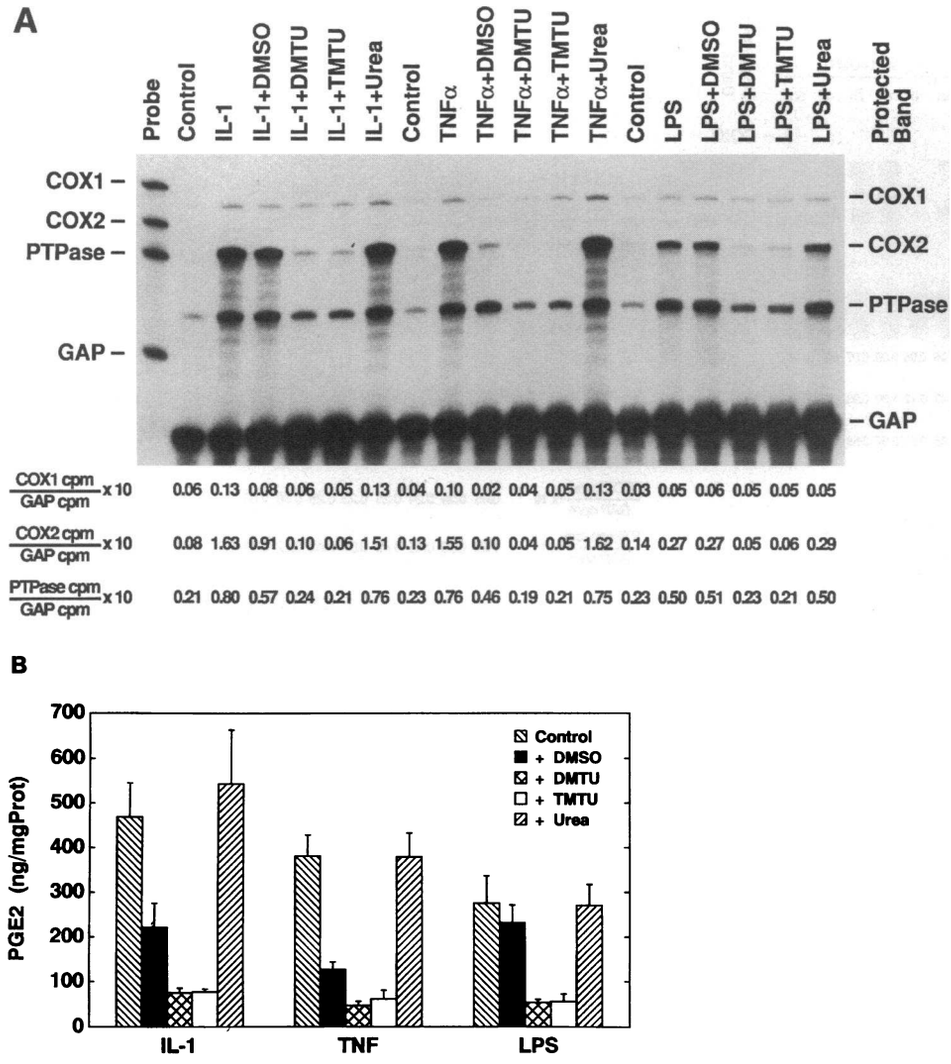

Figure 1. (*A*) RNase protection assay analysis of mRNA for COX-1, COX-2, PTPase, and GAPDH in mesangial cells. After attaining confluency, mesangial cells were incubated in serum-free RPMI 1640 for 48 h and were then further incubated in the serum-free medium with various reagents for 4 h before harvesting. The cells were treated in the presence of IL-1 (1 ng/ml), TNFα (200 U/ml), or LPS (1 μg/ml) with or without DMSO (1%), DMTU (10 mM), TMTU (10 mM), or urea (10 mM) for 4 h. The protected COX-1, COX-2, PTPase, and GAPDH bands are shorter than their respective probes because the unhybridized polylinker regions in the cRNA probes are digested by RNase. The counts per minute of the bands in each lane were factored for GAPDH counts, and the ratio of the two values ×10 is presented below each lane. (*B*) 4 h after the treatment indicated, the mesangial cell supernatants were collected and assayed for PGE_2. The final values were factored relative to protein level (in milligrams) assayed by the Bradford method. Each point represents mean±SE (n = 3).

Induction of COX-2 expression with hydrogen peroxide and superoxide. To determine whether ROIs could directly induce expression of COX-2 in mesangial cells, the cells were incubated with hydrogen peroxide or a superoxide-generating system. The time course for hydrogen peroxide COX-2 induction is shown in Fig. 5. Hydrogen peroxide transiently induced a low level of COX-2 expression that increased at 1 h, peaked at 4 h, and declined thereafter. The time course of ROI-induced COX-2 expression corresponded to the time course of ROI scavenger inhibition of cytokine-induced COX-2 expression. In comparison with COX-2, PTPase was dramatically induced by hydrogen peroxide, with the induction occurring as early as 15 min and lasting for 12 h. COX-2 was markedly induced by superoxide generated by xanthine oxidase hypoxanthine and the superoxide-generated drug menadione.

Induction of COX-2 expression by ROIs generated by NADPH-dependent oxidase. The generation of ROIs by mesangial cells has been linked to NADPH-dependent oxidase (19). The effects of NADPH and the inhibitor of NADPH oxidase 4′-hydroxy-3′-methoxy-acetophenone (HMAP) (19) on COX-2 expression by mesangial cells were examined. NADPH added to the incubation medium increased COX-2 expression in a dose-dependent fashion. The COX-2 expression induced by NADPH was suppressed by DMTU and TMTU (Fig. 6). The NADPH oxidase inhibitor HMAP blocked TNFα-induced COX expression and partially blocked IL-1–induced COX-2 expression while having little affect on LPS-, serum-, or PMA-induced COX-2 expression (Fig. 6). This indicated a role for NADPH in intracellular signaling during TNFα- and probably IL-1–induced expression of COX-2.

The radical scavenger DMTU transcriptionally inhibits COX-2 mRNA expression. The effects of radical scavengers on the transcription of the COX-2 and PTPase genes were examined by nuclear run-off assay. Mesangial cells were stimulated with IL-1 (1 ng/ml) for 30 min or 2 h in the presence or absence of DMTU. There was a marked increase in both COX-2 and PTPase transcripts after stimulation, and this increase was blocked by DMTU at 2 h, but not at 30 min, corresponding to the findings of the scavenger effect on steady-state COX-2 expression (Fig. 7).

COX-2 and PTPase expression responds differently to environmental stress. COX-2 expression induced by hydrogen peroxide and superoxide might be a response of the mesangial cell to environmental stress. The expression of COX-2 was not induced at 45°C in contrast to PTPase, which was strongly induced (Fig. 8). The heat shock induction of PTPase was not

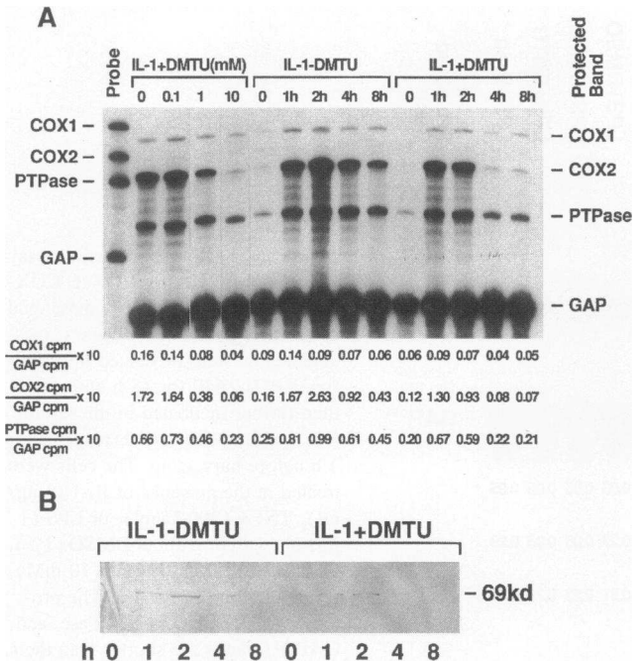

Figure 2. (*A*) Time- and dose-dependent inhibition of COX-2 expression as assessed by RNase protection assay in mesangial cells stimulated by IL-1. Cells preincubated in serum-free medium for 48 h were further incubated in medium containing IL-1 (1 ng/ml) with or without urea or DMTU for the specified times or doses (4-h incubation). Total RNA was extracted and hybridized with riboprobes, as described in Methods. The [α-^{32}P]UTP-labeled COX-1, COX-2, PTPase, and GAPDH riboprobes were protected with the complementary mRNA from mesangial cells. The ratio of counts per minute was calculated as described in Fig. 1. (*B*) Western blot analysis of COX-2 in mesangial cells stimulated by IL-1 with or without DMTU. The mesangial cells were incubated in serum-free RPMI 1640 for 48 h and were then further incubated in RMPI 1640 with IL-1 (1 ng/ml) with or without DMTU (10 mM) for the specified times. Western blot analysis was performed as described in Methods.

inhibited by the scavengers. These results indicate that although the induction of COX-2 and PTPase expression can be mediated through an oxidant-dependent mechanism, they are not coordinately induced by other stress treatments.

Discussion

The hydroxyl radical scavengers DMTU and TMTU, and to a lesser extent DMSO, inhibited the later stages (2–4 h) of IL-1–, TNFα-, and LPS-induced COX-2 expression without affecting COX-1 or GAPDH mRNA expression. The scavengers were less effective in inhibiting COX-2 expression induced by PMA or serum, and the expression of PTPase induced by PMA was not blocked at all by the scavengers. The differential inhibitory effects of the scavengers on the expression of COX-2 and PTPase induced by serum, PMA, cytokines, and LPS implied that the inhibitory effects were specific. By nuclear run-off assay, IL-1 transcriptionally induced COX-2 expression. The scavenger-induced decrease in COX-2 expression was shown to be related to transcriptional inhibition at later time points (2 h), since DMTU decreased transcriptional activity. The delayed transcriptional inhibition correlated with the steady-state COX-

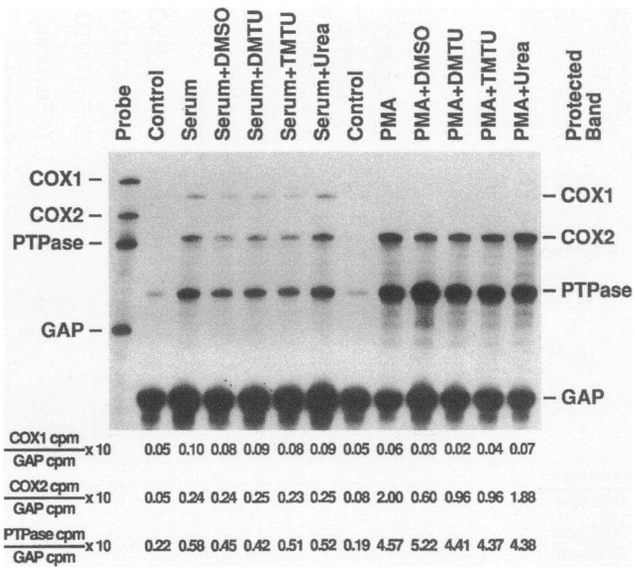

Figure 3. Effects of DMSO (1%), DMTU (10 mM), TMTU (10 mM), and urea (10 mM) on mRNA levels for COX-1, COX-2, and PTPase induced by serum and PMA, as assessed by RNase protection assay. Cells preincubated in serum-free medium for 48 h were further incubated in medium containing 10% FCS or PMA (200 nM). Radioactivity was analyzed by the AMBIS system (see Methods).

2 mRNA level. The transcriptional inhibition of COX-2 resulted in the reduction of COX-2 protein. A decrease in COX activity was also seen in the experiments (Fig. 1 *B*). Marked inhibition occurred at 4 h. DMTU inhibition of COX-2 mRNA expression

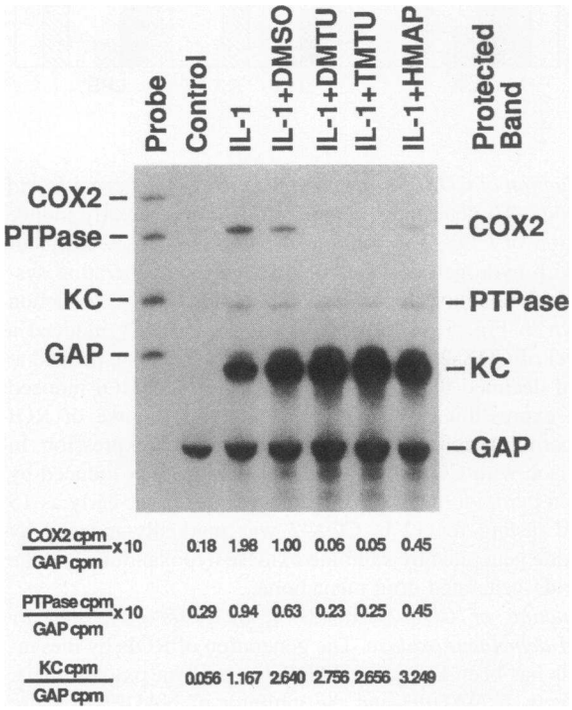

Figure 4. The effects of the scavengers on the mRNA levels for COX-2, PTPase, KC, and GAPDH. After serum starvation for 48 h, the cells were incubated in serum-free medium containing IL-1 (1 ng/ml) with or without DMTU (10 mM) or TMTU (10 mM).

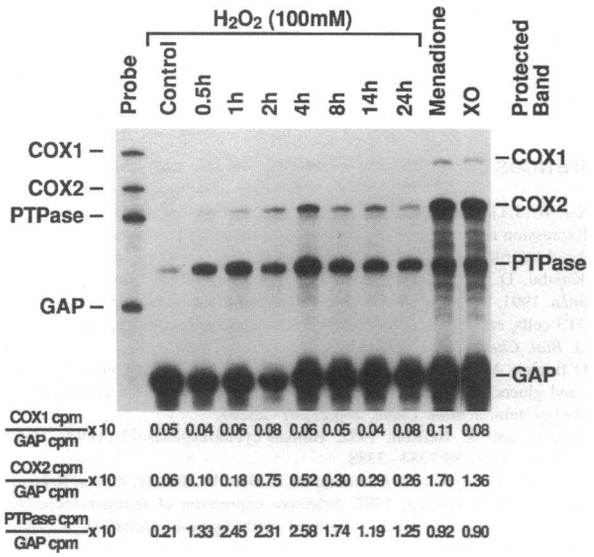

Figure 5. Effects of hydrogen peroxide (100 μM), xanthine oxidase (*X O;* 10 mU/ml)/hypoxanthine (HX, 0.6 mM), and menadione (500 μM) on the mRNA levels for COX-1, COX-2, and PTPase as assessed by RNase protection assay.

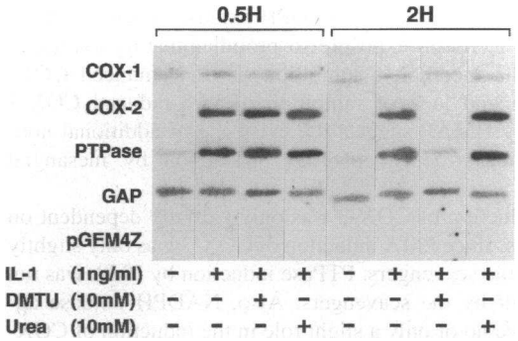

Figure 7. Effects of DMTU and TMTU urea on the transcriptional activity for COX-1, COX-2, and PTPase induced by IL-1, TNFα, and LPS at 30 min and at 2 h. Labeled transcripts (10^7 cpm/ml) generated by nuclei were hybridized with full-length cDNA for COX-1, COX-2, PTPase, GAPDH, and pGEM4Z (as a negative control).

was not affected by CHX, suggesting that ROI-mediated COX-2 gene expression induced by IL-1 might belong to the delayed early response (24).

Since neither COX-1 nor GAPDH was stimulated by the proinflammatory agents and PTPase expression also was regulated through the antioxidant-sensitive mechanism, these three genes may not represent adequate controls. Therefore, another IL-1–inducible gene, KC, was selected as a control. KC belongs to a large superfamily of chemokines and is regulated by proinflammatory cytokines (18). It was recently reported that IL-8 and MCP-1, members of the chemokine family, are regulated by ROIs (20, 25). A homolog of IL-8 in rats has not been identified; however, rat KC has some similarity with IL-8. In the current study, KC expression was not related to oxidant stress, since the scavengers had no effect on its expression (Fig. 4). Therefore, although both COX-2 and KC gene expression are costimulated by IL-1, they are differentially inhibited by the scavengers. This observation further verified the specificity of the inhibitory effects of the scavengers on COX-2.

The observations that the addition of NADPH increased COX-2 expression and the NADPH oxidase inhibitor HMAP dramatically inhibited TNFα-induced COX-2 expression and partially inhibited IL-1– and LPS-induced COX-2 expression indicate the involvement of NADPH oxidase in the generation of ROIs. However, several non-NADPH oxidase–dependent sources of ROI production also exist, including radicals generated during mitochondrial electron transport and arachidonate metabolism (26). Moreover, many cell types, such as endothe-

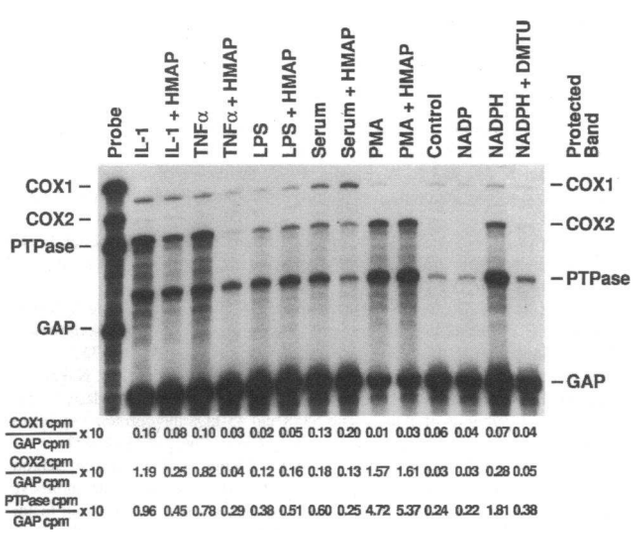

Figure 6. Effects of NADPH (5 mM) and HMAP (100 μg/ml) on COX-1, COX-2, and PTPase mRNA levels. Cells preincubated in serum-free medium for 48 h were further incubated with 10% FCS medium or serum-free medium containing IL-1 (1 ng/ml), TNFα (200 U/ml), LPS (1 μg/ml), or PMA (200 nM).

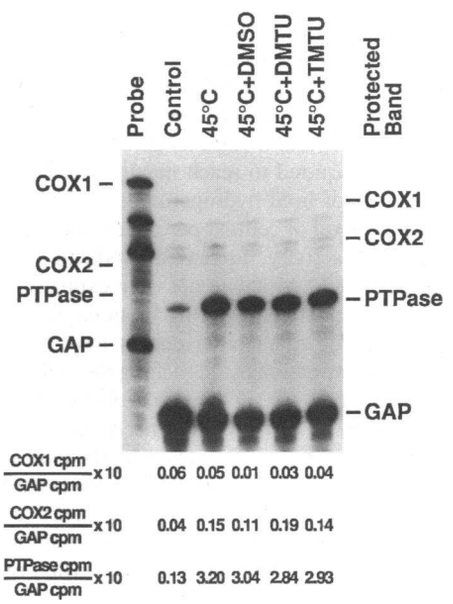

Figure 8. Effects of heat shock (45°C, 5 min) on COX-1, COX-2, and PTPase mRNA levels, as assessed by RNase protection assay. The double bands result from incomplete digestion of the probes.

lial cells and fibroblasts, lack NADPH oxidase systems, but can still produce ROIs on exposure to proinflammatory cytokines (27, 28). The incomplete inhibition of IL-1–induced COX-2 expression and lack of inhibition of LPS-induced COX-2 expression by HMAP suggest the existence of additional non-NADPH oxidase–dependent ROI production by mesangial cells.

PMA induction of COX-2 was only partially dependent on oxidant stress, since PMA induction of COX-2 was only slightly inhibited by the scavengers. PTPase induction by PMA was not blocked at all by the scavengers. Also, NADPH oxidase appeared to have no or only a slight role in the induction of COX-2 by PMA or serum, since there was no or little inhibition by HMAP of either, again suggesting that the PMA/serum signaling pathway of COX-2 expression differed from that of the ROIs.

In contrast to PTPase, heat shock stimuli failed to induce COX-2 expression. These observations suggest that COX-2 induction via ROIs is not just a general response to environmental or cytotoxic stress. Moreover, the differential induction of COX-2 and PTPase by heat shock suggests that the specific responses of these genes to the various stimuli represent different regulatory mechanisms. Indeed, heat shock–induced PTPase expression was not influenced by the scavengers DMTU and TMTU, whereas these scavengers were very potent inhibitors of cytokine-induced PTPase mRNA expression.

ROIs may serve as intracellular signals for gene activation involving specific transcription factors such as NK-κB (29–33). Pyrrolidone-thiocarbamate has been shown to inhibit cytokine-induced activation of the NF-κB in several cell lines (20, 34, 35). It did not effect cytokine-induced COX-2 expression in mesangial cells (data not shown). In addition, an NF-κB–like element has not been identified in the promoter regions of rat or mouse genomic DNA, although it has been found in chicken inducible COX-2 (36–38). These observations suggest that NK-κB may not be involved in cytokine-induced/ROI scavenger–inhibited COX-2 expression.

The pathophysiologic significance of our finding that COX-2 is an oxidant stress–inducible gene may help to explain the enhanced expression of COX-2 during inflammation. The ROIs produced by inflammatory cells could lead to the deleterious amplification of prostanoids during inflammation. Lipid hydroperoxides are important physiological activators of COX (12). An important aspect of COX is that it exhibits autoaccelerative reaction kinetics. The time required to reach maximal velocity is decreased in the presence of lipid hydroperoxides (39, 40). Thus, small increases in the level of cellular peroxides can cause dramatic increases in COX activity. Increased production of ROIs during inflammation could stimulate not only expression of COX-2, but also enzyme activity, resulting in an explosive production of prostanoids. The radical scavengers have been reported to block prostanoid formation by quenching the generation of hydroperoxides, which are the activators of COX (12). Our results suggest that such an interpretation needs to be re-evaluated. The inhibition of prostanoid formation by the radical scavengers may be due to the suppression of COX-2 expression. The findings reported here suggest the potential efficacy of antioxidants in ameliorating inflammatory diseases not only to inhibit their direct effects, but also to aid in the suppression of prostanoids when desirable.

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