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Development of Cyclooxygenases in the Treatment of Pain, Fever and Inflammation.

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Review Article

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ABSTRACT

Cyclooxygenases (COXs) are enzymes that take part in a complex biosynthetic cascade that results in the conversion of polyunsaturated fatty acids (PUFAs) to prostaglandins (PGs) and thromboxanes (TXs). Their main role is to catalyze the transformation of AA into the intermediate PG-H2, which is the procursor of a variety of prostanoids (PTS) with diverse and potent biological actions. COXs have two main isoforms that are called COX-1 and COX-2 (as well as a COX-3). COX-1 is responsible for the synthesis of PG and TX in many types of cells, including the gastro-intestinal tract (GIT) and blood platelets. COX-2 plays a major role in PG biosynthesis in inflammatory cells and in the CNS. PG synthesis in these sites is a key factor in the development of inflammation and hyperalgesia. COX-2 inhibitors have analgesic and anti-inflammatory activity by blocking the transformation of AA into PG-H2 selectively. The impetus for development of selective COX-2 inhibitors was the adverse GIT side-effects of NSAIDs. Soon after the discovery of the mechanism of action of NSAIDs, strong indications emerged for alternative forms of COX. COX enzyme proved to be difficult to purify. The COX-2 enzyme was cloned.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are drugs with analgesic and antipyretic effects and which have, in higher doses, anti-inflammatory effects. The term "nonsteroidal" is used to distinguish these drugs from steroids, which, among a broad range of other effects, have a similar eicosanoid-depressing, anti-inflammatory action. As analgesics, NSAIDs are unusual in that they are non-narcotic. The most prominent members of this group of drugs are aspirin, ibuprofen, and naproxen, all of which are available over the counter in many areas. NSAIDs are usually indicated for the treatment of acute or chronic conditions where pain and inflammation are present. Research continues into their potential for prevention of colorectal cancer, and treatment of other conditions, such as cancer and cardiovascular disease. NSAIDs are generally indicated for the symptomatic relief of the following conditions: Rheumatoid arthritis Inflammatory arthropathies. Low doses of aspirin reduce both pain and fever, whereas the anti-inflammatory action of aspirin requires a much higher dose. It is possible that inhibition of COX-1 is the major action of aspirin involved in its analgesic and antipyretic effects, and inhibition of COX-2 is responsible for its anti-inflammatory action. We compared the analgesic effects of an aspirin-like drug (diclofenac) and a centrally acting analgesic (paracetamol) in the mouse stretching test and confirmed that the analgesic action of the aspirin-like drug was peripheral. Two possible sites have been postulated for the antipyretic action of NSAIDs; inhibition of COX in endothelial cells of hypothalamic blood vessels or inhibition of COX synthesising PGs near sensory receptors of sub-diaphragmatic vagal afferents. The antipyretic action of aspirin may be mediated by inhibition of COX-3 in hypothalamic endothelial cells or by inhibition of COX-1 localised close to sensory receptors of peripheral vagal afferents. It is also possible that both enzymes are involved in the antipyretic action of aspirin. Whereas lipopolysaccharide (LPS)-induced fever is attenuated in COX-2 gene-deleted mice, suggesting that COX-2 is responsible for this type of fever, the COX-1 gene may also be important in temperature regulation and in mediating the pyresis that occurs in the absence of infection [1-13]. Paracetamol or acetaminophen, is a widely used over-the-counter analgesic and antipyretic. It is commonly used for the relief of headaches and other minor aches and pains and is a major ingredient in numerous cold and flu remedies. In combination with opioid analgesics, paracetamol

can also be used in the management of more severe pain such as post-surgical pain and providing palliative care in advanced cancer patients. The onset of analgesia is approximately 11 minutes after oral administration of paracetamol, and its half-life is 1–4 hours. Though acetaminophen is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity. While generally safe for use at recommended doses (and up to for adults), acute overdoses of paracetamol can cause potentially fatal liver damage and, in rare individuals, a normal dose can do the same; the risk is heightened by alcohol consumption. Paracetamol toxicity is the foremost cause of acute liver failure. It is the active metabolite of phenacetin, once popular as an analgesic and antipyretic in its own right, but unlike phenacetin and its combinations, paracetamol is not considered carcinogenic at therapeutic doses. Paracetamol produces analgesia in the mouse writhing test through a central action which is paralleled by a reduction in brain PGE (2) concentrations. In contrast, diclofenac has a peripheral analgesic action in this test. Paracetamol-induced hypothermia is also accompanied by a reduction in brain PGE (2) concentrations in C57/Bl6 mice. This hypothermic effect of paracetamol was reduced in COX-1 but not in COX-2 gene-deleted mice. These results support the view that analgesia and hypothermia due to paracetamol are mediated by inhibition of a third COX isoenzyme (COX-3). In cultured mouse macrophages, COX-2 is induced by treatment with LPS or with high concentrations of diclofenac. Diclofenac-induced COX-2 is inhibited with low concentrations of paracetamol, whereas LPS-induced COX-2 is insensitive to paracetamol inhibition. The mechanisms of induction and possibly the functions of these two COX-2 enzymes are also different [14-22]. Two COX isozymes, COX-1 and -2, are known to catalyze the rate-limiting step of prostaglandin (PG) synthesis and are the targets of NSAIDs. Here we describe a third distinct COX isozyme, COX-3, as well as two smaller COX-1-derived proteins (partial COX-1 or PCOX-1 proteins). COX-3 and one of the PCOX-1 proteins (PCOX-1a) are made from the COX-1 gene but retain intron 1 in their mRNAs. PCOX-1 proteins additionally contain an in-frame deletion of exons 5-8 of the COX-1 mRNA. COX-3 and PCOX mRNAs are expressed in canine cerebral cortex and in lesser amounts in other tissues analyzed. In human, COX-3 mRNA is expressed as an approximately 5.2-kb transcript and is most abundant in cerebral cortex and heart. Intron 1 is conserved in length and in sequence in mammalian COX-1 genes. This intron contains an ORF that introduces an insertion of 30-34 aa, depending on the mammalian species, into the hydrophobic signal peptide that directs COX-1 into the lumen of the endoplasmic reticulum and nuclear envelope. COX-3 and PCOX-1a are expressed efficiently in insect cells as membrane-bound proteins. The signal peptide is not cleaved from either protein and both proteins are glycosylated. COX-3, but not PCOX-1a, possesses glycosylation-dependent COX activity. Comparison of canine COX-3 activity with murine COX-1 and COX-2 demonstrates that this enzyme is selectively inhibited by analgesic/antipyretic drugs such as acetaminophen, phenacetin, antipyrine, and dipyrone, and is potently inhibited by some NSAIDs. Thus, inhibition of COX-3 could represent a primary central mechanism by which these drugs decrease pain and possibly fever [23-27].

Study of cyclooxygenases

Cyclooxygenase (COX) is an enzyme that is responsible for formation of important biological mediators called prostanoids (PTS), including prostaglandins (PGs), prostacyclin (PTN) and thromboxane (TX). Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain. NSAIDs, such as aspirin and ibuprofen, exert their effects through inhibition of COX. The names "prostaglandin synthase (PHS)" and "prostaglandin endoperoxide synthetase (PES)" are still used to refer to COX. COX converts arachidonic acid (AA, an ω-6 PUFA) to PG-H2, the precursor of the series-2 PTS. The enzyme contains two active sites: a heme with peroxidase activity, responsible for the reduction of PGG2 to PGH2, and a COX site, where AA is converted into the hydroperoxy endoperoxide PG-G2. The reaction proceeds through H atom abstraction from by a tyrosine radical generated by the peroxidase active site. Two O2 molecules then react with the AA radical, yielding PGG2. At present, three COX isoenzymes are known: COX-1, COX-2, and COX-3. The COX-2 enzyme that is the target of celecoxib and other COX-2 inhibitors. Daniel L. Simmons discovered the COX-2 and COX-3 enzymes. COX-3 is a splice variant of COX-1, which retains intron one and has a frame shift mutation; thus some prefer the name COX-1b or COX-1 variant (COX-1v). Different tissues express varying levels of COX-1 and COX-2. Although both enzymes act basically in the same fashion, selective inhibition can make a difference in terms of side-effects. COX-1 is considered a constitutive enzyme, being found in most mammalian cells. COX-3 is an enzyme that is encoded by the PTGS1 (COX1) gene, but is not functional in humans. COX-3 is the third and most recently discovered COX isozyme, the others being COX-1 and COX-2. 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 [28-35]. The other two COX isozymes are known to convert Dihomo-gamma-linolenic acid and AA into PGs, and are the targets of NSAIDs. PTGS1 "COX-1" redirects here. COX-1 may also refer to mitochondrial cytochrome c oxidase subunit 1 (COX1). COX-1, also known as PG-G/H synthase 1, PG-endoperoxide synthase 1 or PG-H2 synthase 1, is an enzyme that in humans is encoded by the PTGS1 gene. COX is the central enzyme in the biosynthetic pathway to PGs from AA. There are two isozymes of COX encoded by distinct gene products: a constitutive COX-1 (this enzyme) and an inducible COX-2, which differ in their regulation of expression and tissue distribution. The expression of these two transcripts is differentially regulated by relevant cytokines and growth factors. A splice variant of COX-1 termed COX-3 was identified in the CNS of dogs, but does not result in a functional protein in humans. Two smaller COX-1-derived proteins (the partial COX-1 proteins PCOX-1A and PCOX-1B) have also been discovered, but their precise roles are yet to be described. PG-endoperoxide synthase (PTGS), also known as COX, is the key enzyme in PG biosynthesis. It converts free AA, released from membrane phospholipids at the sn-2 ester binding site by the enzymatic activity of phospholipase A2, to PG-H2. The

reaction involves both cyclooxygenase (dioxygenase) and hydroperoxidase (peroxidase) activity. COX-3 is an enzyme that is encoded by the PTGS1 (COX1) gene, but is not functional in humans. COX-3 is the third and most recently discovered isozyme, the others being COX-1 and COX-2. 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. The other two COX isozymes are known to convert AA into PGs and are the targets of NSAIDs. COX-3 is transcribed from the PTGS1 (COX1) gene, but the resulting mRNA is spliced differently. 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. This mechanism is due to the fact that the spliced intron has 93 bases in dogs, resulting in the loss of 93:3 = 31 amino acids in the COX-3 sequence, which apparently does not impair its functionality. In humans, the intron is 94 bases long, leading to a protein with a completely different amino acid sequence from those of COX-1 or COX-2. The expressed protein does not show COX activity, and it is unlikely to play a role in PG-mediated physiological responses. The original COX-1/COX-2 model did not fully explain the immune responses of fever and inflammation. Even though COX-2 inhibitors are as active as traditional NSAIDs in inflammatory models, there were still some unexplained issues. PG-endoperoxide synthase-2 or COX-2, is an enzyme that in humans is encoded by the PTGS2 gene. COX-2 exists as a homodimer, each monomer with a molecular mass of about 70 kDa. The tertiary and quaternary structures of COX-1 and COX-2 enzymes are almost identical. Each subunit has three different structural domains: a short N-terminal epidermal growth factor (EGF) domain; an α-helical membrane-binding moiety; and a C-terminal catalytic domain. COX enzymes are monotopic membrane proteins; the membrane-binding domain consists of a series of amphipathic α helices with several hydrophobic amino acids exposed to a membrane monolayer. COX-1 and COX-2 are bifunctional enzymes that carry out two consecutive chemical reactions in spatially distinct but mechanistically coupled active sites. COX-3 was actually discovered in 2002, and been found to be selectively inhibited by paracetamol, phenacetin, antipyrine, dipyrone, and some NSAIDs in rodent studies [22,23]. A number of arguments counted against the COX-3 hypothesis: COX-2-selective inhibitors react weakly with the COX-3 enzymatic site, because the site is identical to that in COX-1, but they are as good at reducing fever as older NSAIDs. The fever response has also been clearly associated with a rapid induction of COX-2 expression and an associated increase in PG-E2 production, with no role for COX-1 or a COX-1 gene product (e.g., COX-3). Finally, the sites of COX-3 expression do not appear to fit in well with those sites associated with fever, and the protein should be present within the hypothalamus rather than the cerebral cortex. All these considerations appeared to argue against COX-3 being the site of the antipyretic actions of NSAIDs and COX-2-selective agents. However, the results could be read as showing that paracetamol acts at a different site than the other NSAIDs and that more than one COX isoform contribute to the fever response. Finally, the discovery of the frame-shift mechanism has made it highly unlikely that COX-3 plays a role in inflammation and fever in humans [36-41].

The original COX-1/COX-2 model did not fully explain the immune responses of fever and inflammation. Even though COX-2 inhibitors are as active as traditional NSAIDs in inflammatory models, there were still some unexplained issues. For example, the widespread use of the newer generation of COX-2-selective compounds demonstrated that COX-2 also has other physiological roles, e.g. in the maintenance of fluid balance by the kidneys. In addition, the COX-1/COX-2 model did not explain the properties of paracetamol: although its antipyretic and analgesic effects might be explained by inhibition of COX-2, it is not anti-inflammatory. Daniel et al., suggested this was because of the presence of a variant of COX-1, which they named COX-3 that would be especially sensitive to paracetamol and related compounds. If this enzyme were particularly expressed in the brain, it could explain both the characteristics of paracetamol, which has been reputed for some time of being a centrally-acting antipyretic [22,23]. Mice pretreated with the drugs demonstrated that the antinociception of metamizol and paracetamol is dose-dependent. In addition, the coadministration of metamizol with paracetamol induced a strong synergistic antinociception in the algesiometer assays. Both drugs showed effectiveness in inflammatory pain. These actions can be related to the differential selectivity of the drugs for inhibition of COX isoforms and also to the several additional antinociception mechanisms and pathways initiated by the analgesic drugs on pain transmission. Since the efficacy of the combination of metamizol with paracetamol has been demonstrated in the present study, this association could have a potential beneficial effect on the pharmacological treatment of clinical pain [42]. NSAIDs belong to different chemical groups and thus they can exert different effect on metabolism of biologically important substances. The effect of two NSAIDs: COX-1 inhibitor-resveratrol (trans-3,4,'5-trihydroxystilben) which is a diet supplement, and COX-3 inhibitor-paracetamol (acetaminophen) on blood nitric oxide (NO) concentration in rars. The resveratrol in a dose 2.5 and 10 mg/kg body weight and paracetamol in a dose 36 and 150 mg/kg body weight for three weeks, both paracetamol and resveratrol increased significantly nitric oxide concentration in blood [43]. Members of the COX family are known to catalyze the rate-limiting steps of PGs synthesis and reported to be involved in neuropathic pain. Diabetic neuropathy is a type of neuropathic pain, though it is not clear if COX is relevant to the condition. Spinal COX-2 protein was found to be increasing in streptozotocin-induced rats as compared to the constitutive expression. Intrathecal administrations of the COX-2 inhibitors SC-58125 (7-100μg) and NS-398 (7-60μg), as well as a high dose (100μg) of the COX-1 inhibitor SC-560 attenuated hyperalgesia, whereas intrathecal administrations of a low dose (10μg) of SC-560 and the COX-3 inhibitor acetaminophen (1-7mg) did not. Intrathecal administration of SC-58125 (100μg) did not produce an analgesic effect in normal rats. These results indicate that intrathecal administration of COX-2 inhibitors has an anti-hyperalgesic effect on streptozotocin-induced mechanical hyperalgesia and we concluded that spinal COX-2 is pivotal in streptozotocin-induced hyperalgesia [44]. The clone and sequence COX-1b (COX-3) mRNA and to generate an antibody against the

mouse COX-1b protein and to demonstrate its existence in vivo in mouse tissues. The mouse COX-1b mRNA is a splice variant of the COX-1 mRNA generated by the retention of intron-1. COX-1b mRNA encodes a 127 amino acid protein with no similarity with known COX sequences. We generated an anti-mouse COX-1b antibody and demonstrated the existence of COX-1b protein in vivo with the highest expression in kidney, heart, and neuronal tissues. The COX-1b mRNA and protein expression is in COX-1 knockout mice. In mouse, COX-1b encodes a protein with a completely different amino acid sequence than COX-1 or COX-2; therefore it is improbable that COX-1b in this species plays a role in PG-mediated fever and pain [45].

A novel COX-1 splice variant termed COX-3, sensitive to acetaminophen, was recently discovered by Simmons et al., and is considered to play a key role in the biosynthesis of PTS known to be important mediators in pain and fever. Drugs that preferential block COX-1 also appear to act at COX-3. However the existence of COX-3 at the nucleotide sequence level in humans has been called to question. A functional COX-3 in humans is still to come underlining that the concept of COX-3 is still attractive. Here, we discuss some of the implications drawn from the identification of additional functional COX members in the generation of bioactive autacoids [46]. In randomized groups of Wistar rats, the effect inhibitor of selective NASAID over the COX-1, COX-2 and COX-3. The synchronize inhibition of COX-1 and COX-2, COX-1 and COX-3, COX-2 and COX-3, and COX-1, COX-2 and COX-3 were studied. The conclusions were that the selective inhibition of COX-1, COX-2 and COX-3 no given GI damage; the synchronizes inhibition of COX-1 and COX-2 given preferential gastric damage; in contrast the inhibition of COX-2 and COX-3 given massive necrosis preferential in small intestine [47]. We have detected an expressed mRNA encoding a splice variant of COX-1 in the mouse central nervous system. This isoform, referred to as COX-3, is identical in sequence to COX-1 except for the in-frame retention of intron 1. Like its counterpart COX-1, COX-3 does not generally appear to be induced by acute inflammatory stimulation [48].

DISCUSSION

A new generation of NSAIDs has been described that selectively targets the inducible isoform of cyclo-oxygenase (COX), COX-2. This isoform is expressed at sites of inflammation, which has led to the speculation that its inhibition could provide all the benefits of current NSAIDs, but without their major side-effects on the gastrointestinal system (which are due to inhibition of COX-1) COXs catalyse the key rate-limiting step in PTN and TX biosynthesis and are targets of NSAIDs. Presence of only two isoforms-COX-1 and COX-2-remained question because the potent anti-pyretic and analgesic effects of acetaminophen (paracetamol) could not be explained by either COX-1 or COX-2 blockades [49-53]. We have shown that COX-2 (identified by use of specific antibodies) is induced during the resolution of an inflammatory response, inhibition of COX-2 resulting in persistence of the inflammation due to the prevention of the synthesis of a range of anti-inflammatory PTS. We propose that there is a third isoform of this enzyme family, COX-3, a proposal that will have implication for the prescription of both existing and new generation anti-inflammatory drugs, and might represent a new therapeutic target.

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