Differential regulation of interleukin-1 -induced cyclooxygenase-2 gene expression by nimesulide in human synovial fibroblasts

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ABSTRACT

Osteoarthritic (OA) human synovial fibroblasts (HSF) in culture were treated with the preferential COX-2 inhibitors nimesulide or NS-398, the non-specific COX-1/COX-2 inhibitor naproxen, or dexamethasone, in the presence or absence of IL-1 or LPS. Nimesulide or NS-398 inhibited IL-1 -induced PGE_2 production at all concentrations tested, and in addition they suppressed IL-1 -induced COX-2 mRNA expression and protein synthesis. These suppressive effects were most evident at therapeutic levels of the drugs. Mechanistic studies revealed that the drug-induced inhibition of COX-2 expression and synthesis was not promoter-based, but may be associated with the blockade of IL-1 -dependent calcium flux and increased cellular calcium levels.

Introduction

Nimesulide (NIM, 4-nitro-2-phenoxymethanesulfonanilide) preferentially inhibits cyclooxygenase-2 (COX-2) (1-3) and has marked anti-inflammatory effects (4, 5). It is believed that the main molecular basis for the therapeutic actions of NIM lies in its ability to inhibit the synthesis of pro-inflammatory prostaglandins by inhibiting COX-2 activity (3).

Using human osteoarthritic (OA) synovial fibroblasts in culture, we showed (6) that NIM and naproxen (NAP) reduced the synthesis of urokinase (uPA) and interleukin-6 (IL-6), while increasing the formation of plasminogen activator inhibitor-1 (PAI-1). Furthermore, NIM decreased matrix metalloprotease (MMP) synthesis by cartilage *in vitro* (7). These results suggest that the drug can inhibit cartilage catabolism through mechanisms not associated with the inhibition of COX-2 activity and eicosanoid production. Recently, we demonstrated that NIM stimulates hyperphosphorylation of glucocorticoid receptors (GRs) in human synovial fibroblasts, resulting in activation of the GRs in terms of glucocorticoid response element (GRE) binding and transactivation of GR-sensitive promoters (8).

The aim of the present study was to determine whether preferential COX-2 inhibitors could alter COX-2 expression in connective tissue cells. We report that NIM down-regulated IL-1 - and LPS (endotoxin)-induced COX-2 mRNA expression and protein synthesis in human OA-affected synovial fibroblasts at therapeutically relevant concentrations, in addition to its well-described suppression of PGE_2 formation.

Materials and methods

Synovial lining cells (human synovial fibroblasts, HSF) were isolated from OA and RA patients undergoing arthroplasty. The synovial fibroblasts were released by sequential enzymatic digestion (9, 10) and then cultured until confluence in DMEM supplemented with 10% FCS. The cells were incubated in fresh serum-free medium for 24 hours before the experiments, and only second- or third-passaged HSF were used.

Cellular protein extracts in RIPA buffer (50-100 g) from control and treated HSF were subjected to SDS-PAGE through 10% gels under reducing conditions, and transferred onto nitrocellulose membranes (8). The antibody used was the polyclonal anti-human COX-2 (Cayman Chemical Co., 1:5000 dilution).

Total cellular RNA was isolated and 10-15 g of total RNA were resolved on 0.9% agarose-formaldehyde gel and transferred to Hybond-N™ nylon membranes (Amersham Pharmacia

Biotech Canada Ltd., Oakville, Ontario, Canada). Hybridizations were carried out after pre-hybridization, followed by high-stringency washing (8). The following probes, labelled with digoxigenin (DIG)-dUTP by random priming, were used for hybridization: (a) a human COX-2 cDNA (1.8 kb), and (b) a 780 bp PstI/XbaI fragment from GAPDH cDNA (1.2 kb). The latter probe served as a control of RNA loading as GAPDH is constitutively expressed. All blots were analyzed semi-quantitatively by densitometry.

Confluent HSF from control and treated cells were used to prepare nuclear extracts as described previously (8). Double-stranded oligonucleotides containing consensus sequences for NF- B and c/EBP were end-labeled with [-^{32}P]ATP using T4 polynucleotide kinase (Promega, Madison, WI). Binding reactions were conducted with nuclear extract and ^{32}P-labeled oligonucleotide probe. Binding complexes were resolved by non-denaturing polyacrylamide gel electrophoresis and prepared for autoradiography.

Transient transfection experiments were conducted as previously described (8). Transfections were carried out by the FuGENE6™ method (Boehringer Mannheim, Laval, Quebec) according to the manufacturer's protocol. HSF were re-exposed to a complete culture medium prior to drug treatments. Transfection efficiencies were controlled by co-transfection with pCMV- -gal, a -galactosidase reporter vector under the control of a CMV promoter. The human COX-2 promoter construct used was a 2.04 kb fragment of the promoter [-1840 (EcoRI) to +123 (MspA 1I)] cloned into the HindIII site upstream of the firefly luciferase gene in a pGL3-basic vector (Promega, Madison, WI) after linker ligation (11).

Results

NIM inhibited IL-1 -induced COX-2 expression and protein synthesis in an inverse concentration-dependent fashion: inhibition occurred at a therapeutic concentration of free NIM (0.03 g/mL) and with 0.3 g/mL, but not at higher concentrations (e.g., 3 g/mL). Similar results were obtained with NS-398. The inhibitory effect of NIM was greatest at 16-24 hr. In control studies, the anti-inflammatory steroid dexamethasone (1 mol/L) also inhibited IL-1 /LPS-induced COX-2 expression and synthesis, whereas the non-selective COX inhibitor naproxen did not produce this effect at the high concentration of 90 µg/mL. The inhibition of IL-1 -induced COX-2 mRNA expression at therapeutically relevant concentrations of NIM did not result from a change in the half-life of the COX-2 mRNA. When incubated with quiescent unstimulated cells, NIM provoked COX-2 protein synthesis in a concentration-dependent fashion, and there was no PGE_2 release. Again, similar results were obtained with NS-398.

NIM 0.03, 0.3, or 3 g/mL affected neither the basal nor the IL-1 -induced COX-2 promoter activity in transfected HSF. Interleukin-1 (10 U/mL) strongly induced both c/EBP and NF- B oligonucleotide binding but NIM 0.3 g/mL, a concentration that inhibited COX-2 expression, had no effect on the level of nuclear protein binding to the ^{32}P-oligonucleotides tested.

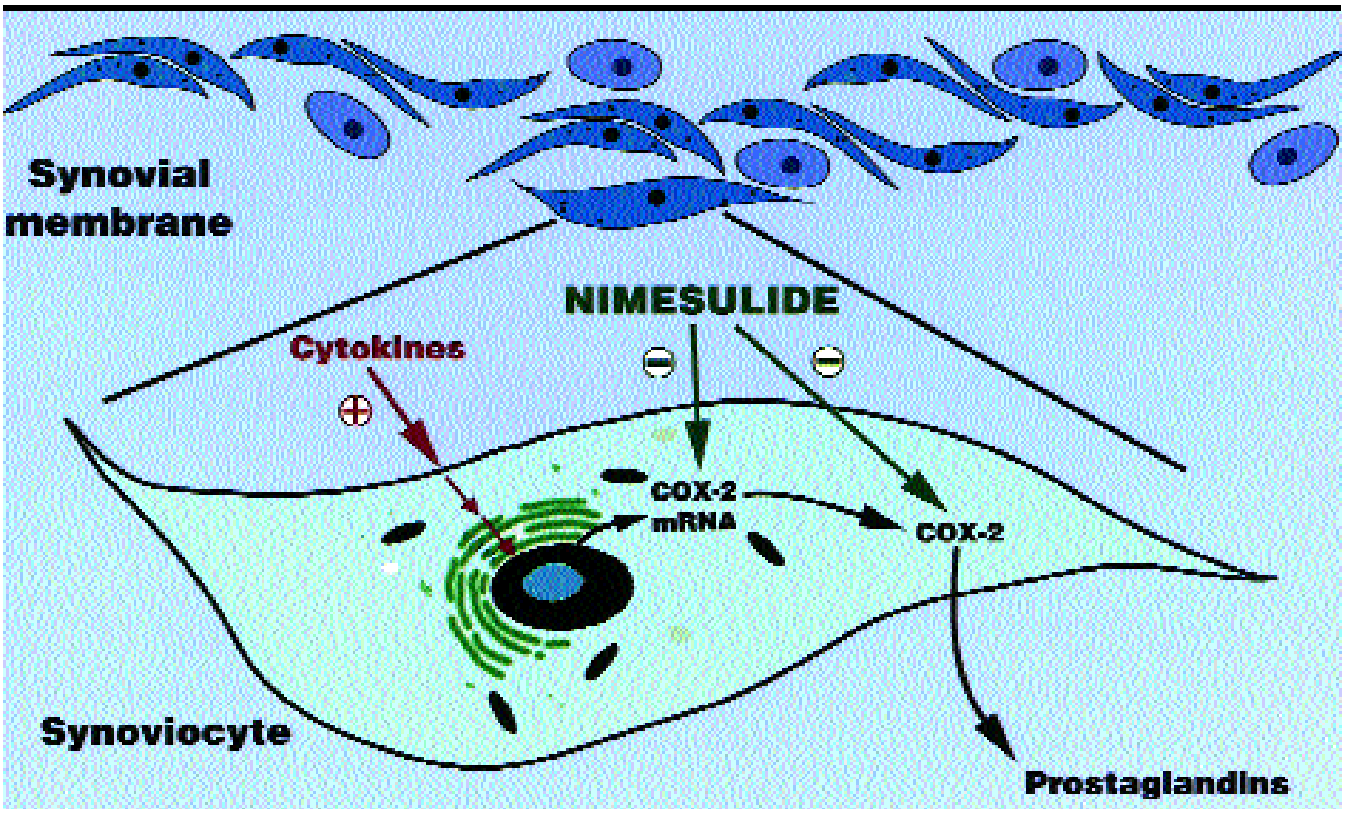

Fig. 1. Schematic representation of the effect of nimesulide on the COX-2 pathway. Nimesulide can inhibit COX-2 expression, synthesis and activity.

Discussion

This study was stimulated by our findings that NSAIDs, in particular preferential COX-2 inhibitors of the methylsulfonamide class (NIM, NS-398), owe their therapeutic efficacy in part to what we have defined as "allo-effects". The latter consist of activities other than the simple inhibition of cyclooxygenase activity and formation of prostaglandins, and can be promoter-based, modulation of signaling cascades, suppression of free-radical generation or modulation of second messengers (e.g. calcium flux) (8, 12-14). Examples of "allo-effects" would be changes in the expression level of target genes (e.g. COX-2) or the recently reported activation of ligand-inducible nuclear receptor systems (e.g. glucocorticoid receptors) with the resultant induction of target gene promoters. In addition, we observed the stimulation of at least one MAPK cascade (ERK1/2) in human connective tissue cells and macrophages (8). The effects of NIM on basal and IL-1 -induced COX-2 suggest differential effects on cell metabolism depending on the concentration of NIM used. At therapeutically relevant concentrations, NIM not only inhibits COX-2 activity but also reduced COX-2 expression and synthesis (Fig. 1). Thus our work has demonstrated an additional "allo-effect" of this distinct methylsulfonamide class of COX-2 preferential inhibitors, and raises the possibility of enlarging their therapeutic usefulness. Additional studies will be required to explain the molecular basis for the inhibition of COX-2 gene expression and synthesis in pathological synovial lining cells.

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