THE INDUCIBLE ISOFORM OF CYCLO-OXYGENASE (COX-2) : NEW TARGET FOR ANTIINFLAMMATORY THERAPY

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ABSTRACT

Prostaglandins (PGs) have numerous cardiovascular and inflammatory effects. Cyclo-oxygenase (COX) is the first enzyme in the pathway in which arachidonic acid is converted to PGs, prostacyclin (PGI_2) and thromboxane (TX) A_2. Recently, two isoforms of COX have been identified, sequenced and cloned. The constitutive isoform (COX-1), encoded by a 2.8kb mRNA, is present in cells under physiological conditions. The other form, mitogen-inducible isoform (COX-2), encoded by a 4.2kb mRNA, is induced by some cytokines, mitogens and endotoxin presumably in pathological conditions, such as inflammation. This review focuses on what is known about the newly described COX-2.

Keywords: COX-2, prostaglandins,inflammation, NSAIDs

INTRODUCTION

PGs have numerous cardiovascular and inflammatory effects.[1] Cyclo-oxygenase (COX) is the first enzyme in the pathway in which arachidonic acid is converted to PGs, prostacyclin (PGI_2) and thromboxane (TX) A_2.[2,3] Recently, two isoforms of COX have been identified, sequenced and cloned. One is a constitutive enzyme (COX-1) producing regulatory prostanoids under physiological conditions, whereas the other (COX-2) is induced by mitogens and proinflammatory cytokines during pathological states such as inflammation.[4,5,6,7]

The possibility that COX could exist as different isoforms was initially suggested by Flower and Vane,[8] based on their observation that in different COX systems extracted from different regions of the body, there were different sensitivities to inhibition by nonsteroidal anti-inflammatory drugs (NSAIDs). Further investigations into enzymatic kinetics have also suggested the existence of different

"pools" of COX.[9-11] These various studies, however, did not determine whether the "multiple forms" of COX being analyzed were actually separate isoenzymes or if the differences seen could have been due to the environment or subcellular location of the protein.[12]

The discovery of COX-2

In the last few years of molecular biological studies, there has been a clear description of a novel, mitogen-inducible isoform of COX,[13,14,15,16] which unlike its constitutive (COX-1) counterpart is regulated by glucocorticoids.[13,16,17] These two proteins (COX-1 and COX-2) are encoded by separate genes,[18,19,20] but have the same molecular weight (approximately 70 kDa) and share about 60% homology at both the cDNA and amino acid level, the structural and enzymic components being the most highly conserved.[21] Moreover, COX-1 and COX-2, purified from mouse, have similar K_m values for AA (3.0 and 2.5 mM, respectively) and appear to have similar V_{max} values (29.5 and 23.0 nmol AA consumed/min per mg, respectively).[22,23]

The two isoforms of COX carry out the same biochemical function but there is a dramatic difference between the two isoenzymes in their patterns of expression and regulation. The COX-1 protein from sheep vesicular glands[24] was cloned and expressed first,[25] and the cDNA shown to encode a 2.8 kb mRNA. Similar analysis of COX-2 yielded a larger mRNA; 4.2 kb in mouse fibroblasts and human monocytes[5] and 3.9 kb in human umbilical vein endothelial cells (HUVEC).[26] Each of these were translated into protein of molecular weight of 70 kDa.

The cDNA for COX-2 differs from COX-1 primarily at the encoding regions responsible for induction.[7] The COX-2 gene is an immediate-early response gene,[27] one of a set of genes associated with control of the cell cycle. This gene was identified in chicken embryo fibroblasts transformed by Rous sarcoma virus,[14] and was identical with an already discovered gene (TIS-10 early response gene) in Swiss 3T3 fibroblasts treated with phorbol ester.[15]

COX-1 is constitutively expressed in most tissues,[28] although the level of expression varies between cell types. Some cells, including vascular endothelial cells[29] and platelets,[30] express relatively large amounts of COX-1. COX-1 appears to be maintained at constant levels, although small increase of about two to four folds can occur after stimulation with hormones and growth factors.[31] In contrast, COX-2 expression increases by as much as 10-80 folds. COX-2 is undetectable in most tissues, but its expression is dramatically increased during inflammation or in cultured cells after exposure to mitogenic stimuli. For example, growth factors, phorbol esters, and IL-1 induce COX-2 in fibroblasts;[5,15,28,32] LPS (lipopolysaccharide, endotoxin) can stimulate COX-2 expression in monocytes and macrophages;[6,33,34] and tumour necrosis factor, phorbol esters, LPS and IL-1 can induce COX-2 in vascular endothelial cells.[4,35] COX-2 is also increased in synoviocytes during inflammation,[36] and a dramatic transient increase in COX-2 occurs in rat ovarian follicles immediately preceding ovulation.[37,38] This latter event can be viewed as a local inflammatory response.

The induction of COX-2

Table 1.1 lists some of the known inducers of COX-2 in a variety of cell types. Other possible known inducers of COX-2 include transforming growth

factor-β (TGF-β), forskolin, colony stimulating factor-1 (CSF-1),[39,40] and hormones such as progesterone, or luteinizing hormone (LH).[12] Although only a limited number of tissues and cell types have been examined, it is possible that COX-2 can be expressed in almost any cells or tissues with the appropiate stimuli. Another important aspect of the regulated induction of COX-2 is that it can be completely inhibited by anti-inflammatory glucocorticoids such as dexamethasone.[5,6,16,17] Thus, COX-2 appears to belong to a family of similarly regulated, glucocorticoid-sensitive, inflammatory-response genes.[41] The ability of dexamethasone to inhibit the induction of COX-2 is a possible explanation of its potent anti-inflammatory activity. While the induction of *de novo* COX-2 synthesis can also be inhibited by protein synthesis inhibitors (cycloheximide), inhibitors of RNA synthesis (actinomycin D) can cause further decreases in PG production,[42] indicating a gene expression regulated at the level of mRMA.[14]

That COX-1 is expressed constitutively in most tissues suggests that it is a "housekeeping enzyme" whose purpose is to produce prostaglandins that regulate normal cellular processes.[21] It is likely that COX-1 is the enzyme which, under physiological conditions, produces the PGs that modulate gastrointestinal and kidney functions and vascular homeostasis. In contrast, COX-2 produces PGs that are involved in inflammation and/or mitogenesis.[27,41,51] Physiological roles for the two COX isozymes will only be determined definitively when specific inhibitors for each isozyme have been developed or when animal models have been constructed that express only one of the two isozymes are available. At present, the idea of the housekeeping COX-1 and the inflammatory COX-2 provides a strong working model for investigating the biology of these enzymes (see Table 1.2 for summary).

Inhibition of prostanoid biosynthesis

The main therapeutic value of inhibition of prostanoid biosynthesis is in the treatment of inflammation. Elevated levels of prostanoids have been observed in almost all models and all acute and chronic inflammatory conditions studied in human. PGs release is associated with the local tissue and blood vessels, while TXs and other eicosanoid mediators such as leukotrienes are released by accumulating leucocytes.[52] PGE_2 and PGI_2 at physiological levels do not appear directly to cause oedema formation and pain, but strongly synergise with the effects of bradykinin and histamine in exudate formation, and pain production by an hyperalgesic mechanism. PGE_2 is also a potent pyrogenic factor.[52]

However, anti-inflammatory properties, especially seen with PGE_2,[53,54] are likely to be involved in resolution and healing. For instance, $PGF_{2\alpha}$ may be involved in the contraction of granulation tissue[55] and PGE_2 inhibits lymphotoxin production in T-cell suggesting a role in immunosuppression.[54,56]

Anti-inflammatory steroids

Glucocorticosteroids, either as exogenous or synthetic drugs or the endogenous forms, are potent anti-inflammatory compounds, but exhibit severe side effects after prolonged use.[57] Their ability to reduce inflammation is at many levels, including suppression of PLA_2 activity and secretion of proinflammatory cytokines and via the production of lipocortins.[58] Another level

Table 1.1 Induction of COX activity in a variety of cell types. Induction was, in certain models, deduced from the observation of increased PG synthesis. In the examples marked,* the induction of COX-2 mRNA or protein was specifically demonstrated. There is a wide range of inducing stimulus including endotoxin, cytokines, growth factors, mitogens and second messenger molecules.

Inducers	Cell types	Ref.
LPS	Endothelial cells Monocyte/Macrophages	26,35 33,34
TNF	Endothelial cells Macrophages	35 16
IL-1	Endothelial cells Macrophages Fibroblast	35 5 5
IL-2	Endothelial cells	43
PDGF	Endothelial cells Fibroblast	44 45
EGF	Endothelial cells Smooth muscle cells Amnion cells Osteoblast	46 47 48 49
PMA or TPA	Endothelial cells Smooth muscle cells Fibroblast	26,35 26 26,32
Serum	Fibroblast	32
cAMP	Osteoblast	50

by which steroids act is to inhibit the induction of the COX-2 isoform.[13,16]

Non-steroidal anti-inflammatory drugs (NSAIDs)

In 1971, Vane showed that aspirin, indomethacin and salicylate exerted their effects by the inhibition of COX.[59] Since then, three different mechanisms of inhibition have been described for the inhibitory action of these drugs. Aspirin noncompetitively and irreversibly inhibits COX by acetylation of the hydroxyl group of serine residues and specifically Ser_{530} to disable the bis-oxygenase activity.[60] Indomethacin noncompetitively inhibits COX without covalent modification of the enzyme while ibuprofen-like drugs inhibit COX by competing in a reversible manner for the AA binding site.[61]

Table 1.2 A comparison of some properties of COX-1 and COX-2

Parameter	COX-1	COX-2
Homology :		
cDNA*	599 amino acids	604 amino acids (60% identical)
mRNA	2.8 kb	3.9-4.2 kb
Protein (MW)	70 kDa	70 kDa
K_m (for AA substrate)	3.0 Mm	2.5 mM
V_{max} (for AA substrate)	29.5 nmol/min/mg	23.0 nmol/min/mg
Regulation	Constitutive	Inducible
Range of expression	Can increase 2-4 folds	Can increase 10-80 folds
Tissue expression	Most tissues	Most tissues but requires suitable stimulation
Effect of glucocorticoids	Little or none	Inhibit expression
Proposed role of enzyme	"Housekeeping gene" to produce PGs in physiological conditions	Inflammatory response gene, immediate early gene, responded to mitogenesis or pathophysiological conditions

* Protein regions believed to be important for enzyme function that are conserved include: EGF homology domain, haem ligand sites, aspirin acetylation "active" site, glycosylation sites, maximum enzymic activity (V_{max}) and affinity for AA (K_m).

Irrespective of the mode of inhibition, the major side effects observed with all NSAIDs used are gastrointestinal ulceration and renal damage.[2,62,63] Both forms of toxicity are associated with a decrease in PG production in the stomach and in the kidney. Experimentally, these side effects of NSAIDs can be reduced by the simultaneous administration of exogenous PGs (PGE_1).[64] Indeed, some NSAIDs are now marketed in combination with a prostaglandin analogue. Following the demonstration of the two COX isoforms, these toxic effects have been re-interpreted as being due to inhibition of the constitutive COX-1 in these tissues. It is implied that PG production in the stomach and the kidney is essential for the normal functioning of these organs and, thus, inhibition of this normal production catalysed by COX-1 leads to the observed pathology.

Future prospect

The differential inhibition between these two enzymes by NSAIDs shows their pharmacological difference and which it is suggested that the inhibition of COX-1 activity ("housekeeping" enzyme responsible for the formation of COX-metabolites under physiological conditions) by NSAIDs accounts for the well-documented side effects (e.g. gastric damage) of these drugs. In contrast, inhibition of COX-2 activity accounts for the potent anti-inflammatory effects of NSAIDs. The development of relatively selective inhibitors of COX-2 activity will lead to better anti-inflammatory drugs, with fewer side effects. As COX-2 is present in many inflammatory conditions and is induced by various inflammatory stimuli, an elucidation of the signal transduction mechanisms involved in the synthesis of COX-2 will gain a better understanding of pathophysiological conditions, such as inflammation.

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