

The Future of Selective COX Inhibitors

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In the preceding article, Dr Low has provided an overview of the history and development of the selective inhibitors of cyclooxygenase-2 (COX-2) which promised so much in the separation of analgesic and anti-inflammatory effects from adverse gastrointestinal effects, but delivered new problems of potentially disastrous prothrombotic effects. The story of the selective COX-2 inhibitors provides some salutary lessons about the detection of uncommon adverse events and the importance of post-marketing surveillance. Looking to the future, this article discusses the potential avenues of development of new selective COX inhibitors which might deliver safer analgesic, anti-inflammatory and antineoplastic therapies.

The function of prostaglandins in the mechanism of action of non-steroidal antiinflammatory drugs (NSAID), and thus the role of COX, was first described by Vane in 1971¹ and the COX enzyme was described as having two isoforms in 1990.² The basic science behind the "coxib" drugs is 15 years old. In the meantime, the state of knowledge about the synthetic pathway of prostaglandins and their regulation have progressed, and a great deal of effort has gone into the further sub-typing of the COX enzymes.

Molecular Biology of the COX enzymes

The genes encoding the COX-1 and COX-2 enzymes have been sequenced and mapped in humans and laboratory animals. They are encoded by genes at two distinct loci, enabling the breeding of mice with deletions of either gene. Soon after the description of the presence of COX-1 and COX-2 genes, the first description of a splice variant of the COX-2 mRNA was published³ and subsequently other splice variants of COX-2 and of COX-1 have been described. These include partial COX-1 (PCOX-1) proteins, which are smaller proteins expressed from the COX-1 gene.

The COX-1 splice variant 1 (COX-1V₁, also called "COX-3" in some papers) is the best described of the splice variants. It is derived from the COX-1 gene with the retention of intron-1 in the mRNA (a part of the COX-1 gene which, unlike the exons, does not code for part of the COX-1 enzyme) and was first described in dogs. The mRNA expresses a functional protein, which differs from COX-1 in its sensitivity to

inhibition by paracetamol and some NSAIDs. Thus, it was thought to be a potential site of action to explain the effects of paracetamol.

In humans, COX1-V₁ is found in the cerebral cortex and cardiac muscle. It has been studied in more detail in rats where the COX1-V₁ mRNA is expressed at the highest levels in the brain microvasculature and appears to be constitutively expressed, as its levels do not change in models of inflammation. The significance of this mRNA expression in rats is uncertain as the length of intron-1 of the COX-1 gene in the rat is such that it introduces a frame-shift error when retained, making it unlikely that this mRNA can express a functional protein the same way as in dogs. Intron-1 of the COX-1 gene in humans is 94 base pairs long and so also introduces a frame-shift error. Studies with antibodies directed against protein expressed by intron-1 confirmed in humans that a COX-1 variant protein including intron-1 is expressed, however.⁴

A COX-2 variant was described in cell culture in 1999.⁵ This is distinguished from COX-2 on the basis of pharmacological differences. It is not inhibited by aspirin, less sensitive to NSAIDs than COX-1 or COX-2 and more sensitive to inhibition by paracetamol. The molecular genetics of this COX variant have not been described and its physiological significance is unknown.

Physiological studies detected the presence of another COX-2-like enzyme expressed in a rat model of inflammation in the resolving stages of inflammation.⁶ This was not associated with inflammation, but rather with an anti-inflammatory effect. This enzyme was also referred to as "COX-3" in some papers⁷ and is inhibited by COX-2 inhibitors and conventional NSAIDs.

Studies of COX expression in models of inflammation serve to illustrate how complex the range of products of the COX genes might be. The expression of COX enzymes with different activities in prostaglandin synthesis, different sensitivities to inhibition and different locations within the cell seem to depend on the cell type, the specific stimulus to COX induction and the time following that stimulus. Thus, in an intact animal there are variations in the timing and anatomical location of induced COX, as well as its activity and the pharmacodynamics of relevant drugs. The work required to tease out the full detail of this multidimensional system will be immense.

A range of point mutations of the COX genes have also been described with around 40 single nucleotide polymorphisms. These polymorphisms appear to have little phenotypic significance; there is a low rate of variation in the COX genes within and between species, reflecting their important role. There is some evidence that there may be a role of COX gene variants in the risk of development of adenomas, but there is no evidence of pharmacological significance.

Partial COX-1 proteins have been detected following analysis of the cDNAs derived from the COX-1 gene.⁴ The PCOX-1 cDNAs lack exons 5-8 of the COX-1 gene, which code for part of the substrate binding site of the enzyme and so the PCOX-1 proteins do not have prostaglandin synthesizing activity. However, they can participate in other reactions. There are two PCOX-1 variants described, PCOX-1a and PCOX-1b which differ in the inclusion of intron-1. Their physiological role remains unexplored.

The range of possibilities therefore for pharmacological intervention extends well beyond a single ratio of inhibition of COX-1 to COX-2 which has thus far described the available drugs. However, the number of potential enzymatic targets is unknown. Of the several variations in COX mRNA discovered, only a subset encodes proteins and possibly only a subset of those proteins are functional. Complete description of the range of functional COX products remains some way in the future.

The label “COX-3” is somewhat confusing in the scientific literature, having been used to describe at least two different enzymes. Most commonly, it has been used to refer to COX1-V₁. The basis for this use is that the original distinction between COX-1 and COX-2 was a physiological and pharmacological one, and so the apparently distinctive pharmacology of the COX1-V₁ enzyme merits the label COX-3. Unfortunately the COX-1/COX-2 distinction is also a genetic one and the COX1-V₁ enzyme is derived not from a third COX gene which might be termed COX-3, but from the COX-1 gene.

Integrated models of COX physiology and pharmacology

The simple model of COX-1 as the constitutive COX responsible for the homeostatic functions of prostaglandins and COX-2 as the inducible form responsible for inflammation and pain has never explained the range of experimental observations, but has a certain appeal. It promised a hope that selective COX-2 inhibitors would provide potent anti-inflammatory and analgesic effects without the adverse effects of the non-selective COX inhibitors. By the time the coxib drugs were marketed, their theoretical potential to cause adverse effects though inhibition of prostacyclin synthesis and a resultant prothrombotic effect, and also their potential interference with renovascular regulation were well understood. Nonetheless, they captured a great market share with the implicit promise of being safe and effective.

The most glaring problem with this simple model of COX physiology is its failure to explain the mechanism of action of paracetamol, which thirty years ago was proposed to operate by a central effect on COX. In vitro studies of COX-1 and COX-2 have demonstrated only weak inhibition by paracetamol, though it shares with the COX inhibitors its antipyretic and analgesic effects. In vitro studies of the effect of paracetamol on COX may have been confounded by its phenol-like chemical structure, which imposes technical methodological difficulties. The history of the search for a mechanism of action for paracetamol is the history of the search for other isoforms of COX.

With each discovery of a potential or actual new COX variant, there has been offered the explanation that the new “COX-3” is the site of action of paracetamol. This provides a simple explanation of three COX enzymes with discrete functions, COX-3 being the isoform in the central nervous system responsible for fever and playing a role in pain perception. However, neither the molecular biology nor the animal research supports such a simple explanation. COX-2 is not exclusively inducible but is constitutively expressed in many tissues, while evidence from gene-knockout mice show COX-1 plays an important role in pain perception.⁸ Paracetamol does have a measurable peripheral antiinflammatory effect,⁹ despite not being a potent COX-1 or COX-2 inhibitor.

The presence of “COX-3” as COX1-V₁, being a CNS variant of COX-1, can explain the apparent paradox of COX-1 knockout mice having impaired hot-plate nociception, while administering NSAIDs (which block COX-1 but are less effective on COX1-V₁) to mice does not affect this test. However, the antipyretic effect of the NSAIDs and paracetamol is probably due to inhibition of COX in the organum vasculosum laminae terminalis, adjacent to the hypothalamus. This response is blocked in COX-2 knockout mice. So, if the antipyretic effect of paracetamol is caused by a “COX-3”, it is likely to be a product of the COX-2 gene (and thus not COX1-V₁).

A less satisfyingly simple explanation, but one which can better explain the

experimental evidence, was proposed by Warner and Mitchell.¹⁰ They describe a “continuum” of at least five COX isoforms derived from the two known COX genes, with both physiological functions and pharmacological spectra spread over multiple subtypes. This is bad news for examination candidates trying to memorize simple physiology, but it does marry the complex actions of the pharmacological agents we use with the bewildering array of identified and proposed COX subtypes.

The future of selective COX inhibition

If the death of a simple two- or three-isoform model for COX is disappointing for those hoping to understand prostaglandin physiology in detail, it is a source of excitement and hope for those wanting the ideal selective analgesic and anti-inflammatory drug devoid of adverse effects. For the pharmaceutical industry, more isoforms means more targets for selective inhibition and more potential for a “magic bullet”.

From the currently marketed drugs, we know that avoiding COX-1 inhibition in the periphery avoids the complications of gastric ulcers and inhibition of coagulation, while retaining almost all of the analgesic, anti-inflammatory and antipyretic effects of the non-selective inhibitors. Unfortunately there is a prothrombotic effect, probably due to lack of inhibition of thromboxane synthesis (a COX-1 function). Adding a small dose of a non-selective inhibitor might reduce the thrombotic risk, but also removes the advantages in the risk of gastric ulceration.

If the other COX-1 gene products or the proposed COX-2 variants play a role in nociception and inflammation, there may be potential for selective inhibitors which could expand our range of effective and safe analgesics. Such drugs are still a long way off, but research in this direction is continuing and the dramatic rise and fall of the coxibs will only stimulate further studies. A drug as effective as rofecoxib, but as safe as paracetamol, would be very attractive to the pharmaceutical industry.

Drugs such as antipyrine and aminopyrine, which selectively inhibit COX1-V₁, are in use in animal studies and selective COX1-V₁ inhibitors may eventually make their way into clinical practice. We might expect such drugs to be analgesic and antipyretic based on animal evidence, but they would not be expected to have significant anti-inflammatory effects or systemic side effects. Indeed, this line of research may provide side-effect free analgesics or may turn up nothing better for clinical practice than paracetamol, which has been in use since 1893 and is still one of the safest analgesics available.

References

1. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol* 1971; 231(25): 232-235.
2. Fu JY et al. The induction and suppression of prostaglandin H2 synthase (cyclooxygenase) in human monocytes. *J Biol Chem* 1990; 265(28):16737-16740.
3. Xie WL et al. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. *Proc Natl Acad Sci USA* 1991; 88(7):2692-2796.
4. Chandrasekharan NV et al. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci USA* 2002; 99(21):13926-13931.
5. Simmons DL et al. Induction of an acetaminophen-sensitive cyclooxygenase with reduced sensitivity to nonsteroid antiinflammatory drugs. *Proc Natl Acad Sci USA* 1999; 96(6):3275-8280.
6. Gilroy DW, Tomlinson A, Willoughby DA. Differential effects of inhibitors of cyclooxygenase (cyclooxygenase 1 and cyclooxygenase 2) in acute inflammation. *Eur J Pharmacol* 1998; 355(2-3):211-217.

7. Willoughby DA, Moore AR, Colville-Nash PR. COX-1, COX-2, and COX-3 and the future treatment of chronic inflammatory disease. *Lancet* 2000; 355(9204):646-648.
8. Ballou LR et al. Nociception in cyclooxygenase isozyme-deficient mice. *Proc Natl Acad Sci USA* 2000; 97(18):10272-10276.
9. Skoglund LA, Eidsaunet W, Pettersen N. The anti-oedematous efficacy of oxindanac equals that of paracetamol in acute postoperative inflammation; are weak cyclooxygenase inhibitors more effective than strong inhibitors? *Int J Clin Pharmacol Res* 1989; 9(6):371-375.
10. Warner TD, Mitchell JA. Cyclooxygenase-3 (COX-3): filling in the gaps toward a COX continuum? *Proc Natl Acad Sci USA* 2002; 99(21):13371-13373.