Clinical Implications of Cyclooxygenase Enzymes: COX-1/COX-2 Role of the New NSAIDs

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Introduction

Professor John Vane\(^1\) won the Nobel Prize for elucidating the mechanism of action of aspirin. He reported that this was accomplished by blocking the enzyme called cyclooxygenase (COX-1) that was responsible for the conversion of arachidonic acid to prostaglandins. Prostaglandins liberated from arachidonic acid by cyclooxygenase are short-lived substances that act as local hormones (autocoids) important in normal physiology and pathologic conditions. Prostaglandins E\(_2\) (PGE\(_2\)) is the principal eicosanoid in inflammatory conditions.\(^2\) Since that discovery, more than 30 nonsteroidal anti-inflammatory drugs (NSAIDs) have come on the market, and they represent the most widely prescribed class of drugs in the world. Along with their benefits, several side effect involving the gastrointestinal, renal, and hemopoietic systems have emerged that have limited their usefulness. Recently, a second cyclooxygenase enzyme system that appears to be only upregulated in inflammation has been described.\(^3\) Inhibition of this second enzyme (COX-2) appears to confer anti-inflammatory effects without inhibiting the prostaglandins that are important for normal physiologic function of the gastrointestinal, renal, and hemopoietic systems.

These two enzyme systems differ in terms of genetics, biochemistry, and function. Celecoxib, a selective inhibitor of COX-2, was recently approved by the Food and Drug Administration. Other inhibitors of the COX-2 system are now in clinical testing. Proven to be as efficacious as COX-1 inhibitors but with a better safety record, they will represent an important addition to our therapeutic armamentarium against pain.

Discovery of COX-2

In studying a rabbit kidney model of inflammation, Reingold et al\(^4\) noted that when one ureter was tied off causing inflammation, that kidney produced high levels of prostaglandins above the normal baseline production of the contralateral normal kidney. They also observed that when dermal fibroblasts were stimulated with lipopolysaccharide (LPS) or endotoxin in tissue culture, the same increase in prostaglandin production above baseline was observed. This excess prostaglandin could be inhibited by glucocorticoid and indomethacin.\(^5\) The postulation that a second enzyme system existed was based on the fact that excess prostaglandin could be produced in models of inflammation, that it was inhibited by inhibitors of protein synthesis as well as glucocorticoid, and that the baseline prostaglandin production was not affected by glucocorticoid. This second enzyme system — COX-2 — was considered to be inducible and upregulated by inflammatory stimuli such as cytokines. The baseline production of prostaglandins not inhibited by glucocorticoids was considered to be the constitutive or "housekeeping" prostaglandins important in the protection of the gastrointestinal tract, regulators of renal blood flow, and functioning in platelet aggregation. The radiographic crystallographic structure of both enzymes has been identified,\(^6\) and specific monoclonal antibodies to each entity have been developed\(^7\) together with DNA probes to obtain the cDNA necessary to express mRNA and protein in tissue culture.\(^7\) With these tools, researchers produced quantities of both enzymes and began to look for specific inhibitors. In summary, COX-1 and COX-2 enzymes exhibited major differences in regulation and expression. COX-1 present in most tissue-synthesized prostaglandin is important in regulating cell function, whereas COX-2 is generally undetectable in most tissues but increases its expression during acute inflammation or in response to cytokine stimulation-producing prostaglandins found at sites of inflammation. Inhibition of COX-2 by NSAIDs would give an anti-inflammatory effect, whereas inhibition of COX-1 would result in adverse effects such as gastrointestinal toxicity and nephrotoxicity. All of the present NSAIDs inhibit both COX-1 and COX-2 to varying degrees depending on the assay system used. Development of a pure COX-2 inhibitor should provide anti-inflammatory effects without host toxicity.

Genetics and Structure: Evidence for Two Separate Enzyme Systems

Molecular Biology

The genes for COX-1 and COX-2 are located on separate chromosomes, with COX-1 on chromosome 9 and COX-2 on chromosome 1. The COX-2 gene is smaller than COX-1. Exons 1 and 2 of COX-1 (containing the translation site and original peptide) are condensed into a single exon in COX-2. The introns of COX-2 are smaller than COX-1. COX-2 has a TATA box promoter and COX-1 lacks a TATA box. Lastly, the mRNA of COX-2 contains long 3\(^\prime\) untranslated regions containing several different polyadenylation signals and multiple "AUAUA" instability sequences that act to mediate rapid degradation of the transcript. These features differentiate the gene for COX-1 into a gene consistent with rapid transcription and mRNA processing for processing a continuously transcribed stable message. The provides for a constant level of enzyme in most cell types to synthesize prostaglandins responsible for homeostatic functions. In contrast, the features of the COX-2 gene are those of an "immediate-early" gene that is not always present but is highly regulated and upregulated during inflammation or pathological processes.

Protein Structure

Although there are clear differences in DNA mRNA structure and function between COX-1 and COX-2, there is less difference between the protein structure and function of these enzymes. The core sequences of both enzymes, as well as their crystal structures, are 60% identical.\(^8\) Both enzymes have similar kinetics for arachidonic acid. Despite these similarities, evidence indicates that COX-1 and COX-2 function as separate enzyme systems. COX-1 is localized to the endoplasmic reticulum, whereas COX-2 is localized to the endoplasmic reticulum and the nuclear membrane. In addition, COX-1 and COX-2 use different pools of arachidonate that are mobilized in response to different cellular stimuli for prostaglandin synthesis.

Tissue Expression

COX-1 is constitutively present in virtually all tissues under basal conditions. COX-2 is constitutively expressed under basal conditions in many areas of the central nervous system (CNS). Most information on localization is based on animal studies. The highest level in the CNS have been found in the hippocampus associated with granule and pyramidal cell layers.\(^9\) Moderate levels have been found in pyramidal cell, piriform cortex, neocortex, and amygdala. Lower levels have also been found in caudate-putamen, thalamus, hypothalamus, striatum, and preoptic levels.\(^10\) Increased levels have also been found in cortical neurons in response to natural N-methyl D-aspartic acid (NMDA) receptor-mediated neuronal activity.\(^11\) Expression may be involved in modulation of pain. This is important because it suggests that blocking COX-2 centrally may be important in control of pain.

Data relating to the kidney, and there are differences between human and animal data. Animal studies demonstrate that COX-1 is constitutively located in medullary collecting ducts and medullary interstitial cells with less expression in ascending collecting tubules. There is none in the macula densa or cortical thick ascending limbs. COX-2 is constitutively expressed in the cortex and medullary interstitial cells, particularly at the renal papilla. COX-2 is also expressed in the macula densa of the juxtaglomerular (JG) apparatus and in the epithelial cells of the cortical ascending limb. In animal models, salt restriction leads to increased COX-2 levels in the JG apparatus with increased renin and decreased salt excretion. In the medulla, the opposite occurs, with increased COX-1 expression seen with increased salt intake in accord with the physiologic action of medullary prostaglandins to promote salt excretion. Medullary COX-1 produces prostaglandins that are important for renal adaptation to states of high
Localization of COX-1 and COX-2 is somewhat different in humans (Table). This may result in different actions on renal function depending on which species is examined. In rats, COX-2 in the macula densa increases with salt depletion and medullary COX-1 increases with salt repletion. In humans, COX-2 is seen in podocytes and arterioles. Low salt intake stimulates prostaglandin E synthesis in the JG apparatus with secretion of renin. COX-2 stimulates thromboxane and with resultant vasoconstriction could cause podocyte contraction with a possible decreased single nephron glomerular filtration rate.

<table>
<thead>
<tr>
<th>COX-1/COX-2 Renal Localization</th>
<th>Rats</th>
<th>Humans</th>
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<tbody>
<tr>
<td>COX-1</td>
<td>Concentrated in medulla papilla</td>
<td>Collecting ducts</td>
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<tr>
<td>Over cortex</td>
<td>Interstitial cells</td>
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<tr>
<td></td>
<td>Arterial smooth muscle</td>
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<tr>
<td></td>
<td>None in macula densa</td>
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<tr>
<td>Thick ascending limb</td>
<td>Podocytes</td>
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<tr>
<td></td>
<td>Endothelial cells</td>
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Predicting differences in renal function based on anatomic localization of COX-1/COX-2 is not possible from the data presently available. Interspecies differences may be significant.

COX-2 is upregulated during inflammation and cellular transformation and is decreased by glucocorticoids. When looking at animal models of inflammation, COX-2 was found in multiple cell types in the joint including synovial lining cells, fibroblast-like cells, vascular endothelial cells, infiltrating mononuclear cells, chondrocytes, and adjacent bone marrow. All this expression could be suppressed by treating the animals with glucocorticoids.13 Anderson et al14 demonstrated the time-dependent appearance of COX-2 mRNA but not COX-1 with induction of adjuvant induced arthritis followed by protein synthesis (increased levels of PGE2) and its blockade with a selective COX-2 inhibitor. In human synovial tissue, expression of COX-2 but not COX-1 occurred in patients with rheumatoid arthritis, ankylosing spondylitis, and psoriatic arthritis compared with patients with osteoarthritis.15 In human polymorphonuclear (PMNs) exposed to several cytokines, increased expression of COX-2 and PGE2 was found. There was no increased expression of COX-2 in human PMNs not exposed to these mediators.16 Lastly, monosodium urate crystals (that occur in gout) induce COX-2 and PGE2 in monocytes. This activity could be blocked by colchicine, tyrosine kinase, or herbimycin A.17

NSAID Selectivity

All of the currently available NSAIDs have the ability to inhibit both COX-1 and COX-2 by binding reversibly or irreversibly to the enzyme. The major toxicities of NSAIDs are thought to be due to their ability to block synthesis of the housekeeping prostaglandins (those in the kidney, stomach, and platelets) by inhibition of COX-1. Inhibition of COX-2 does not affect these prostaglandins but stops the synthesis of prostaglandins involved in inflammation. Most of the current NSAIDs exhibit some degree of selectivity in their ability to differentially block COX-1 or COX-2.18 For example, etodolac has been shown to inhibit COX-2 tenfold more than COX-1, and 6-MNA (the active moiety of nabumetone) inhibited COX-2 seven times more efficiently than COX-1. The major problem with all of the experimental studies that attempt to rank order NSAIDs in terms of COX-1/COX-2 selectivity is that they are dependent on the assay used. Assay systems for studying the amount of inhibition of either COX-1 or COX-2 by inhibitors include whole blood systems, whole cell systems, and even microsomal membrane (pure enzyme) systems. Each NSAID may have different ratios for inhibition of COX-1 to COX-2 depending on the system used.21

Newly developed COX-2 inhibitors are many times more potent against COX-2 than COX-1. At recommended therapeutic doses, they block only COX-2. Therefore, comparisons of currently available NSAIDs with regard to COX-1/COX-2 ratios are probably not of clinical value.

COX-2 Beyond Inflammation

Since the discovery that COX-2, although structurally similar to COX-1, is derived from a different gene and represents a new enzyme system, many new roles for its gene product have come to light.

Carcinogenesis — COX-2 may be upregulated in some forms of cancer including colon cancer. Epidemiologic studies have demonstrated a decreased rate of colon cancer in patients taking NSAIDs. Research is ongoing to determine if COX-2 inhibition may be beneficial in prevention of cancer.22 In an aneural model of multiple prognosis, COX-2 is upregulated in the precancerous areas compared to normal tissue.

Apoptosis — Programmed cell death, apoptosis, is a natural event in many cells. Overexpression of COX-2 in some cell lines is associated with the expression of Bcl-2, a protein that acts to make cells resistant to apoptosis. In addition, overexpression of COX-2 is associated with decreased expression of transforming growth factor-beta (TGF-beta). TGF-beta is important for transducing signals that inhibit cell growth. Both of these effects were blocked with NSAIDs. It is hypothesized that if cells were resistant to apoptosis and/or allowed to grow indefinitely, such cells may be responsible for either autoimmune disease or neoplasia.

Conclusions

NSAIDs are the most widely prescribed drugs on the market and are effective for decreasing pain and inflammation. Originally, their mechanism of action was discovered to be the inhibition of cyclooxygenase. A second form of cyclooxygenase has been discovered, the gene cloned, and inhibitors synthesized. Prostaglandins derived from the action of COX-1 are considered to be the constitutive or "housekeeping" prostaglandins present in all tissues. They are important for platelet aggregation, renal blood flow in the impaired kidney, and cytoprotection in the stomach. Prostaglandins derived from COX-2 are inducible and upregulated in areas of inflammation. They do not exist in the basal state. A review of the genetics and biology of COX-1 and COX-2 shows that although they share 60% structural homology, they constitute two completely different systems with different functions in the human body. Inhibition of COX-1 is not necessary to achieve the anti-inflammatory and analgesic effects of NSAIDs, but doing so significantly contributes to the risk of GI ulceration, bleeding, inhibition of renal blood flow (in the previously impaired kidney), and inhibition of platelet aggregation. Inhibition of COX-2 with new selective agents yields equal anti-inflammatory and analgesic effects without the above-mentioned side effects. New and exciting roles in humans for inhibition of COX-2 synthesis other than pain control are now being investigated.

References


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