Related article on page 1064

American Journal of Pathology, Vol. 168, No. 4, April 2006

DOI: 10.2353/ajpath.2006.060023

Commentary

Commonality of Defensive Roles of COX-2 in the Lung and Gut

John L. Wallace

From the Mucosal Inflammation Research Group, University of Calgary, Calgary, Alberta, Canada

The discovery of a second isoform of cyclooxygenase (COX), COX-2, led to an enormous burst of activity in the pharmaceutical industry aimed at developing selective inhibitors of this isozyme.[1] This was based largely on the notion that COX-2 was expressed solely at sites of inflammation, whereas COX-1 was regarded as the isozyme responsible for "housekeeping" functions in most tissues.[2] Selective inhibition of COX-2 would, therefore, produce the beneficial effects of nonsteroidal anti-inflammatory drugs (NSAIDs) without the detrimental effects. As Oscar Wilde said, "The pure and simple truth is rarely pure and never simple." In the decade since the arrival of selective COX-2 inhibitors in research laboratories and in the marketplace, it has become clear that several tenets of the "COX-2 hypothesis" are, at best, over-simplifications and, at worst, invalid.[3] In this issue of *The American Journal of Pathology*, Bonnans et al[4] describe a series of elegant experiments that provide compelling evidence for an essential role of COX-2 in airway epithelial responses to acid-induced injury. The similarity of COX-2-dependent airway responses to those in the stomach are striking, possibly reflecting a programmed defensive response existing in all mucosal (and possibly other) tissues. These studies also underscore the therapeutic potential of compounds that mimic the actions of endogenous mediators of resolution of inflammation.

Selective COX-2 Inhibitors

Selective COX-2 inhibitors were developed as replacements of conventional NSAIDs: comparable in efficacy but without the gastrointestinal toxicity. However, subsequent to their entry to the marketplace, large clinical trials have revealed that some selective COX-2 inhibitors (eg, celecoxib) exhibited no gastrointestinal safety advantage over conventional NSAIDs.[5,6] Others (eg, rofecoxib) were found to exhibit as much as a 50% reduction in serious gastrointestinal adverse events,[7] but possibly much less.[6] Toxicity in other organs, such as the kidney and cardiovascular system, appears to be at least as common with selective COX-2 inhibitors as with conventional NSAIDs.[8]

The gastric mucosa is exposed regularly to acid of pH less than 1. Although hydrochloric acid at these concentrations can cause considerable damage when applied to skin, ulceration in the stomach is a rare event. The ability of the stomach to resist damage by acid and by digestive enzymes is attributed to a number of adaptations and responses collectively referred to as "mucosal defense."[9] This includes such elements as bicarbonate and mucus secretion by the gastric epithelium and a neurally mediated hyperemic response to diffusion of acid into the mucosa. It is also clear that the apical membrane of the gastric epithelium is adapted to resist damage induced by acid.[10] Nevertheless, circumstances arise in which damage does occur to the epithelium, and in those circumstances, there is a rapid response to limit tissue injury and to facilitate repair. These mechanisms appear to be quite similar in the stomach and lung. For decades, it has been recognized that prostaglandins (PGs) make an important contribution to these responses, and this is part of the reason that NSAIDs cause ulcers.[11] When COX-2 was first cloned, it was soon-after reported that the predominant isoform of COX expressed in the stomach was COX-1.[12] The assumption was made that it was inhibition of COX-1 that accounted for the detrimental effects of NSAIDs on the gastric mucosa. However, there is considerable evidence that gastric COX-2 is also an important target in terms of the induction of ulcers by NSAIDs, and this also explains why selective COX-2 inhibitors did not achieve the level of gastric safety that their proponents envisaged.

Accepted for publication January 11, 2006.

This commentary relates to Bonnans et al, Am J Pathol 2006, 24: 1064–1072, published in this issue.

Dr. Wallace is an Alberta Heritage Foundation for Medical Research Senior Scientist and holds a Canada Research Chair in Inflammation Research.

Address reprint requests to Dr. John L. Wallace, Department of Pharmacology and Therapeutics, University of Calgary, 3330 Hospital Dr. NW, Calgary, Alberta, T2N 4N1 Canada. E-mail: wallacej@ucalgary.ca.

Defensive Role of COX-2 in the Stomach

Although expressed in low levels in the healthy stomach of laboratory animals and humans, COX-2 contributes significantly to gastric mucosal defense. Selective inhibition of COX-1 results in a marked reduction in gastric PG synthesis in rats but, surprisingly, does not produce gastric damage.[13] However, when a selective COX-2 inhibitor is co-administered with a selective COX-1 inhibitor, rats develop extensive gastric erosions, comparable in severity to what was induced with a conventional NSAID.[13] These observations are consistent with the finding that COX-1-deficient mice do not exhibit spontaneous gastric damage, despite negligible gastric PG synthesis, but do develop erosions when given an NSAID.[14] There are other situations in which COX-2 contributes to mucosal resistance to damage. For example, the gastric damage associated with ischemia reperfusion in the rat stomach was found to be markedly exacerbated by pretreatment with selective COX-2 inhibitors.[15] These observations were somewhat paradoxical, given the fact that COX-2 expression in the stomach was not detectable. However, although the expression of COX-2 in the healthy stomach is low, it can be very rapidly up-regulated. For example, COX-2 expression in the rat stomach is markedly increased within 1 hour of oral administration of aspirin or indomethacin.[16] Others have reported the same in the stomach and intestine after administration of selective COX-1 inhibitors.[17] The induction of COX-2 could be prevented by administration of PGs, suggesting that this up-regulation was a compensatory response to diminished mucosal PG levels.[16,17] In the context of the study of Bonnans et al[4] in airway epithelium, it is interesting that intragastric instillation of hydrochloric acid has been found to cause marked up-regulation of COX-2 but not COX-1 in the stomach.[18]

Defensive Role of COX-2 in the Lung

Like the stomach, the epithelium of the airways forms a continuous barrier that restricts access of luminal substances, including microbes, to the systemic circulation. Unlike the stomach, however, the epithelium of the lung does not appear to be specially adapted to withstand exposure to high concentrations of acid. Aspiration of gastric acid can directly damage this layer of cells, leading to impaired barrier function and acute inflammation. This damage can progress to severe clinical illnesses such as acute respiratory distress syndrome. As in the stomach, repair of a damaged epithelium in the lung requires restitution of the epithelial layer and resolution of the acute inflammatory response. The studies of Bonnans et al[4] suggest that COX-2 plays a crucial role in these responses, and there is considerable commonality with the responses to acid-induced injury in the stomach.

COX-2 is constitutively expressed in the lung.[19] In this issue of the *AJP*, Bonnans et al[4] show that acid-induced epithelial injury resulted in rapid and marked up-regulation of COX-2 mRNA and protein (ie, within 2 hours). In parallel, there was a marked increase in PGE_2 synthesis that could be suppressed by a selective COX-2 inhibitor. Over the ensuing 72 hours, the tissue injury and acute inflammation were resolved, and the expression of COX-2 and synthesis of PGE_2 returned to basal levels. Interestingly, COX-2-derived PGE_2 synthesis appeared to coordinate several other aspects of the response to acid-induced epithelial injury, which involved another important anti-inflammatory mediator, lipoxin A_4 (LXA_4). LXA_4 exerts a number of effects that contribute to resolution of inflammation, including inhibition of neutrophil function, T-cell activation, and cytokine generation as well as stimulation of the clearance of apoptotic neutrophils by macrophages.[20] Many of these actions are mediated via the "ALX" receptor, which is expressed on leukocytes[21] and both airway[19] and intestinal[22] epithelial cells. In the study of Bonnans et al,[4] expression of ALX mRNA was increased in the bronchial epithelium within 2 hours of induction of injury with acid. This expression was driven by COX-2-derived PGE_2, because it could be prevented with a selective COX-2 inhibitor and could be mimicked in cultured airway epithelium by exposure to PGE_2. When COX-2 expression and PGE_2 synthesis normalized 72 hours after induction of epithelial damage, so too did ALX expression.

The anti-inflammatory effects of LXA_4 in the acid-induced airway epithelium model included suppression of cytokine generation. IL-6 is a key acute phase reactant that is produced by epithelial cells in various tissues in response to injury and inflammation. Acid-induced injury to cultured bronchial epithelial cells resulted in significant release of IL-6 that could be suppressed by LXA_4 and by PGE_2.[4] LXA_4 also markedly suppressed TNFα-induced IL-8 release from cultured bronchial epithelial cells. These inhibitory effects of LXA_4 on cytokine generation were dependent on the expression, by the epithelium, of the ALX receptor. Interestingly, inhibitory effects of LXA_4 (via ALX) on TNFα-induced IL-8 secretion have also been observed in human intestinal epithelial cells.[22]

Resolution of airway epithelial injury after exposure to acid involves down-regulation of the acute inflammatory response and repair of the damaged epithelium. Interestingly, LXA_4 exhibits the capacity to contribute to the latter as well as the former. LXA_4 concentration-dependently stimulated proliferation of cultured airway epithelial cells, exhibiting similar potency to two known bronchial epithelial proliferation agonists, PGE_2 and epidermal growth factor.

Summary and Future Directions

Although initially proposed as an enzyme expressed predominantly at sites of inflammation and producing prostaglandins that generated some of the key features of inflammation (pain and edema), COX-2 is increasingly recognized for its role in the resolution of inflammation. For example, COX-2-derived PGs down-regulate experimental pleuritis,[23] paw inflammation in COX-2-deficient mice fails to resolve as it does in normal mice,[24] and suppression of COX-2 synthesis results in exacerbation of inflammation of the colon.[25] Crucial roles for COX-2 in

the defensive responses of mucosal epithelia continue to be elucidated, as in the study of Bonnans et al.[4] Of particular interest was the finding that COX-2-derived PGE_2 drove the production of a potent anti-inflammatory substance, lipoxin A_4, and the expression of its cognate receptor, ALX. This finding has very important therapeutic implications for the future. Lipoxin A_4 mimetics or other ALX agonists may have considerable utility in conditions in which inflammation is dysregulated. Asthma and cystic fibrosis have been reported to be associated with impaired lipoxin A_4 biosynthesis.[26,27] In a mouse model of cystic fibrosis-like airway inflammation and infection, administration of a stable lipoxin A_4 mimetic resulted in markedly decreased neutrophil infiltration, bacterial burden, and disease activity.[27] Stable lipoxin analogs have also been shown to be effective in experimental models of colitis.[28–30]

Novel therapeutics that accelerate resolution of inflammation have considerable potential utility. They also offer the possibility of efficacy with a marked reduction of toxicity relative to more broadly acting anti-inflammatory drugs, such as NSAIDs. By acetylating COX-2, aspirin triggers the production of a lipoxin that contributes significantly to the anti-inflammatory activity of aspirin[31] but also increases the resistance of the gastric mucosa to aspirin-induced damage.[32] Finally, in addition to promoting the resolution of inflammation, lipoxin-based therapies can promote tissue repair, thereby facilitating resolution of tissue function.

References

1. Xie W, Chipman JG, Robertson DL, Erikson RL, Simmons DL: Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci USA 1991, 88:2692–2696
2. Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG, Isakson PC, Seibert K: Selective inhibition of inducible cyclooxygenase 2 in vivo is anti-inflammatory and non-ulcerogenic. Proc Natl Acad Sci USA 1994, 91:3228–3232
3. Wallace JL: Selective COX-2 inhibitors: is the water becoming muddy? Trends Pharmacol Sci 1999, 20:4–6
4. Bonnans C, Fukunaga K, Levy MA, Levy BD: Lipoxin A_4 regulates bronchial epithelial cell responses to acid injury. Am J Pathol 2006, 168:1064–1072
5. Jüni P, Rutjes JP, Dieppe PA: Are selective COX 2 inhibitors superior to traditional non-steroidal anti-inflammatory drugs? BMJ 2002, 324:1287–1288
6. Hippisley-Cox J, Coupland C, Logan R: Risk of adverse gastrointestinal outcomes in patients taking cyclo-oxygenase-2 inhibitors or conventional non-steroidal anti-inflammatory drugs: population based nested case-control analysis. BMJ 2005, 331:1310–1316
7. Bombardier C, Laine L, Reicin A, Shapiro D, Burgosvargas R, Davis B, Day R, Ferraz MB, Hawkey CJ, Hochberg M, Kvien TK, Schnitzer TJ: Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. N Engl J Med 2000, 343:1520–1528
8. Wang D, Wang M, Cheng Y, Fitzgerald GA: Cardiovascular hazard and non-steroidal anti-inflammatory drugs. Curr Opin Pharmacol 2005, 5:204–210
9. Wallace JL, Granger DN: The cellular and molecular basis of gastric mucosal defense. FASEB J 1996, 10:731–740
10. Sanders MJ, Ayalon A, Roll M, Soll AH: The apical surface of canine chief cell monolayers resists H+ back-diffusion. Nature 1985, 313:52–54
11. Wallace JL: Nonsteroidal anti-inflammatory drugs and gastroenteropathy: the second hundred years. Gastroenterology 1997, 112:1000–1016
12. Kargman S, Charleson S, Cartwright M, Frank J, Riendeau D, Mancini J, Evans J, O'Neill G: Characterization of prostaglandin G/H synthase 1 and 2 in rat, dog, monkey, and human gastrointestinal tracts. Gastroenterology 1996, 111:445–454
13. Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD: Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell 1995, 83:483–492
14. Wallace JL, McKnight W, Reurter BK, Vergnolle N: NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. Gastroenterology 2000, 119:706–714
15. Maricic N, Ehrlich K, Gretzer B, Schuligoi R, Respondek M, Peskar BM: Selective cyclooxygenase-2 inhibitors aggravate ischaemia-reperfusion injury in the rat stomach. Br J Pharmacol 1999, 128:1659–1666
16. Davies NM, Sharkey KA, Asfaha S, MacNaughton WK, Wallace JL: Aspirin causes rapid up-regulation of cyclo-oxygenase-2 expression in the stomach of rats. Aliment Pharmacol Ther 1997, 11:1101–1108
17. Tanaka A, Hase S, Miyazawa T, Takeuchi K: Upregulation of cyclooxygenase-2 by inhibition of cyclooxygenase-1: a key to nonsteroidal anti-inflammatory drug-induced intestinal damage. J Pharmacol Exp Ther 2002, 300:754–761
18. Gretzer B, Maricic N, Respondek M, Schuligoi R, Peskar BM: Effects of specific inhibition of cyclo-oxygenase-1 and cyclo-oxygenase-2 in the rat stomach with normal mucosa and after acid challenge. Br J Pharmacol 2001, 132:1565–1573
19. Fukunaga K, Kohli P, Bonnans C, Fredenburgh LE, Levy BD: Cyclooxygenase 2 plays a pivotal role in the resolution of acute lung injury. J Immunol 2005, 174:5033–5039
20. McMahon B, Godson C: Lipoxins: endogenous regulators of inflammation. Am J Physiol 2004, 286:F189–F201
21. Takano T, Fiore S, Maddox JF, Brady HR, Petasis NA, Serhan CN: Aspirin-triggered 15-epi-lipoxin A_4 (LXA_4) and LXA_4 stable analogues are potent inhibitors of acute inflammation: evidence for anti-inflammatory receptors. J Exp Med 1997, 185:1693–1704
22. Gronert K, Gewirtz A, Madara JL, Serhan CN: Identification of a human enterocyte lipoxin A_4 receptor that is regulated by interleukin (IL)-13 and interferon gamma and inhibits tumor necrosis factor alpha-induced IL-8 release. J Exp Med 1998, 187:1285–1294
23. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA: Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med 1999, 5:698–701
24. Wallace JL, Bak A, McKnight W, Asfaha S, Sharkey KA, MacNaughton WK: Cyclooxygenase 1 contributes to inflammatory responses in rats and mice: implications for gastrointestinal toxicity. Gastroenterology, 1998, 115:101–109
25. Reuter BK, Asfaha S, Buret A, Sharkey KA, Wallace JL: Exacerbation of inflammation-associated colonic injury in rat through inhibition of cyclooxygenase-2. J Clin Invest 1996, 98:2076–2085
26. Levy BD, Bonnans C, Silverman ES, Palmer LJ, Marigowda G, Israel E: Diminished lipoxin biosynthesis in severe asthma. Am J Respir Crit Care Med 2005, 172:824–830
27. Karp CL, Flick LM, Park KW, Softic S, Greer TM, Keledjian R, Yang R, Uddin J, Guggino WB, Atabani SF, Belkaid Y, Xu Y, Whitsett JA, Accurso FJ, Wills-Karp M, Petasis NA: Defective lipoxin-mediated anti-inflammatory activity in the cystic fibrosis airway. Nat Immunol 2004, 5:388–392
28. Gewirtz AT, Collier-Hyams LS, Young AN, Kucharzik T, Guilford WJ, Parkinson JF, Williams IR, Neish AS, Madara JL: Lipoxin A_4 analogs attenuate induction of intestinal epithelial proinflammatory gene expression and reduce the severity of dextran sodium sulfate-induced colitis. J Immunol 2002, 168:5260–5267
29. Fiorucci S, Wallace JL, Mencarelli A, Distrutti E, Rizzo G, Farneti S, Morelli A, Tseng JL, Suramanyam B, Guilford WJ, Parkinson JF: A beta-oxidation-resistant lipoxin A_4 analog treats hapten-induced colitis by attenuating inflammation and immune dysfunction. Proc Natl Acad Sci USA 2004, 101:15736–15741

30. Arita M, Yoshida M, Hong S, Tjonahen E, Glickman JN, Petasis NA, Blumberg RS, Serhan CN: Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. Proc Natl Acad Sci USA 2005, 102:7671–7676
31. Claria J, Serhan CN: Aspirin triggers previously undescribed bioactive eicosanoids by human endothelial cell-leukocyte interactions. Proc Natl Acad Sci USA 1995, 92:9475–9479
32. Fiorucci S, de Lima OM Jr, Mencarelli A, Palazzetti B, Distrutti E, McKnight W, Dicay M, Ma L, Romano M, Morelli A, Wallace JL: Cyclooxygenase-2-derived lipoxin A_4 increases gastric resistance to aspirin-induced damage. Gastroenterology 2002, 123:1598–1606