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REVIEW

Emerging roles for cyclooxygenase-2 in gastrointestinal mucosal defense

*,[1]John L. Wallace & [2]Pallavi R. Devchand

[1]Mucosal Inflammation Research Group, University of Calgary, Calgary, Alberta, Canada and [2]Centre for Excellence in Vascular Medicine, Cardiovascular Division, Harvard Medical School and Brigham & Women's Hospital, Boston, MA, U.S.A.

The development of selective inhibitors of cyclooxygenase-2 (COX-2) was based on the concept that this enzyme played little, if any, role in modulating the ability of the gastrointestinal (GI) tract to resist and respond to injury. There is now overwhelming evidence that this is far from true. Indeed, COX-2 mediates several of the most important components of 'mucosal defense', contributes significantly to the resolution of GI inflammation and plays a crucial role in regulating ulcer healing. COX-2 also contributes to long-term changes in GI function after bouts of inflammation.
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Introduction

'Never ignore a gut feeling, but never believe that it is enough'
—Kermit ('The Muppets')

The gastrointestinal (GI) tract is a dynamic environment that serves primarily as an entry site to the body for nutrients, while restricting the uptake of harmful antigens, toxins and microbes. Pivotal to this role is the ability to respond appropriately to potentially damaging luminal agents, a process referred to as 'mucosal defense'. Failure of mucosal defense can lead to sustained mucosal tissue injury and chronic inflammation, and in extreme cases, to systemic infection.

Over a decade ago, cyclooxygenase-2 (COX-2) was cloned and described as an inducible enzyme involved in the generation of potent lipid mediators (i.e., prostaglandins (PGs)) during inflammation. Today, medications designed to selectively inhibit the activity of this enzyme are widely prescribed for the treatment of osteoarthritis and rheumatoid arthritis, as well as for acute pain. Selective inhibition of the COX-2 isotype with 'coxibs', it was proposed, would achieve anti-inflammatory and analgesic effects comparable to those of conventional nonsteroidal anti-inflammatory drugs (NSAIDs), without causing damage in the GI tract. However, it is now clear that, at best, coxibs cause significant ulceration at about half the rate of conventional NSAIDs (Bombardier *et al.*, 2000), while also exhibiting significant toxicity in the renal and cardiovascular systems (Cheng & Harris, 2004). The

cardiovascular toxicity of rofecoxib, first suggested as early as 1998 (Mitchell & Evans, 1998), was cited as the reason for its withdrawal from world markets on September 30, 2004. Reports of increased cardiovascular complications in trials of rofecoxib, celecoxib, etoricoxib, paracoxib and valdecoxib support the notion that this toxicity is a feature of the entire class of selective COX-2 inhibitors (Bombardier *et al.*, 2000; Crofford *et al.*, 2000; Mukherjee *et al.*, 2001; The Pink Sheet, 2001; Ott *et al.*, 2003; Mamdani *et al.*, 2004). The advent of the selective COX-2 inhibitor has stimulated considerable research into the role of this enzyme in mucosal defense and the associated impact on human health and disease. Here, we review some recent findings on COX-2 in the context of GI mucosal defense with a focus on cellular and molecular mechanisms, as well as some genetic considerations.

COX-2 and resistance to damage by luminal irritants

The lining of the GI tract is exposed regularly to a wide range of potentially damaging substances, including those that we ingest (alcohol, aspirin, etc.) and endogenous secretions (acid, bile salts). Perhaps most notable about the GI mucosa is not simply its ability to resist damage by these substances but also the impressive reparative capacity when damage is produced. PGs, particularly PGE_2 and PGI_2, play a very important role in modulating GI mucosa defense and repair (Wallace & Granger, 1996; Wallace & Ma, 2001). Among the many important effects of these PGs, with respect to mucosal defense, are stimulation of mucus and bicarbonate secretion, and maintenance of mucosal blood flow. Thus, inhibition of PG synthesis by NSAIDs is one of the primary mechanisms

*Author for correspondence at: Department of Pharmacology & Therapeutics, University of Calgary, 3330 Hospital Drive NW, Calgary, Alberta, Canada T2N 4N1; E-mail: wallacej@ucalgary.ca

through which this class of drugs produces injury in the GI tract (Wallace, 1997). NSAIDs reduce mucus and bicarbonate secretion, as well as reduce mucosal blood flow. These drugs also trigger an increase in adhesion of leukocytes (most notably neutrophils) to the vascular endothelium in the GI microcirculation, which has been shown to be an early and critical event in the pathogenesis of NSAID-induced mucosal ulceration (Wallace, 1997).

Early studies of selective COX-2 inhibitors in healthy rats and mice confirmed the predictions of their manufacturers that these drugs would not produce gastric injury. Since mice with targeted disruption of COX-2 did not develop spontaneous gastric lesions (Morham *et al.*, 1995), the production of the PGs that mediate gastric mucosal defense, at least under normal conditions, was attributed to COX-1 activity. Surprisingly, COX-1 knockout mice also did not spontaneously develop gastric lesions, despite negligible gastric PG synthesis, but were susceptible to lesion formation when given an NSAID (Langenbach *et al.*, 1995). Other studies demonstrated that suppression of gastric COX-1 activity in the rat with a selective inhibitor (SC-560) failed to elicit gastric damage (Wallace *et al.*, 2000). When both COX-1 and COX-2 were inhibited, as would occur with conventional NSAIDs, gastric damage was elicited (Wallace *et al.*, 2000). A similar situation was observed in models of NSAID-induced small intestinal injury; that is, selective inhibition of COX-1 or COX-2 did not result in injury, but suppression of both isoforms of COX led to significant damage (Tanaka *et al.*, 2002). It can therefore be concluded that PGs derived from both COX-1 and COX-2 contribute to mucosal defense. Indeed, there is good evidence that the two COX isoforms may influence different components of mucosal defense: suppression of COX-1 accounts for the reduction in mucosal blood flow that is observed following NSAID administration, while suppression of COX-2 accounts for the increase in leukocyte adherence to vascular endothelium that is observed following NSAID administration (Wallace *et al.*, 2000) (Figure 1).

The studies described above involved administration of COX inhibitors to healthy animals, in which the only 'challenge' of the mucosa was the COX inhibitor itself. We are only beginning to understand the contribution of COX-2 to the ability of the GI mucosa to resist and respond to luminal insults. For example, exposure of the rat stomach to 70% ethanol results in the formation of hemorrhagic lesions. Exposure of the rat stomach to a 'mild irritant', such as 20% ethanol, results in a marked reduction in damage induced by subsequent exposure to 70% ethanol. This phenomenon has been termed 'adaptive cytoprotection' (Robert *et al.*, 1979). Pretreatment with anti-inflammatory doses of selective COX-2 inhibitors (NS-398, DFU, L-745,337) led to inhibition of the adaptive cytoprotective response (Gretzer *et al.*, 2001). Another circumstance in which COX-2 appears to play a role in mucosal resistance to injury is that in which the stomach is subjected to a period of ischemia followed by reperfusion. In a rat model of ischemia–reperfusion, hemorrhagic lesions consistently form in the stomach, particularly if acid is present in the lumen during the period of ischemia. Ischemia–reperfusion leads to a marked upregulation of COX-2 expression in the stomach, and treatment with a selective COX-2 inhibitor prior to the period of ischemia resulted in a significant worsening of gastric injury (Maricic *et al.*, 1999). Also, inhibition of the upregulation of COX-2 expression, through prior administration of a gluco-
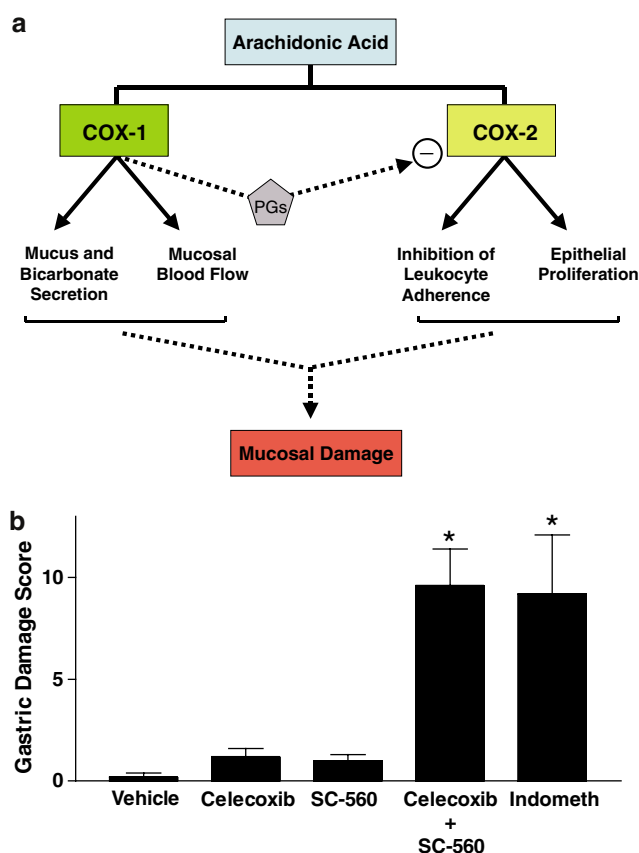
Figure 1 Contributions of COX-1 and COX-2 to GI mucosal defense. Panel a: PGs derived from the two COX isoforms influence different aspects of mucosal defense. COX-1 appears to produce the PGs that regulate mucosal blood flow and epithelial secretion of mucus and bicarbonate, while PGs from COX-2 influence epithelial proliferation and endothelial-leukocyte adherence. PGs from COX-1 also tonically suppress COX-2 activity in the GI tract. COX-2 is rapidly upregulated when COX-1 is inhibited, when the GI mucosa is exposed to a potentially damaging agent or when mucosal injury occurs. Suppression of COX-2 activity in these circumstances leads to enhanced mucosal injury and delayed repair. Panel b: Selective suppression of COX-1 (with SC-560) or of COX-2 (with celecoxib) does not result in mucosal injury in healthy rats, while suppression of both isoforms of COX leads to such damage. Indomethacin is a nonselective inhibitor of COX-1 and COX-2. This figure was constructed using previously published data (Wallace *et al.*, 2000).

corticoid, resulted in a significant exacerbation of ischemia–reperfusion-induced gastric damage (Maricic *et al.*, 1999). As ischemia–reperfusion injury is primarily a neutrophil-driven response (Hernandez *et al.*, 1987), it is possible that the ability of selective COX-2 inhibitors to increase leukocyte adherence to the vascular endothelium (Muscará *et al.*, 2000) contributed to the production of injury in the stomach in this setting.

Emerging evidence indicates that COX-2 plays an expanded role in modulating resistance to luminal irritants when other mediators of mucosal defense are pharmacologically or genetically depressed. For example, nitric oxide is an important mediator of many components of mucosal defense (Wallace & Miller, 2000). Studies in the rat have demonstrated that when nitric oxide synthesis is inhibited, administration of selective COX-2 inhibitors results in significant gastric damage (Ehrlich *et al.*, 2004). Sensory afferent nerves also contribute significantly to the ability of the GI mucosa to resist injury, mainly by regulating mucosal blood flow. When sensory

afferent nerves are chemically ablated, administration of selective COX-2 inhibitors results in formation of hemorrhagic lesions (Ehrlich *et al.*, 2004). Thus, at the molecular level, mucosal resistance to luminal irritants involves significant cross-talk among COX-2-derived signals and other endogenous signalling pathways.

A major focus of the research on this enzyme in the context of mucosal injury and defense in the upper GI tract is largely a consequence of manufacturers' claims that selective COX-2 inhibitors did not cause ulceration. However, there is convincing evidence for physiological and beneficial roles for COX-2 in maintenance of mucosal integrity in more distal parts of the GI tract. For example, COX-2 has been suggested to be an essential factor in immune tolerance. Newberry *et al.* (1999; 2001) reported that lamina propria stromal cells constitutively express COX-2 and produce PGE_2 *via* this enzyme in a continuous manner. Given the known immunomodulatory effects of PGE_2, these authors suggested that COX-2 contributes to ongoing downregulation of the intestinal immune response. Further evidence of COX-2 involvement in maintenance of mucosal integrity was the report that COX-2 knockout mice spontaneously developed peritonitis, presumably related to deterioration of intestinal barrier function (Morham *et al.*, 1995). These consequences of either chronic suppression of COX-2 activity or absence of COX-2 expression may be related to a role for products of this enzyme in mediating GI epithelial proliferation (Sawaoka *et al.*, 1997; Erickson *et al.*, 1999). No doubt, further studies will delineate the molecular mechanisms behind beneficial roles of COX-2 in maintenance of mucosal integrity during responses to luminal irritants along the GI tract.

Dynamic regulation of GI COX-2 expression

The observation that COX-2 expression is low in the GI tract of healthy humans and animals (Kargman *et al.*, 1996; Davies *et al.*, 1997; Zimmerman *et al.*, 1998) contributed to the notion that COX-2 played little, if any, role in mediating physiological events in the GI tract. However, as outlined in the section above, it is now clear that COX-2 expression is dynamic and required for maintenance of homeostasis. At the transcriptional level, *COX-2* is an early-response gene to several stimuli. For example, significant induction of COX-2 in the rat stomach was detected as early as 1 h after administration of aspirin or indomethacin (Davies *et al.*, 1997). The observation that induction of COX-2 could be prevented by concomitant administration of a PG suggested that diminished mucosal PG levels were responsible for triggering the upregulation of COX-2 expression (Davies *et al.*, 1997). Indeed, Tanaka *et al.* (2002) observed that selective inhibition of COX-1 in the rat intestine led to rapid upregulation of COX-2 expression. Significant upregulation of COX-2 in the rat stomach has also been observed within 40 min of oral challenge with acid (Sawaoka *et al.*, 1997). Taken together, these data suggest that rapid COX-2 induction is a common response to luminal irritation aimed at enhancing mucosal resistance to injury and at priming the mucosa to be prepared for repair in the event that injury does occur.

The specific role of COX-2 in resolution of inflammation is discussed later in this article. Studies in a rat model of colitis indicate that COX-2 also contributes to long-term changes in colonic function after resolution of colitis. As colitis resolves, COX-2 expression increases significantly, and, in parallel, there is increased synthesis of the downstream product PGD_2. The elevated COX-2 expression and activity contributes to impaired epithelial secretion and increased bacterial translocation (Zamuner *et al.*, 2003). Thus, selective inhibition of COX-2 causes a rapid normalization of colonocyte function and of epithelial barrier function. The prolonged increase in COX-2 expression and PGD_2 synthesis following a bout of colitis might also contribute significantly to an increased susceptibility of the post-colitis rats to colon cancer (Zamuner & Wallace, 2004), mimicking the human situation in which patients with ulcerative colitis are at much greater risk of developing colon cancer (Itzkowitz & Yio, 2004). Selective COX-2 inhibitors have been suggested to have some utility for chemoprevention of colon cancer (Mann & DuBois, 2004). Thus, the dynamic nature of COX-2 expression is an important determinant in GI homeostasis and susceptibility to disease.

Lipoxin A_4 (LXA_4): a gastroprotective lipid

Concomitant administration of aspirin and selective COX-2 inhibitors results in the production of significantly more gastric damage than with either drug alone. This synergistic interaction has been observed in rodent (Fiorucci *et al.*, 2002; Souza *et al.*, 2003; Wallace *et al.*, 2004) and human studies (Fiorucci *et al.*, 2003b; Laine *et al.*, 2004). An initial interpretation of these findings was that combined inhibition of COX-1 and COX-2 would produce more gastric damage because PGs from both isoforms of COX contribute to mucosal defense (Wallace *et al.*, 2000). However, it now seems that products of arachidonic acid metabolism other than PGs may hold the key. In the presence of aspirin, COX-2 is acetylated and the enzyme is no longer able to catalyze the conversion of arachidonic acid to PGs. However, acetylated COX-2 can still metabolize arachidonic acid to 15-*R*-hydroxyeicosatetraenoic acid (15-*R*-HETE), which can be converted *via* 5-lipoxygenase to 15-*epi*-LXA_4 (or 'aspirin-triggered lipoxin' (ATL)) (Serhan, 1994). Like its epimer, ATL is a potent inhibitor of various neutrophil functions and exhibits a wide range of anti-inflammatory effects, as well as effects that stimulate resolution of inflammation (Serhan, 1994; Devchand *et al.*, 2003).

The aspirin-dependent switch in COX-2 activity, leading to elevated levels of ATL, does occur in the stomach (Fiorucci *et al.*, 2002). Co-administration of a selective COX-2 inhibitor with aspirin blocks the elevated formation of ATL, confirming that COX-2 activity is required for its synthesis. This is associated with a significant increase in the severity of gastric damage (Fiorucci *et al.*, 2002; Wallace *et al.*, 2004). LXA_4 has potent protective effects in the stomach, being capable of significantly reducing the extent of aspirin-induced gastric damage in the rat when administered parenterally in the low nanomolar range (Fiorucci *et al.*, 2002). Moreover, blockade of ALX, the G-protein-coupled LXA_4 receptor, with peptide antagonists was found to significantly exacerbate aspirin-induced gastric damage, in a manner similar to the effects of selective COX-2 inhibition (Fiorucci *et al.*, 2002).

COX-2-derived ATL may also mediate, at least in part, the well-recognized ability of the stomach to adapt to repeated

exposure to aspirin. In humans and animals, many studies have demonstrated that the gastric mucosa also becomes progressively more resistant to injury during chronic ingestion of aspirin (Wallace, 1997). In parallel with this increased resistance to damage, there is marked upregulation of COX-2 expression in the gastric mucosa, and increased generation of ATL (Fiorucci *et al.*, 2003a). Inhibition of ATL generation, with a COX-2 inhibitor, reversed this adaptive response, returning the susceptibility of the stomach to injury to its basal level (Fiorucci *et al.*, 2003a). The studies above identify the aspirin-triggered LXA$_4$ pathway as a protective mechanism involved in gastric resistance to damage caused by synthetic COX modulators like aspirin.

The studies described above have focussed on understanding the role of aspirin-triggered LXA$_4$ derived from the acetylated-COX-2 pathway as a lipid mediator of gastroprotection. Both 15-*R*-LXA$_4$ and 15-*S*-LXA$_4$ can trigger downstream signalling effects to the nucleus *via* circuits that have significant overlap (Qiu *et al.*, 2001). It is important to remember that these two isomers can also be derived *via* mechanisms independent of aspirin and COX-2 (Claria *et al.*, 1996).

COX-2 in the inflamed GI tract

Inflammation is a key element of mucosal defense. It is aimed at limiting entry of foreign material and microbes to the systemic circulation, as well as facilitating the repair of damaged tissue. Dysregulated inflammation can itself cause significant damage to host tissue. For instance, a dysregulated inflammatory response is thought to contribute to ulcer formation associated with use of NSAIDs, infection with *Helicobacter pylori* and in IBD. Resolution of inflammation is therefore a crucial process for restoring homeostasis, and one in which COX-2 plays a key role.

The importance of COX-2-derived PGs in resolving peripheral inflammation was evident from studies we performed using COX-2-deficient mice (Figure 2a). When carrageenan was injected into the hindpaw of normal mice, significant inflammation was induced, including significant edema formation, and it had resolved within 24–48 h. When carrageenan was injected into the hindpaw of COX-2-deficient mice, a similar acute inflammatory response was observed. However, even 7 days later the inflammatory response, including granulocyte infiltration and edema, was still evident (Wallace *et al.*, 1998). Thus, in the absence of COX-2, the acute inflammatory response to carrageenan was dysregulated. This important role of COX-2 in resolution of inflammation has been observed in a range of inflammatory models. For example, Gilroy *et al.* (1999) demonstrated a key role for COX-2-derived PGs, in particular PGD$_2$ and its metabolites, in the resolution of inflammation in a pleurisy model in rats. These metabolites appeared to produce the resolution *via* induction of apoptosis of infiltrating neutrophils and macrophages (Gilroy *et al.*, 2003).

An anti-inflammatory role of COX-2 has been observed in studies of the colon. There is a rapid and substantial upregulation of COX-2 after induction of colitis in rats. This is accompanied by a sharp increase in colonic generation of PGD$_2$, which has a dampening effect on granulocyte infiltration (Ajuebor *et al.*, 2000). Consistent with these observations, treatment with selective COX-2 inhibitors during the early
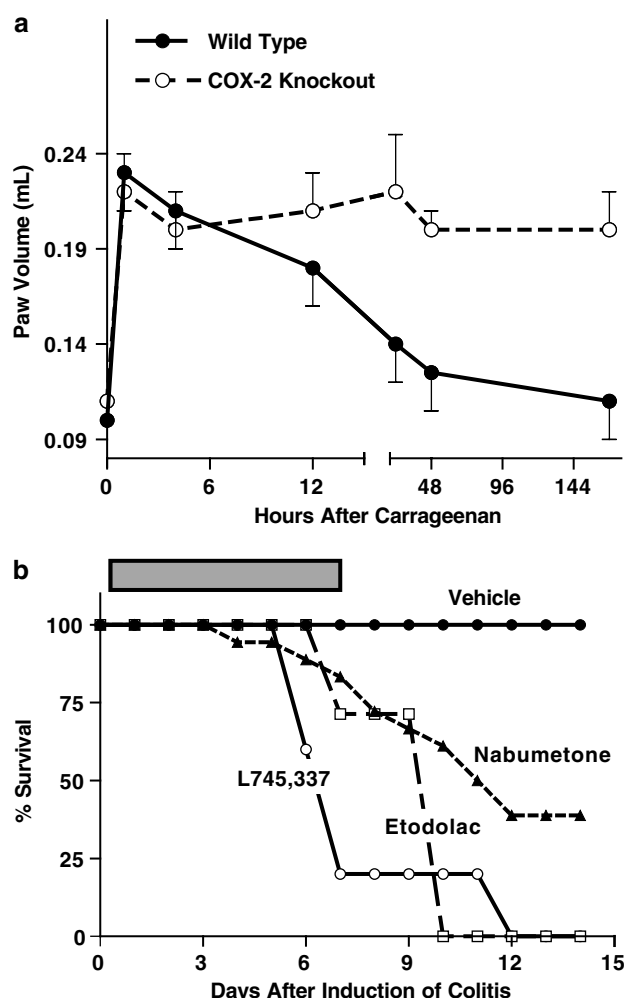
Figure 2 COX-2 is crucial in resolution of inflammation. Panel a shows the change in paw volume in wild-type and COX-2-deficient mice of a 1-week period after subplantar injection of carrageenan. Note that the edema resolves in the wild-type mice, but not in the COX-2-deficient mice (Wallace *et al.*, 1998). Panel b shows survival of rats after induction of colitis with trinitrobenzene sulfonic acid. The rats were treated with vehicle or a COX-inhibitor twice daily for 1 week after induction of colitis (Reuter *et al.*, 1996). Equieffective anti-inflammatory doses of the COX inhibitors were used. The rank order of selectivity for COX-2 is L745,337 > etodolac > nabumetone.

phase of experimental colitis leads to enhancement of granulocyte infiltration and, if continued for several days, to further penetration of ulcers deeper into the wall of the bowel and, eventually, to perforation and death (Figure 2b) (Reuter *et al.*, 1996). Mice deficient in COX-2 (or in COX-1) were similarly shown to be more susceptible to chemically induced colitis (Morteau *et al.*, 2000). There are also clinical reports of NSAIDs exacerbating colitis (Bonner, 2001; Matuk *et al.*, 2004), in some cases with such events described as occurring with 'high incidence' (Matuk *et al.*, 2004).

At least one-half of the world's population has ongoing gastric inflammation, attributable to colonization of the stomach by *H. pylori*. This infection is associated with significantly elevated expression of COX-2 in the stomach (Tatsuguchi *et al.*, 2000). Moreover, while acute administration of selective inhibitors of COX-2 to *H. pylori*-negative individuals does not result in mucosal injury, the same

treatment of individuals with *H. pylori*-associated gastritis resulted in significant damage (Takahashi *et al.*, 2000). Thus, there is an increased contribution of COX-2 to mucosal defense in a setting of *H. pylori*-associated inflammation. This has also been demonstrated in a rat model of gastric inflammation (Souza *et al.*, 2003). Interestingly, the inflamed stomach was found to have a greater capacity to produce LXA_4 in response to aspirin administration, and an increased resistance to aspirin-induced damage (Souza *et al.*, 2003).

COX-2 and ulcer healing

Damage to the GI epithelium likely occurs on a daily basis, but true 'ulcers' (which penetrate deeper than the mucosal layer) only rarely develop. Superficial damage to the mucosa can be healed in a few hours or days. When damage penetrates into the submucosa and muscularis, repair can take several weeks or months, and involves formation of granulation tissue at the ulcer base, formation of new blood vessels (angiogenesis) and and re-establishment of the glandular architecture. As outlined above, COX-2 expression in the normal stomach is low. However, at the margins of ulcers, COX-2 expression is very strong (Mizuno *et al.*, 1997). It is at the margins of ulcers that epithelial proliferation primarily occurs, which is critical for re-establishment of glands. COX-2 is also strongly expressed in endothelial cells in the ulcer bed (Mizuno *et al.*, 1997), which is the site of new vessel growth. Perhaps not surprisingly, selective COX-2 inhibitors, like conventional NSAIDs that also suppress COX-2 activity, significantly delay gastric ulcer healing (Mizuno *et al.*, 1997; Schmassmann *et al.*, 1998; Halter *et al.*, 2001; Ma *et al.*, 2002; Perini *et al.*, 2003). Both selective and nonselective NSAIDs inhibit angiogenesis through direct effects on endothelial cells. Jones *et al.* (1999) demonstrated that this action involved inhibition of mitogen-activated protein kinase (ERK2) activity and interference with ERK nuclear translocation. This process occurred independently of protein kinase C and was partially PG-dependent and partially PG-independent.

The inhibition of ulcer healing associated with inhibition of COX-2 activity may be in part related to effects on serum levels of growth factors that regulate angiogenesis. Growth factors released from platelets and contained within serum can profoundly affect ulcer healing (Ma *et al.*, 2001). When gastric ulcers are induced in rats, a shift in the serum and platelet levels of growth factors occurs such that the balance between pro- and antiangiogenic factors is tilted in favour of promotion of angiogenesis, thereby assisting ulcer healing (Ma *et al.*, 2001). When rats with pre-established gastric ulcers were treated with a selective COX-2 inhibitor (celecoxib) or a conventional NSAID (flurbiprofen), the balance of pro- and antiangiogenic factors in serum was altered in the opposite direction, favoring inhibition of angiogenesis (Ma *et al.*, 2002). Moreover, both celecoxib and flurbiprofen significantly inhibited ulcer repair in this model.

Genetic considerations

The human *COX-2* gene is located on Chromosome 1 at q31.1 and is organized in 10 exons and introns (Figure 3; http://www.ensembl.org/Homo_sapiens/). Mutation databases re-
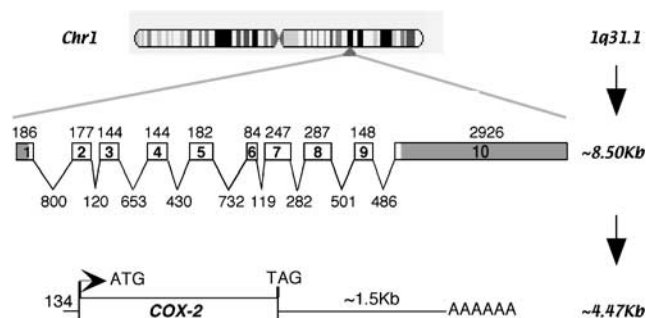

Figure 3 *COX-2* gene. Human COX-2 is located on Chromosome 1 at q31.1. The transcript ~8.5 kb consists of 10 introns and exons. Splicing results in a ~4.47 kb mRNA, including a long 3'-untranslated region. This hCOX-2 message codes for a 604 amino-acid protein.

port at least 35 single-nucleotide polymorphisms (SNPs) in both coding and noncoding (5'-upstream, introns and 3'-untranslated) regions (http://www.ncbi.nlm.nih.gov/projects/SNP/). When translated, the 16 SNPs in the coding region result in both synonymous and nonsynonymous changes but, to date, no phenotypes, GI-related or otherwise, have been associated with these hCOX-2 polymorphisms. Based on genetic ablation experiments in mice, one could speculate that, in the adult, the lack of phenotypic mutations in COX-2 is because a fully functional COX-2 is required for fetal implantation and embryonic development. However, rigorous analyses of larger population data sets might still reveal SNP-associated phenotypes.

Processing of the ~8.5 kb hCOX-2 transcript results in a spliced message of ~4.47 kb (Figure 3a; Appleby *et al.*, 1994). Particularly noteworthy are the short 5'- and long 3'-untranslated regions (O'Banion *et al.*, 1992). Control of COX-2 expression is in part mediated *via* sequences in the ~1.5 kb 3'-untranslated region. For example, studies using gamma irradiation on HT-29 colon cancer epithelial cells have deduced that control of COX-2 translation can be mediated by two AU-rich sequences in the first 60 nucleotides of the 3'-untranslated region. The molecular mechanism involves interaction of the AU-rich sequences with a RNA-binding protein previously identified for its editing activity at CUG sequences (CUGBP), to increase mRNA stability and inhibit translation of COX-2 (Mukhopadhyay *et al.*, 2003). This dynamic, antagonistic relationship between COX-2 and CUGBP2 is relevant in intestinal epithelial crypt cells, as demonstrated by recent studies examining endotoxin-induced protection against radiation damage using mouse loss-of-function models for COX-1 and COX-2 (Murmu *et al.*, 2004).

Human COX-2 is a 604 amino-acid heme protein that localizes to the nuclear membrane. Better understanding of the function of COX-2, including its role in GI mucosal defense, may be gained by studies of lower vertebrates. COX-2 is highly conserved in vertebrates, with recent reports of a zebrafish COX-2 with 67% identity to hCOX-2, as compared to 84% identity between COX-2 of rodents and hCOX-2 (Grosser *et al.*, 2002). Comparison of COX-2 primary sequences from human (Hla & Neilson, 1992), rat (Kennedy *et al.*, 1993), mouse (Dewitt *et al.*, 1990) and zebrafish (Grosser *et al.*, 2002) show that the greatest variability lies in the N- and C-terminals of the protein, while the highest conservation is in the epidermal growth factor and catalytic domains. Specific amino acids

associated with post-translational modification (glycosylation sites at $Asn^{53, 130, 396 \text{ and } 580}$), heme coordination ($His^{193 \text{ and } 374}$) and catalytic residues (Arg^{106}, Tyr^{341} and Tyr^{371}) are conserved from human to zebrafish. Notably, the aspirin-acetylation site (Ser^{516}) is also conserved (Lecomte *et al.*, 1994). Moreover, several fish species have been characterized for their ability to generate and respond to LXs (Rowley, 1991). In particular, LXs have been shown to modulate migration of fish leukocytes, as they do in mammals. Thus, in the future, comparative studies in fish might be a useful approach to better understanding the role of COX-2 in human health and disease.

Conclusions

It is somewhat ironic that the development of selective COX-2 inhibitors, whose *raison d'être* was to reduce inflammation but spare the GI tract of injury, resulted in a series of discoveries that strongly suggest a crucial role of COX-2 in GI mucosal defense and repair. While expressed in low levels in the healthy GI tract, COX-2 nonetheless contributes significantly to mucosal immunity and to the ability of the mucosa to resist injury induced by luminal irritants. The *COX-2* gene rapidly responds to stress, and the downstream products of this enzyme are potent lipid mediators that enhance resistance to injury and regulate dynamics of both inflammation and resolution. Emerging evidence implicates COX-2 in mediating some of the long-term consequences of inflammation in the GI tract, including the generation of symptoms in conditions such as irritable bowel syndrome, and the predisposition to cancer in individuals with colitis.

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