Regulation by reactive oxygen species of interleukin-1β, nitric oxide and prostaglandin E₂ production by human chondrocytes


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Summary

Objectives: To determine the effects of two drugs, N-monomethyl-L-arginine (L-NMMA) and N-acetylcysteine (NAC), on interleukin-1β (IL-1β), nitric oxide (NO) and prostaglandin E₂ (PGE₂) production by human chondrocytes. The effect of aceclofenac (ACECLO), a non-steroidal antiinflammatory drug (NSAID), was also examined.

Methods: Human chondrocytes were enzymatically isolated from osteoarthritic knee cartilage and then maintained in culture in suspension for 48 h in the absence or in the presence of lipopolysaccharide (LPS) (10 μg/ml), L-NMMA (0.5 mM), NAC (1 mM) or ACECLO (6 × 10⁻⁶ M). IL-1β and PGE₂ productions were quantified by specific immunoassays. Nitrite was measured in the culture supernatants by a spectrophotometric method based upon the Griess reaction. Cyclooxygenase-2 (COX-2), inducible NO synthase (iNOS) and IL-1β gene expressions were quantified by transcription of mRNA followed by real time and quantitative polymerase chain reaction. COX-2 protein expression was analysed by Western blot.

Results: LPS markedly increased the expression of IL-1β, iNOS and COX-2 genes. In parallel, NO₂ and PGE₂ amounts found in the culture supernatants were significantly enhanced whereas IL-1β was immunologically undetectable. The addition of L-NMMA (0.5 mM) fully blocked LPS-induced NO production but greatly increased PGE₂ production, suggesting a negative effect of NO on PGE₂ synthesis. Inversely, NO production was stimulated by NAC while PGE₂ production was not affected. Interestingly, NAC increased the IL-1β and iNOS mRNA levels but did not significantly modify COX-2 mRNA expression. L-NMMA did not significantly affect the expression of IL-1β, iNOS and COX-2. The amount of COX-2 protein did not change in the presence of the antioxidants. Finally, ACECLO fully blocked the production of PGE₂ by chondrocytes without affecting the levels of COX-2 mRNA.

Conclusions: The stimulation of IL-1β, NO and PGE₂ production by LPS is differentially controlled by reactive oxygen species (ROS). In fact, L-NMMA and NAC have different mechanisms of action on the regulation of NO and PGE₂ productions. L-NMMA fully inhibits NO but increases PGE₂ production whereas NAC up-regulates NO but does not modify PGE₂ synthesis. The stimulating effect of L-NMMA on PGE₂ production is not controlled at the transcriptional level. These findings suggest that antioxidant therapy could have different effects according to the oxygen radical species targeted. © 2002 OsteoArthritis Research Society International. Published by Elsevier Science Ltd. All rights reserved.

Key words: Reactive oxygen species, Chondrocyte, Nitric oxide, Cytokines, Arthritis.

Introduction

NO is a gaseous free radical, which is transformed to nitrate and nitrite in the presence of oxygen. NO is synthesized through L-arginine oxidation by a family of NO synthases. It rapidly reacts with superoxide anions (O₂⁻) to form peroxynitrite (ONOO⁻) which is a strong oxidant. It is well accepted that chondrocytes possess a NADPH oxidase and produce both ROS and NO¹,². In vitro, ROS and NO productions are strongly stimulated by IL-1β, which is a key cytokine in the pathogenesis of arthritis³. Nitric oxide is presented as a catabolic agent since it was demonstrated that intraarticular injection of L-NIL (n-iminoethyl-L-lysine), a selective inhibitor of inducible nitric oxide synthase, reduced the progression of cartilage erosion in an experimental osteoarthritic dog model⁴. In vitro, the inhibition of endogenous NO production by L-NMMA, another well established NO synthase inhibitor, led to the suppression of IL-1β stimulating effects on metalloproteases⁵ and partially reversed the IL-1β inhibitory effect on glycosaminoglycan synthesis by chondrocytes⁶. Furthermore, NO inhibits IL-1 receptor antagonist (IL-1ra) production by chondrocytes, a mechanism possibly responsible for the enhancement of IL-1 biological activity⁷.

Although there is a general agreement that NO is involved in cartilage destruction, the literature is unclear about the role played by NO in the initiation and progression of synovial membrane inflammation. NO was originally identified as the endothelium-derived relaxing factor with potential proinflammatory effects, including increase of vascular permeability, enhancement of PGE₂ synthesis⁸–¹⁰ and the induction of catabolic cytokines like IL-1β and...
TNFα. Further evidence that NO may be antiinflammatory under certain circumstances is provided by its ability to down-regulate PGE\(_{15-14}\) IL-6 and IL-8 production by chondrocytes and liver kupffer cells\(^1\) and to suppress the production of O\(_2\) by neutrophils through a direct action upon NADPH-oxidase\(^6\). These conflicting results obtained with NO synthase inhibitor could be explained by the fact that intracellular signalling pathways are very sensitive to intracellular fluctuation of ROS levels. This hypothesis gains strong support from the finding that the two well studied nuclear transcription factors, NF-kB and AP-1, are activated when intracellular ROS are increased or decreased, respectively\(^7\). These factors are involved in rapid induction of gene transcription in response to a variety of pathogenic conditions, specifically chronic inflammation. Another explanation could be that NO reactivity could be strongly different according to its ability to react with other oxygen species to form new oxidative molecules. In fact, NO interacts easily with O\(_2\) to form not only the most potent peroxynitrite but also derivative active free radicals such as peroxynitrous acid, hydroxyl radical and nitrogen dioxide\(^8\).

This complex interdependent regulation of NO, IL-1β and PGE\(_2\) creates a dilemma for pharmaceutical intervention of NO. In pathological context, such as arthritis where both inducible NOS and COX-2 are activated, therapeutical strategies focused on regulating COX activity by NO may be of some benefit.

In view of these observations, this study was designed to investigate the effects of antioxidant molecules on IL-1β and PGE\(_2\) productions by human chondrocytes and then to identify the regulatory role played by NO or derived reactive species.

**Materials and methods**

**SHORT TERM SUSPENSION CULTURE OF HUMAN CHONDROCYTES**

Cartilage specimens were obtained from the knees of eight donors shortly after death (four males and four females) with the mean age of 49 (47–52) years old, being excised from the superficial and medium layers and avoiding the calcified layer. Upon dissection, femoral and patellar articular surfaces were evaluated for the severity of the macroscopic cartilage lesions using a personal scale. Four different grades were considered: 0, normal white cartilage; II, irregular surface with deep fibrillations on one or more articular surfaces; III, ulcers penetrating to subchondral bone on one or more articular surface; IV, shallow ulcers, lesions of grade III and four cartilage samples had lesion of grade I. Each culture was run with chondrocytes from a single patient. The cartilage slices were cut into small fragments and then subjected to sequential enzymatic digestions with hyaluronidase, pronase and collagenase as previously described\(^9\). The cells were then filtered through a nylon mesh (70 μm), washed three times, counted (1.10\(^6\) cells/ml) and resuspended in 1 ml of adequate culture medium: DMEM without red phenol (BioWhittaker, Brussels, Belgium) supplemented with 10 mM HEPES, penicillin (100 U/ml), streptomycin (0.1 mg/ml) and 1% of ITS\(^+\) (ICN, Doornveld, Belgium). ITS\(^+\) is a culture supplement containing in 1 ml: 0.526 mg of insulin, 0.625 mg of transferrin, 0.625 μg of selenious acid, 125 mg of bovine serum albumin (BSA) and 0.535 mg of linoleic acid. The cells recovered after collagenase digestion of the tissue were 95% viable (trypan blue test). Cells were then kept in culture medium for 48 h in order to eliminate in vivo contamination with drugs that the donors may have taken before death.

After this washout period, cells (1 · 10\(^6\) cells/ml in culture medium) were seeded in 15 ml polypropylene Falcon tubes and maintained under agitation on a gyratory shaker (100 rpm) in a 95% air/5% CO\(_2\) environment. Chondrocytes were cultured for 48 h in the absence or in the presence of LPS (10 μg/ml, serotype 026:B6; Sigma-Aldrich, Bornem, Belgium) and with or without L-NMMA (0.5 mM; Calbiochem, San Diego, U.S.A.) NAC (1 mM); Sigma-Aldrich, Bornem, Belgium) or ACECLO (6 · 10\(^{-6}\) M; Prodesfarma, Spain). ACECLO was first dissolved in N,N-dimethylethamidate (DMF, Sigma-Aldrich, Bornem, Belgium), and then diluted in culture medium to achieve the required concentration. The final concentration of DMF was 0.1%. A similar amount of the vehicle was added to the controls. Cells and supernatant were then separated by centrifugation (1000 rpm; 5 min). Other chemicals were of the purest commercial grade available.

**NITRIC OXIDE ASSAY**

NO production was determined by quantifying its derived product nitrite in conditioned medium using a spectrophotometric method based on the Griess reaction\(^1\). Briefly, 150 μl of conditioned culture medium or sodium nitrite (NaNO\(_2\)) standard dilutions were mixed with 100 μl of Griess reagent (0.5% sulfanilamide, 0.05% naphthylethylene-diamine dihydrochloride, 2.5% H\(_3\)PO\(_4\)) and incubated for 5 min at 37°C. The limit of detection was 2 μM of nitrite. Before adding the Griess reaction, nitrate was converted to nitrite by treating sample with nitrate reductase (0.2 U/mL, Roche, Brussels, Belgium). The absorbance was measured at 540 nm.

**PGE\(_2\) RADIOIMMUNOASSAY**

PGE\(_2\) level was determined in conditioned culture medium according to a previously described radioimmunoassay (RIA)\(^2\) using a polyclonal antiserum, obtained from rabbit. The 3\(^\text{H}\)-labeled PGE\(_2\) was purchased from New England Nuclear (Brussels, Belgium) and the standard molecule PGE\(_2\) from Sigma-Aldrich (Bornem, Belgium). PGE\(_2\) was measured in triplicate in a 100-μl aliquot of each sample. After 48 h of incubation at 4°C, free antigen was separated from antibodies-antigen complex by charcoal precipitation and centrifugation (20 min at 1000 g, 4°C). The supernatant was counted by liquid scintillation. Intra- and interassay coefficients of variation were 6 and 10%, respectively. The recovery of known amounts of PGE\(_2\) added to the culture medium was satisfactory (90 to 97% for the amounts of PGE\(_2\) ranging from 2500 to 312 pg/ml). The limit of detection of the RIA was 20 pg/ml.

**IL-1β ASSAYS**

IL-1β production by chondrocytes was assayed in the culture media by two different enzyme amplified sensitivity immunoassays (EASIA) designed by RD System (Abingdon, U.K.) and by Genzyme (Cambridge, U.S.A.). The assays were directly performed in the culture media.
without previous extraction. The limits of detection of the two assays were 1 and 0.1 pg/ml, respectively.

DNA ASSAY

Chondrocyte DNA content was directly correlated to the cell number of each culture. DNA content was measured in the cell pellet extracts using a fluorimetric method. This measurement ensures elimination of result variations due to the different number of chondrocytes.

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

RNA was isolated by a single step guanidinium thiocyanate-phenol-chloroform method. Cells (3·10⁶) were lysed using TRI REAGENT (Sigma-Aldrich, Bornem, Belgium) and the samples were processed following the manufacturer’s protocol. RNA (2 μg) was reverse transcribed in a volume of 30 μl containing 1× RT buffer, 10 mM DTT, 1 mM dNTPs, 500 ng oligo(dT)₁₅ primer, 140 U M-MLV reverse transcriptase and 40 U RNaseOUT (Life Technologies, Merelbeke, Belgium) for 60 min at 140 U M-MLV reverse transcriptase and 40 U RNaseOUT (Life Technologies, Merelbeke, Belgium) for 60 min at 37°C. The reaction was halted by an incubation at 95°C for 5 min and by adding 100 μl of H₂O.

The polymerase chain reaction was carried out in an automatic DNA thermal cycler (Hybaid, Doornmond, Belgium). Primer sequences used to amplify the desired cDNA are as follows: hβ-actin forward and reverse primers: 5′-GGGAGCTCTTGAACATCCC AAATAC-3′ and 5′-TGCTATCATATTTGGCAGGTTC-3′; hGAPDH forward and reverse primers: 5′-TGTTATGCTGGGAAGAAGCTGA-3′ and 5′-TGATCATCATATTGCGAGGTTT-3′; hiNOS forward and reverse primers: 5′-CCATGGAACATCCC AAATAC-3′ and 5′-CACCTTGGAACATCCC AAATAC-3′ and 5′-TCTGATATCTGATAGAAGG-3′; hCOX-2 forward and reverse primers: 5′-TTCAATAGGATTTGGGA AAA-3′ and 5′-AGATCATCTCTGCTGATCTTCTT-3′; h IL-1β forward and reverse primers: 5′-TCTCCAGACC ACCACTACAGC A-3′ and 5′-GGGAACTTGGCGACA CTCA A-3′.

The reaction mixture contained 2 μl cDNA (equivalent to 30 ng of RNA), 0.5 μl sense and antisense primer, 250 μM dNTPs, 1× PCR buffer, 1.5 mM MgCl₂ and 1× Taq DNA polymerase (Life Technologies, Merelbeke, Belgium) in a final volume of 30 μl. The cycle program was set to denature at 95°C for 45 s, to anneal at 55°C for 45 s, and to extend at 72°C for 90 s. The number of cycles was selected in the exponential phase of the amplification curve (20 cycles for β-actin, 26 for iNOS, COX-2 and IL-1β).

Electrophoresis of the PCR products was performed on 1.5% ethidium bromide stained agarose gel. The estimated size of the amplified fragments matched the calculated size.

WESTERN-BLOT ANALYSIS

After incubation with the different drugs, chondrocytes (5·10⁶ cells) were washed three times with PBS and lysed by boiling for 5 min in 150 μl of lysis buffer (10 mM Tris-HCl, pH 7.4, 1% SDS, 0.1 mg/ml PMSF). Equal amounts of protein (20 μg) estimated by bicinchoninic acid reagent (Pierce, Rockford, U.S.A.) were loaded onto 10% SDS-polyacrylamide gel and were transferred to nitrocellulose membrane (Bio-Rad, Nazareth, Belgium). Sheep COX-2 purified enzyme (Cayman Chemical, Ann Arbor, U.S.A.) was also loaded on the gel as positive control. The membrane was saturated with 5% fat-free dry milk in phosphate-buffered saline (80 mM Na₂HPO₄, 25 mM NaH₂PO₄, 100 mM NaCl, pH 7.5) with 1% (v/v) Tween 20 (PBST) overnight at 4°C. The membrane was incubated with purified polyclonal rabbit IgG antibody against a peptide corresponding to amino acids 567–599 of human COX-2 (Cayman Chemical, Ann Arbor, U.S.A.) at 1:1000 dilution in PBST for 1 h at room temperature. The blot was washed in PBST three times (10 min each). The blot was further incubated for 1 h at room temperature with goat anti-rabbit IgG antibody coupled to horseradish peroxidase (Biosource, Nivelles, Belgium) at 1:20,000 dilution in PBST. After three washing (10 min) in PBST, the blot was incubated with chemiluminescence substrates (ECL Plus, Amersham, Rosendael, The Netherlands) according to the manufacturer’s instructions. The blot was exposed to a radiographic film for 15 s.

CALCULATION AND STATISTICAL ANALYSIS

The results were expressed as amounts of NO₂ plus NO₃ and PGE₃ into supernatants per μg of DNA. The mean±standard deviation (s.d.) of each variable was calculated. A multivariate repeated measures ANOVA model with calculation of contrast was performed on all of the experiments (MANOVA; SAS system; general linear models).
Fig. 1. Effects of L-NMMA, NAC and ACECLO on the productions of NO₂/NO₃ and PGE₂ by human chondrocytes. Chondrocytes were cultured for 48 h in the absence (A), (B) or the presence (C), (D) of LPS (10 µg/ml) and with or without with L-NMMA (0.5 mM), NAC (1 mM), L-NMMA (0.5 mM)+NAC (1 mM) or ACECLO (6 · 10⁻⁶ M). NO₂/NO₃ [white columns, (A) and (C)] and PGE₂ [hatched columns, (B) and (D)] concentrations were measured in conditioned culture supernatants and are normalized to the production obtained in the absence of the drug. Each condition was tested in triplicate. Results are the mean±S.D. of seven experiments performed with cartilage specimens of different donors. Comparison of mean values was performed by ANOVA analysis. Drug treated groups are significantly different from the untreated groups: ***P<0.001.
Results

EFFECTS OF L-NMMA, NAC AND ACECLO ON NO AND PGE2 PRODUCTIONS BY HUMAN CHONDROCYTES

Chondrocytes were isolated from seven different donors. As previously described, NO and PGE2 synthesis were significantly stimulated in the presence of LPS. In both basal and LPS-stimulated conditions the NO and PGE2 productions varied markedly according to the donor considered. After 48 h of incubation, the mean basal production of NO2/NO3 and PGE2, calculated on seven different chondrocyte cultures, were 2.39 nmoles/μg DNA/48 h and 10.41 pg/μg DNA/48 h, respectively. LPS increased NO and PGE2 productions by mean 2.8 and 7.2 times, respectively. Whichever donor was considered, IL-1β was undetectable.

As expected, L-NMMA reduced by 81 and 87% the basal and LPS-stimulated NO production, respectively [Fig. 1(A), (C)] (P<0.001). As previously described, the addition of L-NMMA resulted in a significant enhancement of LPS-stimulated PGE2 production [Fig. 1(D)] (P<0.001), suggesting a negative effect of NO on PGE2 synthesis.

Inversely, NAC increased both the basal and LPS-stimulated NO production [Fig. 1(A), (C)] without significant effect on PGE2 synthesis [Fig. 1(B), (D)]. When NAC and L-NMMA were added simultaneously, NO and PGE2 productions varied in the same order of magnitude that when L-NMMA was added alone (Fig. 1). ACECLO, a non-steroidal antiinflammatory drug, strongly inhibited
PGE$_2$ production by both untreated and LPS-treated chondrocytes but did not significantly modify NO production (Fig. 1).

**EFFECTS OF L-NMMA, NAC AND ACECLO ON IL-1$\beta$, INOS AND COX-2 mRNA EXPRESSION**

Real-time RT PCR was performed to quantify the drug effects on gene expression (Fig. 2). Under basal condition, both iNOS and COX-2 mRNA were expressed at low levels while IL-1$\beta$ mRNA was undetectable. As expected, LPS (10 $\mu$g/ml) induced a marked increase of IL-1$\beta$, iNOS and COX-2 mRNA levels. After 48 h of incubation, LPS-stimulated chondrocytes expressed until 19 863±642, 112 600±2326 and 11 283±1138 cDNA copies number per 3 ng of total RNA of IL-1$\beta$, iNOS and COX-2 respectively.

L-NMMA (0.5 mM) did not significantly modify LPS-stimulated gene expressions (Fig. 2). NAC (1 mM) enhanced IL-1$\beta$ ($P<0.001$) and iNOS mRNA levels ($P<0.01$) [Fig. 2(A),(B)] without affecting the COX-2 gene expression [Fig. 2(C)]. Finally, ACECLO (6 $\cdot$ 10$^{-6}$ M) had a significant inhibitory effect on IL-1$\beta$ gene expression ($P<0.01$) [Fig. 2(A)] but did not affect COX-2 and iNOS mRNA levels. These results were confirmed by classical RT PCR followed by agarose electrophoresis (Fig. 3).

**EFFECTS OF L-NMMA AND NAC ON COX-2 PROTEIN**

To determine the mechanism of action of L-NMMA on PGE$_2$ production, we studied the COX-2 protein expression by Western blot analysis (Fig. 4). In the basal condition, COX-2 protein level was very low and was not modified by L-NMMA and NAC treatments. An LPS-inducible expression was observed. L-NMMA and NAC added separately or in combination had no significant effect on COX-2 protein level.

**Discussion**

We have demonstrated that the inhibition of NO$_2$ production by L-NMMA was accompanied by an increase of PGE$_2$ production suggesting a negative feedback of NO on PGE$_2$ synthesis. Our results are in accordance with previous studies showing by the inhibition of NO synthase or the incubation of cells with NO donors, that NO down-regulated PGE$_2$ production in cell line murine macrophages, in rat peritoneal macrophages, in human and bovine chondrocytes and in human osteoarthritic cartilage explants. In contrast, others have reported that NO enhanced COX-2 activity and PGE$_2$ production in different cell types. Interestingly, Stadler demonstrated a dual role of L-NMMA on PGE$_2$ production in rabbit chondrocytes culture. When low or high levels of NO were produced by cells activated by, respectively, IL-1$\beta$ alone or by a combination of IL-1$\beta$ plus LPS, an inhibition or an enhancement of the PGE$_2$ production was obtained. Stadler's study provides a possible explanation for the discrepancy reported in the literature about the relation between NO and PGE$_2$ synthesis, suggesting a biphasic effect of NO according to its production level.

Furthermore, we have shown that L-NMMA did not modify both the COX-2 mRNA level and protein synthesis. These observations suggested that the overproduction of PGE$_2$ resulting from the L-NMMA treatment was directly related to the control of the enzymatic activity of cyclooxygenase. This control could be performed by nitrogen-derived oxidant molecules that could regulate by tyrosine nitration and oxidation the cyclooxygenase activity. This mechanism was reported for other enzymes. For example, manganese superoxide dismutase was inactivated by ONOO$^-$ through a mechanism involving not only nitration of critical tyrosine residues but also tyrosine oxidation and subsequent formation of dityrosine. Roy et al. demonstrated that NO reacts rapidly with ribonucleotide.
reductase, silencing its electronic paramagnetic resonance tyrosyl radical signal and inhibiting its enzymatic activity. COX-2 also produces a tyrosyl radical critical to its catalytic activity. In previous studies, we and others have shown that phorbol myristate acetate, LPS, anoxia–reoxygenation cycles and several cytokines (IL-1β, TNFα, IFNγ), induced the production of ROS by chondrocytes including O₂⁻, H₂O₂, and ·OH. Furthermore, using RT-PCR, we have observed that in our experimental conditions chondrocytes expressed the p22 sub-unit which is a regulatory element of the NADPH oxidase (data not shown). These data suggest that ROS, produced intracellularly by LPS-stimulated chondrocytes, could react with NO to form ONOO⁻, which is presented as a potential enzyme inhibitor. Finally, we can also suggest that NO or a nitric-oxide derived oxidant reacts with the phospholipase A2 and inhibits the release of arachidonic acid, the substrate of cyclo-oxygenases. Nevertheless, Kosonen and colleagues did not confirm this latter hypothesis, showing that the addition of exogenous arachidonic acid to endothelial cell cultures did not alter the inhibitory effect of NO donors on prostacyclin production, suggesting that phospholipases are not the target of NO action. On the other hand, Martinez et al. reported an increase of PLA2 activity in the membrane fraction of murine peritoneal macrophages submitted to SIN-1, a NO/O₂⁻ donor. This stimulating effect was markedly inhibited in the presence of SOD, while L-NMMA had no effect.

In our study, we have also demonstrated that NAC increased NO synthesis and iNOS gene expression but was without effect on PGE₂ production and COX-2 mRNA levels. These findings corroborate the results of Jiang and Brecher demonstrating that N-acetyl-cysteine facilitated IL-1β-induced nitric oxide synthase (iNOS) expression in rat vascular smooth muscles cells through a reduction/oxidation-related mechanism involving potentiation of cytokine activation of the p44/42 MAPK signaling pathway. Another explanation was given by Huang and colleagues, who reported that ascorbic acid enhanced endothelial nitric-oxide synthase activity by increasing intracellular tetrahydrobiopterin BH₄ content. Concerning the regulation of COX activity by antioxidants, our study was in disagreement with other studies which reported that known antioxidants, such as butylated hydroxyanisol or diphenylamine, were also potent inhibitors of arachidonic acid metabolism.

ACECLO is a NSAID of the phenylacetic acid class, commonly used in the treatment of arthritis. Interestingly, the mechanism of action of ACECLO is multifactorial. Indeed, this NSAID acts not only by inhibiting preferentially the COX-2 activity but also by decreasing proinflammatory cytokines synthesis and by scavenging reactive oxygen species. At 1 · 10⁻⁵ M, ACECLO decreased luminol-enhanced chemiluminescence generated by PMA-activated neutrophils and accelerated the decomposition of ONOO⁻, suggesting a potential capacity of this drug to scavenge ONOO⁻. In contrast, and by comparison with other classical NSAID, ACECLO did not reduced lipid peroxydation induced by Fe²⁺/ascorbate system and did not significantly reduced HOCl induced chemiluminescence. Furthermore, we have recently demonstrated that, at therapeutic concentration (ranged among 6 to 10 10⁻⁶ M) ACECLO did not react with ·OH or O₂⁻ (submitted data). Taken together, these findings demonstrated that ACECLO preferentially inhibited ONOO⁻ but not O₂⁻, ·OH or HOCl. Considering these particular antioxidant properties, we have tested this drug on the expression of COX-2, IL-1β and iNOS genes, and on the synthesis of PGE₂ and NO. Interestingly, ACECLO fully blocked PGE₂ synthesis without affecting COX-2 gene expression. Furthermore, we have previously reported that ACECLO strongly inhibited COX-2 activity (IC₅₀=8 · 10⁻⁷ M) in the whole blood test but showed an anecdotal effect on purified cyclooxygenases. These observations suggested that ACECLO probably exerts its inhibitory action on PGE₂ synthesis at the post-transcriptional level but not by a direct interaction with the catalytic site of COX.

Taken together, these data clearly demonstrate that NO down-regulates PGE₂ synthesis at the post-transcriptional level.
level and that antioxidants, such as N-acetyl-cysteine, increase NO synthase activity and expression. These findings suggest that antioxidant therapy could have contradictory effects as a function of the molecule administered, especially the oxygen radical targeted. To block NO could result in an increase of PGE₂ production, which was clearly identified as an inflammatory and catabolic factor. Increasing the antioxidant status of the cell could induce an overproduction of NO.

References


