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Cyclooxygenase-3: A Review

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ABSTRACT

A continued need to develop safe and effective analgesics and anti-inflammatory drugs fuels the ongoing investigations of cyclooxygenase (COX). Since the early 1990s, it has been appreciated that there are two cyclooxygenase enzymes, cyclooxygenase-1 and cyclooxygenase-2, responsible for the production of prostaglandin H2, the first step in prostanoid biosynthesis. Cyclooxygenase-1 was responsible for the physiological production of prostanoids and cyclooxygenase- 2 was responsible for the elevated production of prostanoids that occurred in sites of disease and inflammation COX-3 is an enzyme that is encoded by the PTGS1 (COX1) gene and is the third and most recently discovered cyclooxygenase (COX) isozyme. The COX-3 isozyme is encoded by the same gene as COX-1, with the difference that COX-3 retains an intron that is not retained in COX-1. In dogs the resulting protein resembles the other two COX enzymes, but in mice and humans it does not, owing to a frame-shift mechanism.

Key words: Cyclooxygenase, COX-3, Prostaglandins, Inflammation, NSAIDs

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INTRODUCTION

Humans have been using non steroid anti inflammatory drugs (NSAIDs) in various forms for more than 3,500 years. After 3,500 years, the first real progress in our understanding of the mechanism of action of the NSAIDs came few years ago, when it was revealed that these chemically varied drugs reduce the formation of prostaglandins.[1] The prostaglandin field has been one of the most exciting areas of research in recent times because among the many mediators of inflammation, the prostaglandins (PGs) are of great importance. They are released by almost any type of chemical or mechanical stimulus. The key enzyme in their synthesis is prostaglandin endoperoxide synthase (PGHS) or cyclooxygenase (COX) which possesses two catalytic sites. The first, a cyclooxygenase active site, convert arachidonic acid to the endoperoxide PGG2. The second, a peroxidase active site, then converts the PGG2 to another endoperoxide, PGH2. [2] PG-H2 is a common substrate for prostaglandin synthase and the first committed step in the metabolism of arachidonic acid into a cascade of signalling lipids, such as PG-D2 (CNS), PG-E2 (vascular beds), PG-F2α(smooth muscle), PG-I2 (vascular endothelium) and thromboxane(platelets).[3]

Cyclooxygenase activity has long been studied in preparations from sheep seminal vesicles and a purified enzymatically active COX was isolated in 1976.[4] Two distinct isoforms of the cyclooxygenase enzyme were recognized in the early 1990s: COX-1 and COX-2. The concentration of the COX-1enzyme largely remains stable, but small (2- to 4-fold) increases in expression can occur in response to stimulation with hormones or growth factors.[5, 6] COX-1 performs a 'housekeeping' function to synthesise PGs which regulate normal cell activity. The COX-1 activation leads to the production of prostacyclin which when released by the vascular endothelium is anti-thrombogenic[7] and when released by the gastric mucosa is cytoprotective.[8] It is also COX-1 in platelets that lead to thromboxane A_2 production, causing aggregation of the platelets to prevent inappropriate bleeding.[9]

Normally, little or no COX-2 is found in resting cells but its expression can be increased dramatically after exposure of cells to bacterial lipopolysaccharide (LPS), phorbol esters, cytokines or growth factors. However, 'constitutive' levels of COX-2 have been detected in some organs such as the brain and kidney. COX-2 also had physiological roles, being involved, for instance, in the maintenance of fluid balance by the kidney.[10]

The induction of COX-2 generates PGF2a to contract the uterus at the end of pregnancy to initiate birth.[2] COX-2 was rapidly up-regulated at inflammatory sites and appeared responsible for the formation of proinflammatory prostanoids.

EVOLUTION OF COX -3

Although providing a much-needed leap in our understanding, the COX-1-2 model did not appear to explain everything. Paracetamol belongs to a group of drugs known as antipyretic analgesics which also includes its precursor, phenacetin, aminopyrine and dipyrone. Most of these drugs have fallen out of use because of their toxicity for leukocytes, but dipyronecan still be obtained in some countries. Paracetamol is a weak inhibitor of isolatedCOX-1 and COX-2 and its mechanism of action has not yet been resolved. Similarly to other drugs in this group, it has only weak anti-inflammatory effects. In 1972, Flower and Vane[11] postulated the existence of a COX in dog brain more sensitive to inhibition with paracetamol than the COX in rabbit spleen. Simmons and his colleagues characterised and cloned a COX enzyme in dog brain which, unlike COX-1 and COX-2, was sensitive to inhibition with paracetamol.[12] This was a splice variant of COX-1 termed by the authors, COX-3, which consists of a COX-1 mRNA that retains intron-1. In dogs, intron-1 is 90nucleotides in length and represents an in frame insertion into the portion of the COX-1 open reading frame encoding the N-terminal hydrophobic signal peptide.[2] Since then, COX-3 mRNA is found in many tissues such as canine and human cerebral cortices, human aorta, and rodent heart, endothelium, kidney and neuronal tissues.[13]

The difference at the protein level between cyclooxygenase-3 and cyclooxygenase-1 is the insertion of 30 to 34 aa, depending on the mammalian species, into the hydrophobic signal peptide. In cyclooxygenase-3, this signal peptide is not cleaved, and the protein is glycosylated and displays cyclooxygenase activity.[1]

The initial report of cyclooxygenase-3 showed that, in comparative assays using canine cyclooxygenase-3, murine cyclooxygenase-1, and murine cyclooxygenase-2 expressed by transfected insect cells, cyclooxygenase-3 was selectively inhibited by analgesic/antipyretic drugs such as acetaminophen, phenacetin, antipyrine, and dipyrone. Inhibition of cyclooxygenase-3, it was suggested, could represent a primarycentral mechanism by which these drugs decrease pain and possibly fever.[1]The existence of COX-3 as a brain-specific COX isoform,which is mainly responsible for pain and fever, has already been speculated for years.[2] Chandrasekharan et al.[12] successfully isolated and cloned COX-3 from the dog. The canineCOX-3 is identical to the full-length form of COX-1, with the exception that it retains intron 1. This

new isoform is most abundant in the cerebral cortex and shows higher sensitivity towards acetaminophen than COX-1 and COX-2. Analgesic/antipyretic drugs penetrate the blood–brain barrier well and accumulate in the CNS at high enough concentrations to inhibit COX- 3. Carboxylate-containing NSAIDs, on the other hand, cross the blood–brain barrier poorly. Because COX-3 is so sensitive to some carboxylate NSAIDs, COX-3 in the CNS maybe an essential target of both analgesic/antipyretics and standard NSAIDs. Furthermore, the sensitivity of COX-3 to analgesic/antipyretic drugs and NSAIDs observed suggests that highly selective inhibitors can be made for COX-3.[12]

Despite having a signal peptide and intron-1-encoded sequence retained, COX-3 comigrates with COX-1 in SDS/PAGE gels. It also appears to enter the endoplasmic reticulum where it is glycosylated, and its glycosylation is required for activity. In insect cells, COX-3 shows approximately 20% of the activity of COX-1, which in turn exhibits about 20% of the activity of COX-2. Studies show COX-3 to be sensitive to drugs that are analgesic/antipyretic, but which have low anti-inflammatory activity. Pain and fever have many etiologies that employ complex cellular and biochemical pathways. The finding that COX-3 is sensitive to analgesic/antipyretic drugs suggests that the COX-1 gene plays an integral role in pain and/or fever. COX-3 also appears from Northern blot studies to be expressed in specific regions of the human brain, in particular cerebral cortex.

Further, COX-2 functions in resolution of acute inflammatory responses and that “COX-3” is turned on later in inflammation and may be involved in the biosynthesis of endogenous anti-inflammatory mediators. It is speculated that such an enzyme may induce cyclopenetanone prostaglandins and resolvins, which can be triggered by aspirin treatment.[14,15]

It should be noted that a number of scientists prefer calling this newly described protein COX-1b or COX-1 variant (COX-1v), rather than COX-3, because the mRNA is encoded by the COX-1 gene, and other than the retained intron, the mRNA is indistinguishable from COX-1. Both COX-1 and COX-3, but not COX-2 mRNA are expressed in the dorsal root ganglion in mice exhibiting experimental inflammatory pain, supporting that COX-1 and its splice-variant, like COX-2, may be inducible in certain types of pain and that at least in mice.[16] A splice variant of COX-1 was isolated in 2002 and has been called cyclooxygenase-3, (COX-3), COX-1b or COX-1v. The expression of COX-3 mRNA was detected in human cerebral cortex and this protein is pharmacologically different to COX-1 and COX-2 even it is derived from the COX-1 gene.[13] COX-3 in temperature regulation.[17]Cyclooxygenase-3 is termed a splice variant of COX-1. It was isolated by Chandrasekharan et al. in 2002 from the heart and the cerebral cortices of the

dog.7 The expression of COX-3 mRNA in humans was found to be expressed as an about 5.2 kb transcript and it was found to be most abundant in cerebral cortex and heart.[1]

The inducible COX-3 is less potent and produces less PGE2 than either COX-1 or COX-2 and NSAIDs exhibit different inhibition potencies for COX-3 depending on their polarity and capacity to penetrate the blood brain barrier. Antipyretic analgesic drugs (e.g. paracetamol), which are weak inhibitors of COX-1 and COX-2, and penetrate easily into the central nervous system, could explain their pharmacological action of inhibiting COX-3. Research revealed that COX-3 may have a role in remission periods in chronic inflammatory disease and may be involved in the development of ovarian, cervical, colonic cancer and leukaemia.[13, 17, 18]

CONCLUSION

The present treatise highlights the many physiological systems in which the COX enzymes play functional roles. The cyclooxygenase (COX) super family of prostaglandin synthase genes encode a constitutively expressed COX-1, an inducible, highly regulated COX-2, and a COX-3 isoform whose RNA is derived through the retention of a highly structured, G + C-rich intron 1 of the COX-1 gene. Cyclooxygenase (COX)-3, a novel COX splice variant, was suggested as the key to unlocking the mystery of the mechanism of action of acetaminophen. Although COX-3 might have COX activity in canines, and this activity might be inhibited by acetaminophen, its low expression level and the kinetics indicate unlikely clinical relevance. In rodents and humans, COX-3 encodes proteins with completely different amino acid sequences than COX-1 or COX-2 and without COX activity; therefore, it is improbable that COX-3 in these species plays a role in prostaglandin-mediated fever and pain.

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