Cyclooxygenase-2 Polymorphism
Putting a Brake on the Inflammatory Response to Vascular Injury?
Francesco Cipollone, Carlo Patrono

Prostaglandin endoperoxide H synthase (PGHS) catalyzes the conversion of arachidonic acid to PGH₂, the first committed step in the biosynthesis of a range of lipid mediators, termed prostaglandins (PGs) and thromboxanes.¹ PGHS has both cyclooxygenase (COX) and hydroperoxidase activities.² Aspirin and a variety of nonsteroidal antiinflammatory drugs (NSAIDs) inhibit the COX activity of PGHS³ (Figure 1).

Before 1991, only the isoform called PGHS-1, COX-1, or the constitutive enzyme had been described. At that time, Xie et al³ and Kujubu and Herschman⁴ discovered mRNAs whose expression was induced in chicken and mouse fibroblasts in response to src and tumor-promoting phorbol esters, respectively, and that encoded proteins having 60% amino acid sequence identity with COX-1. Subsequent work has shown that the new protein, called PGHS-2, COX-2, or the inducible isofrom, is very similar to COX-1 in structure but differs substantially from COX-1 with respect to its pattern of expression and its biology.¹ In particular, COX-2 can be upregulated by cytokines, growth factors, and tumor promoters,⁴⁻⁵ suggesting its relevance in inflammation and cancer.

Although initially characterized as an isozyme inducible in response to inflammatory or mitogenic stimuli, COX-2 is also expressed constitutively in many tissues (eg, brain and kidney).¹ Moreover, the vast majority of human cell types that have been carefully examined for COX-2 expression do in fact express both COX-isozymes under appropriate circumstances. Paradoxically, COX-2 induction has been described in association with physiological bone marrow stem cell differentiation⁶ as well as with neoplastic transformation of intestinal epithelial cells.⁷

The expression of both COX-1 and COX-2 is increased in the synovia of inflamed joints⁸ and in carotid atherosclerotic plaques.⁹ In addition, COX-2 is considered a critical gene in carcinogenesis, as animal studies have shown that a selective COX-2 inhibitor, celecoxib, has chemopreventive properties, suppressing the incidence and multiplicity of adenocarcinomas of the colon.¹⁰ In addition, in patients with familial adenomatous polyposis, prolonged therapy with celecoxib led to a significant reduction in the number and size of colorectal polyps.¹¹

The human COX-1 gene, mapped to chromosome 9q32-q33.3, is ≈22 kilobase pairs in size and contains 11 exons.¹² The human COX-2 gene, mapped to chromosome 1q25.2-q25.3, is ≈8.3 kilobase pairs in size and contains 10 exons.¹³ The possibility that single nucleotide polymorphisms (SNPs) may be useful in identifying candidate disease genes and individuals at risk of disease has led to extensive projects aimed at discovery and organization of SNPs into databases. At this time, millions of candidate SNPs are available in the public dbSNP databases.¹⁴ In the case of COX-2, differences from the published sequence (GenBank accession number U04636) have been detected. However, these polymorphisms are intronic, or synonymous changes where functional effects are not likely. Far less has been done to functionally characterize polymorphisms in COX-2 coding regions. However, a limited number of polymorphisms in the COX-2 promoter region, with potential impact on the COX-2 phenotype, have been identified.¹⁵,¹⁶ Unfortunately, these polymorphisms were not located in any known transcription factor binding site that could potentially impact transcription, did not result in an inactive COX-2 enzyme, or change the NSAID susceptibility and the metabolite profile. Altered function of COX-2 had been hypothesized to change the number of colonic polyps in patients with an inherited predisposition to colon cancer, and two COX-2 gene sequencing studies in patients with adenomatous polyposis coli (APC) have been reported.¹⁵,¹⁷ Among APC patients, a silent G/C polymorphism in exon 3 was reported, but no association with polyp number was observed.¹⁷ Similarly, there was no suggestion that other SNPs in the coding region of COX-2 gene might play a role in the inherited predisposition to colon cancer.¹⁵ Failure to characterize such an association may reflect the variable contribution of other functionally important events operating upstream or downstream of COX-2 expression and inhibition.¹⁸

The substantial degree of interindividual variability in the pharmacokinetic/pharmacodynamic relation in response to oral dosing with selective COX-2 inhibitors¹⁹ suggests that COX-2 polymorphism might contribute to the individual patient’s response to such treatment. However, no studies addressing this question have been reported yet.

In the current issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Papafili and coworkers²⁰ report the identification of a new variant in the COX-2 promoter, −765G>C, and show that this variant locates within a putative binding site for Sp1, and has significantly lower (−30%) promoter activity compared with the −765G allele. The 5′ flanking region of the human COX-2 gene, principally

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Editorial
involved in regulating gene transcription, contains canonical TATA box and several putative transcription-factor binding sites, including CRE, NF-κB, NF-IL-6, GRE, PEA-3, AP2, C/EBP, TGF-β, and multiple Sp1 response elements. In particular, Sp1 is considered a positive activator of COX-2 transcription and acts through G-rich elements. Interestingly, deletion and forced mutation experiments altering this sequence have identified critical regions involved in inducing COX-2 gene transcription.

Notably, in the present study, Papafili and coworkers reported that, among patients undergoing elective coronary artery bypass graft (CABG) surgery, those carrying the −765C allele had significantly lower plasma levels of C-reactive protein (CRP) compared with patients homozygous for −765G. CRP, a sensitive marker of low-grade inflammation, has been shown to aid cardiovascular risk prediction in a variety of clinical settings, including patients undergoing CABG or percutaneous coronary intervention. Thus, patients with baseline CRP levels ≥3 mg/L had significantly increased risk of recurrent ischemia at 1 to 6 years after CABG surgery. In the study by Papafili et al., baseline CRP levels of patients carrying −765GC or −765CC were only marginally and not significantly lower as compared with carriers of −765GG (1.8±0.3 vs 2.1±0.2 mg/L). However, mean CRP values were significantly lower for carriers of −765GC or −765CC at all times after bypass surgery, with peak levels recorded on day 3 of 150±9 (GC+CC) and 174±6 mg/L (GG), respectively. Although the 14% difference in average peak levels of CRP was statistically significant (P<0.05), some caution is warranted in interpreting these results. Thus, first, carriage of the rare allele (GC+CC combined) did not prevent an 80-fold increase in post-surgery CRP levels versus baseline. Second, individual CRP levels of the three genotypes showed substantial overlap after CABG with no clear-cut separation of carriers of the rare allele (data not shown). As the authors rightly point out, it remains to be seen if this common promoter variant in COX-2 will have clinical relevance. In a variety of colon cancer cell lines, the presence of either G or C at −765 made no difference in the level of expression of COX-2, and a direct role of this prevalent polymorphism in colon carcinogenesis seems unlikely (SM Prescott, Huntsman Cancer Institute, University of Utah, written communication, 2002).

In the meantime, what sort of additional studies should be done to address the many open questions raised by this interesting study? First of all, we need to know whether a 30% lower COX-2 promoter activity is associated with lower prostanooid production in human cells expressing this isozyme in response to pathophysiologic stimuli. This could be accomplished by investigating COX-2 expression and PGE2 production in whole blood monocytes challenged with LPS in vitro. This whole blood assay can also allow assessing the individual susceptibility to COX-2 inhibition by traditional NSAIDs or coxibs, either in vitro or ex vivo, in patients carrying the −765C allele. This type of investigation should be complemented with the measurement of in vivo PGI2 production, a process largely dependent on COX-2 activity in humans.

Mechanistically, reduced COX-2 expression possibly associated with the −765C variant should not be equated with a cardioprotective phenotype given the multifaceted aspects of COX-2 in vascular and renal biology. Moreover, it should be considered that COX-2 is but one of at least 10 different proteins involved in the biosynthesis and cellular actions of a single lipid mediator such as PGE2 (Figure 1). Thus, multiple sites of regulation and potential polymorphisms should be evaluated in future studies.

Finally, we are beginning to understand that the functional read-outs of COX-2 expression (eg, regulation of matrix metalloproteinases in inflammatory cells) may be importantly
modulated by the variable expression of downstream PGH-isomerases (eg, PGD-synthase versus PGE-synthase) that may preferentially couple to COX-isozymes in different pathophysiologic settings relevant to dynamic plaque instability (Figure 2).9,29

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