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Biochemical Pharmacology 65 (2003) 153–159

Biochemical Pharmacology

Commentary

Advances in the pathophysiology of constitutive and inducible cyclooxygenases: two enzymes in the spotlight

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Abstract

The aim of this commentary is to discuss recent data on the role of prostaglandins generated by both constitutive and inducible cyclooxygenases (COXs). According to a popular hypothesis, COX-1 generates 'good' prostaglandins for physiological 'housekeeping' functions like gastrointestinal (GI) mucosal integrity and regulation of renal blood flow, while COX-2 forms the 'bad' prostaglandins responsible for inflammatory symptoms. However, recent data show that the biological functions of prostanoids formed by the two enzymes are much more complex and interrelated than previously appreciated. Experimental evidence indicates that a full inflammatory response is likely sustained by prostanoids generated by both enzymes, and an effective anti-inflammatory effect requires the inhibition of the two enzymes. Similarly, the selective inhibition of either COX-1 or COX-2 does not elicit GI damage, but inhibition of both enzymes is necessary for GI mucosal damage to develop. Prostaglandins generated by both enzymes contribute to normal renal function by regulating the vascular tone and the normal blood flow. The synthesis of endothelial prostacyclin is mainly driven by COX-2, so that the selective COX-2 inhibition may bias vascular prostaglandin synthesis in favour of COX-1-derived thromboxane A_2 in platelets, leading to a prothrombotic outcome. Moreover, prostaglandins formed by COX-2 appear to have a major role in myocardial protection. We propose that the complexity of the situation in the field of COX-derived mediators should be borne in mind when anti-inflammatory therapy is required.

Keywords: Cyclooxygenase; Prostaglandins; Inflammation; NSAIDs

1. Introduction

The dissection of arachidonic acid metabolic pathways undoubtedly was one of the major scientific achievements of the last century that will cast its effects well into this century in many fields of biology. Arachidonic acid is a member of the ω-6 series of essential fatty acids contained in membrane phospholipids. Activation of the enzyme phospholipase A_2 releases arachidonic acid that can be further metabolised by at least two major enzymatic complexes (Fig. 1).

(i) PGH synthase. This enzyme, colloquially known as COX, has two related catalytic functions, a cyclooxygenase activity that catalyses the formation of PGG_2 and a peroxidase activity catalysing a two-electron reduction of PGG_2 to PGH_2. The unstable PGH_2 is then transformed into prostanoids like PGI_2, TXA_2, PGE_2, and $PGF_{2\alpha}$ by specific synthases in different cells.

(ii) 5-LOX. Through a 5-hydroperoxy intermediate this enzyme produces the leukotrienes, so-called for their occurrence in leukocytes and a characteristic conjugated triene structure. The first compound to be formed is LTA_4, an unstable metabolite containing an epoxide moiety. Derived from LTA_4 are LTB_4 by enzymatic hydrolysis and LTC_4 by addition of the tripeptide glutathione catalysed by glutathione-*S*-transferase. From LTC_4, LTD_4 and LTE_4 are produced. The three compounds form the group known as the cysteinyl-leukotrienes.

Prostaglandins mediate several key pathophysiological functions, from host inflammatory response to regulation of blood flow, in many body sites. In recent years, two different cyclooxygenases have been described [1,2].

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Abbreviations: COX, cyclooxygenase; PG, prostaglandin; TX, thromboxane; LT, leukotriene; NSAIDs, nonsteroidal anti-inflammatory drugs; 5-LOX, 5-lipoxygenase.

0006-2952/02/$ – see front matter
PII: S0006-2952(02)01422-3

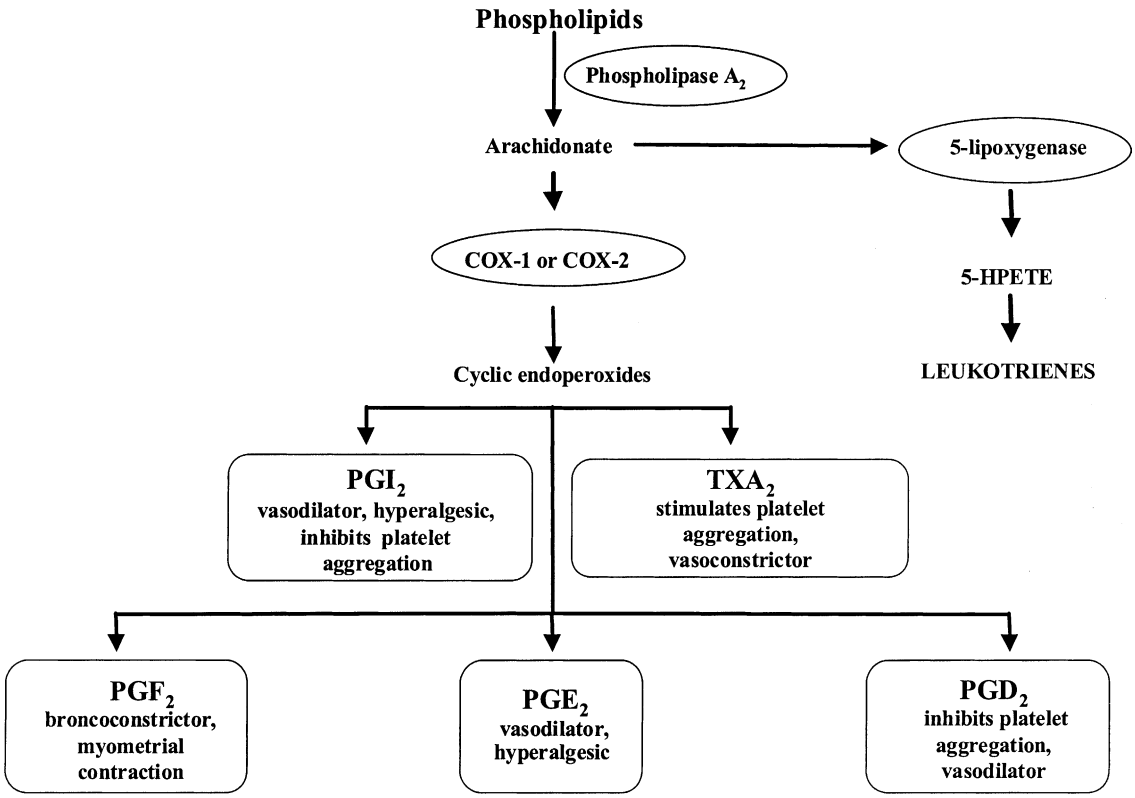

Fig. 1. Metabolism of arachidonic acid.

COX-1 can be detected in most tissues and is typically expressed at constant levels throughout the cell cycle. COX-2 is undetectable in most mammalian tissues, but its expression can be induced rapidly in cells involved in inflammation like fibroblasts, monocytes, and vascular endothelium in response to growth factors, tumour promoters, hormones, bacterial endotoxin, and cytokines. Therefore, COX-1 has become known as the constitutive isoform and COX-2 as the inducible one. This appears to be an oversimplification inasmuch as COX-1 expression can be inhibited in endothelial cells in response to acidic fibroblast growth factor and up-regulated in mast cells by stem cell factor plus dexamethasone, while COX-2 is constitutively expressed in several tissues (see below) [1]. However the predominant constitutive nature of COX-1, together with the observations that expression of COX-2 can be up-regulated by inflammatory stimuli and that prostanoids are produced by COX-2 in much larger amounts compared with COX-1, has led to the hypothesis of the existence of 'good' versus 'bad' prostaglandins. According to this hypothesis, COX-1 generates 'good' prostaglandins for physiological 'housekeeping' functions, including platelet-dependent homeostasis, gastric mucosal integrity, and regulation of renal blood flow, while COX-2 forms the 'bad' prostaglandins involved in inflammatory reactions and responsible for inflammatory signs like fever, pain, capillary edema, and vasodilatation. As a direct consequence, the specific inhibition of COX-2 is expected to cause significant anti-inflammatory relief without interfering with prostaglandin-mediated physiological processes, especially gastrointestinal and renal functions [3]. On these bases, the pharmaceutical industry has made a considerable effort to develop and market highly selective COX-2 inhibitors with little or no COX-1 inhibitory activity. Two of these new drugs, celecoxib and rofecoxib are already available in many countries [4,5], while others are in the pipeline [6,7].

As happens very often in science, things are never as simple as they appear. Recent evidence shows that both COX-1 and COX-2 are indeed involved in physiological as well as pathological processes (Fig. 2). The broad interest of the issue has prompted many excellent and comprehensive reviews [3,8,9], so that this commentary will focus on the effects of COX-1 and COX-2 on inflammation and related pathologies as well as on the role of the enzymes in the physiopathology of those cells and organs that are the target of the main toxic effects of anti-inflammatory drugs. This discussion may contribute to a better understanding of the mechanism of action and a more rational use of classical and newer NSAIDs.

2. Inflammation and hyperalgesia

There is compelling evidence that COX-2-derived prostaglandins play a major role in inflammatory reactions. A number of laboratories have shown that COX-2 is induced

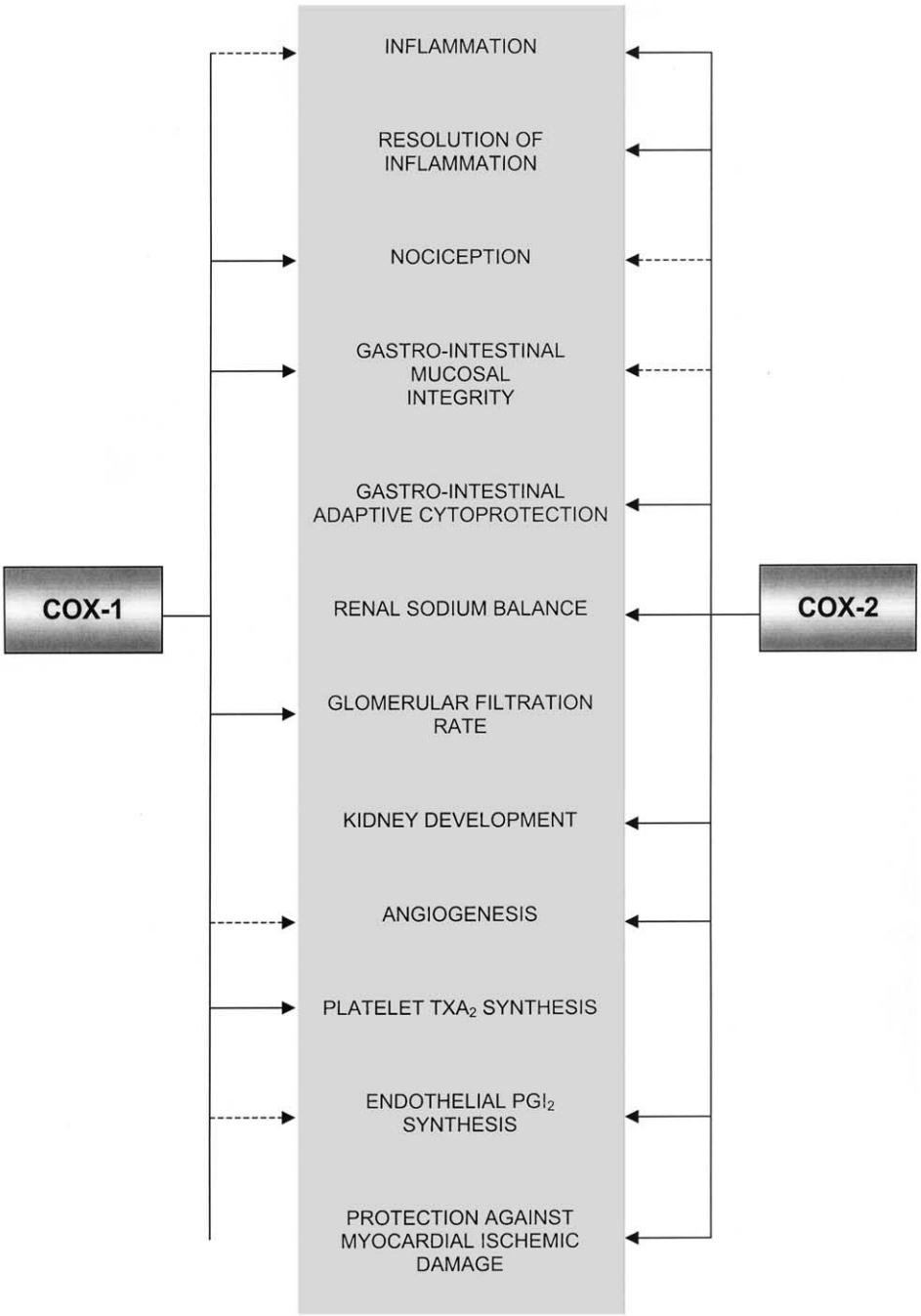

Fig. 2. Involvement of COX-1 and COX-2 in pathophysiological processes. Key: (→) indicates full importance in function; and (----▶) indicates partial importance in function. See text for details and references.

during various experimental inflammatory processes [10] and in human rheumatoid synovial tissues [11] and that prostaglandins produced by COX-2 are indeed responsible for inflammatory signs [12]. It has also been shown that the selective inhibition of the inducible enzyme leads to anti-inflammatory effects in a number of inflammatory models in the rat such as adjuvant arthritis [13], carrageenin-induced air pouch [14], and footpad edema [15]. In this last model, celecoxib was anti-inflammatory and analgesic, whereas a selective COX-1 inhibitor was ineffective.

On the other hand, a number of studies from John Wallace's group in Calgary indicate that COX-1 contributes to inflammatory responses in the same experimental models, challenging in this manner the concept that COX-2 *only* plays an exquisite 'negative' role. In carrageenin-air pouch inflammation, COX-2 blockage results in profound suppression of PGE_2 synthesis, but it does not affect leukocyte migration. Inhibition of exudate volume and leukocyte infiltration was observed only at drug doses affecting both COX-1 and COX-2 activities [16]. Also,

in carrageenin-induced edema, COX-2 blockade was less effective in reducing inflammation compared with the effect achieved with combined inhibition of both enzyme isoforms. Moreover, indomethacin, but not a selective COX-2 inhibitor, was able to suppress inflammation in COX-2-deficient mice [17]. The role of COX-1 in inflammation is confirmed by studies using mice in whom the *Ptgs1* gene encoding COX-1 was disrupted [18]. These animals showed a decreased ear inflammatory response to arachidonic acid. Interestingly, these mice were less sensitive to indomethacin-induced stomach damage. Parallel studies on COX-2-deficient mice [19] have shown that these animals have a normal inflammatory response, but develop a severe nephropathy (see below) and are susceptible to peritonitis [19]. Very recently, Ochi and Goto [20] have shown that a selective COX-1 inhibitor blocked the formation of prostanoids and the development of inflammation in rat collagen-induced arthritis, but not in rat adjuvant-induced arthritis, whereas indomethacin was effective in both arthritis models. These data underline the well-known but often neglected fact that the role of cyclooxygenases (and, needless to say, of any other enzyme or endogenous mediator) in inflammation should be studied in different experimental inflammatory models since the results from a single model may be misleading.

Other studies [21] have investigated the time-course of COX-2 expression in experimental inflammation. In carrageenin-induced pleurisy in rats, two peaks of COX-2 activity were observed, at 2 and 48 hr. The second peak of expression, which was 350% greater than that at 2 hr, was associated with minimal PGE_2 synthesis and coincided with the resolution of the inflammatory reaction. In contrast, at 48 hr a peak of synthesis of anti-inflammatory prostanoids like 15-deoxy-Δ^{12-14}-PGJ_2 was observed. Indomethacin and a selective COX-2 inhibitor reduced inflammation at 2 hr, but significantly exacerbated inflammation at 48 hr. The authors conclude that COX-2 may be pro-inflammatory during the early phases of the inflammatory process, but may contribute to its resolution at a later phase by generating anti-inflammatory prostanoids [21].

It has been known for some time that PGE_2 and PGI_2 are hyperalgesic, so that they do not cause pain directly, but rather potentiate the nociceptive response induced by bradykinin or histamine [22,23]. A more recent study has shown that in rat carrageenin-induced hyperalgesia the synthesis of PGE_2 was accompanied by an increased COX-2 immunoreactivity in the epidermis, skeletal muscle, and inflammatory cells. The same study also suggests that PGE_2 may be involved in a positive feedback loop in inducing more COX-2 expression, since this induction is blocked by indomethacin [12]. Other studies have shown that COX-1-deficient mice exhibited less nociception in two different tests of hyperalgesia: the hot plate and the acetic acid-induced writhing response. Interestingly, COX-2-deficient mice showed a compensatory up-regulation of COX-1 mRNA in the spinal cord, which explains the normal responses observed in these animals. The authors conclude that prostaglandins generated from COX-1 mediate nociception in both the central transmitted pain caused by thermal stimulation (the hot plate test) and in the peripheral pain (the writhing response) [24]. It has also been shown that the acetic acid writhing response may be blocked by a selective COX-1 inhibitor, but not by a selective COX-2 inhibitor [25].

In summary, prostaglandins generated by COX-2 do have pro-inflammatory properties; however, a full inflammatory response is likely sustained by prostanoids generated by both constitutive and inducible cyclooxygenases. Furthermore, COX-1-derived prostaglandins appear to have an important role in inflammatory hyperalgesia.

3. The gastrointestinal system

It has been shown that COX-1, but not COX-2, is constitutively expressed throughout the gastrointestinal tract in several species including humans [26]. Prostaglandins (especially PGI_2 and PGE_2) formed by COX-1 have important cytoprotective effects on the gastrointestinal mucosa. The cytoprotective action is complex and multifactorial. Both PGI_2 and PGE_2 reduce gastric acid secretion from stomach parietal cells, increase mucosal blood flow, and stimulate the release of viscous mucus [27]. It is generally believed that the gastrointestinal toxicity of classical NSAIDs is due to inhibition of COX-1 activity. Highly selective COX-2 inhibitors have been developed on the premise that the selective COX-2 inhibition should cause anti-inflammatory effects without affecting the physiology of the gastrointestinal mucosa. Recent clinical trials have shown that the use of two of these inhibitors, celecoxib and rofecoxib, is associated with effective anti-inflammatory relief in osteoarthritis and rheumatoid arthritis as well as with a significantly lower incidence of ulcer complications [28,29].

As often occurs in science, there is another side of the coin. A recent report has demonstrated the constitutive expression of COX-2 in healthy human and rabbit gastric mucosa [30]. The authors speculate that COX-2 may be an important enzyme generating vasodilatory and cytoprotective prostanoids in the gastric mucosa. Other studies in the rat have shown that selective inhibition of either COX-1 or COX-2 did not elicit gastric or intestinal damage; rather, inhibition of both isoforms of COX was required for NSAID-induced damage to develop both in the stomach [31] and in the small intestine [32]. It has also been suggested that prostaglandins formed by COX-2 may be involved in the so-called ‘adaptive cytoprotection’ response in gastrointestinal mucosa [33]. COX-2 is rapidly induced during gastrointestinal ulcerative processes where it generates large amounts of prostaglandins, which contribute to the healing process. The administration of selective COX-2 inhibitors results in a reduction in mucosal

prostaglandin synthesis and significant inhibition of gastric ulcer healing in both mice [34] and rats [35]. On a similar note, it has been shown that the selective inhibition of the induced COX-2 exacerbates both ischaemia–reperfusion injury in the rat stomach [36] and inflammation-associated injury in the rat colon [37]. These data are reinforced by a recent report on the role of cyclooxygenase in angiogenesis [38]. The process of angiogenesis is essential for the healing of both acute injury and chronic ulceration of the gastrointestinal mucosa. Jones *et al*. [38] have shown that indomethacin as well as selective COX-2 inhibitors blocked angiogenesis through direct effects on the endothelial cells by inhibiting mitogen-activated protein kinase activity. The authors conclude that these findings challenge the premise that selective COX-2 inhibitors will not interfere with gastrointestinal mucosal defense or ulcer/wound healing. Interestingly, it is likely that this anti-angiogenetic effect contributes to the anti-arthritic properties of this class of drugs [39].

In summary, although there is little doubt that COX-1-derived prostaglandins are important for the maintenance of gastrointestinal mucosal integrity, it appears that COX-2-derived prostanoids may have some role in mucosal cytoprotection too.

4. The kidney

Prostaglandins contribute to normal renal function by regulating vascular tone and normal blood flow [3]. Maintenance of normal kidney functions is dependent upon prostaglandins, mainly PGE_2, in animal models of diseases and in patients with congestive heart failure, liver cirrhosis, or renal insufficiency [8]. These prostaglandins are likely to be generated by both COX-1 and COX-2. In humans, COX-1 is constitutively expressed in the vasculature, the collecting ducts, and the thin loop of Henle [4]. Studies in rodents have shown that low but measurable concentrations of COX-2 mRNA are constitutively expressed in the macula densa, epithelial cells lining the cortical ascending limb of the loop of Henle, and medullary interstitial cells of the renal papillae. Moreover, under conditions of sodium restriction, the level of COX-2 in the macula densa is tripled, and the total number of COX-2 immunoreactive cells in the cortex rose nearly 7-fold [40]. More recently, COX-2 expression has been detected in the human macula densa [41]. COX-2 is also involved in normal renal development. COX-2 protein and mRNA peak during postnatal weeks 2 and 3, and then decline to adult levels by week 12 [42]. In light of the role of COX-2 in renal physiology and development, it is not surprising that COX-2-deficient mice develop a severe nephropathy [19,43]. Interestingly, a normal inflammatory response is observed in these $COX\text{-}2^{-/-}$ animals. The involvement of both enzyme isoforms in renal physiology is confirmed by data suggesting that NSAID-induced acute sodium retention in healthy elderly subjects is mediated by the inhibition of COX-2, whereas depression of the glomerular filtration rate is associated with inhibition of COX-1 [44].

The role of the two isoforms in renal function explains the nephrotoxic effects of traditional non-selective NSAIDs and possibly predicts renal toxicity associated with the clinical use of selective COX-2 inhibitors. Interestingly, recent clinical trials have shown that patients taking either rofecoxib or celecoxib had a slight increase in the incidence of edema, a condition often resulting from kidney dysfunction [28,29]. The use of rofecoxib has also been associated with the development of tubulointerstitial nephritis [45].

5. The cardiovascular system

A major clinical achievement from research on arachidonic acid metabolism and the mechanism of action of NSAIDs has been the prophylactic use of aspirin and other non-selective inhibitors against thromboembolic disease [46]. In the platelet, the only isoform detectable is COX-1, which leads to the formation of pro-aggregatory TXA_2. On the other hand, the synthesis of anti-aggregatory PGI_2 in endothelial cells is mainly driven by COX-2 [47]. Aspirin at low doses acetylates COX-1 in platelets, irreversibly blocking TXA_2 synthesis for their lifetime in the circulation. At the same doses, aspirin has little effect on the synthesis of PGI_2. The overall effect of low-dose aspirin use is a reduced risk of thrombosis. At variance with aspirin, selective COX-2 inhibitors have been shown to reduce PGI_2 synthesis markedly [44,47]. Since these compounds do not inhibit COX-1, they may bias vascular prostaglandin synthesis in favour of platelet TXA_2 production, a prothrombotic outcome. On a similar note, the combined inhibition of both COX isoforms, but not the selective COX-2 blockade, retarded atherogenesis in low-density lipoprotein receptor knockout mice [48].

The risk of thrombosis associated with the use of selective COX-2 inhibitors should be small, because of the endothelial release of other anti-thrombotic substances, like nitric oxide [4]. However, the incidence of thrombotic events may rise in patients who are already at increased risk because of other underlying conditions, such as autoimmune diseases. As a matter of fact, the use of celecoxib has been associated with arterial thrombosis in four patients with connective tissue diseases [49]. There are also indications that PGI_2 and PGE_2 formed by COX-2 may exert protective effects against oxidative damage of rat primary cardiomyocytes [50] and against ischemic preconditioning in conscious rabbits [51].

In summary, both COX-1- and COX-2-derived prostanoids appear to have profound regulatory roles in vascular homeostasis. In addition, prostaglandins formed by COX-2 substantially contribute to myocardial protection.

As pointed out by a recent commentary [52], ‘cardioprotection competes with gastroprotection’ in the clinical

usage of both non-selective and selective COX-2 inhibitors. The authors recommend the use of non-selective drugs, such as aspirin or naproxen together with gastroprotective agents, for patients at risk of a vascular event and the use of selective COX-2 inhibitors in patients with risk factors for gastrointestinal side-effects.

6. Conclusions

The importance of cyclooxygenase-derived mediators in the induction and maintenance of many human pathological conditions is beyond any scientific doubt, since validated not only by several experimental observations but also by the profound therapeutic efficacy of cyclooxygenase inhibitors, as appreciated over the decades (if not centuries!).

Top level research in the past 20 years has allowed the dissection of the prostaglandin synthetic pathways and highlighted the distinct role that two enzymes, COX-1 and COX-2, may play in inflammatory and cardiovascular diseases. It is clear that these new findings have allowed the development of more selective and safer drugs, the COX-2 inhibitors. Also, the impact of this new class of drugs in the therapeutic management of arthritis and other inflammatory conditions has been quite impressive.

However, more recent data briefly summarised in this commentary indicate that the biological functions are not a clear-cut division between COX-1 and COX-2, and that the biological roles of prostanoids formed by the two enzymes are much more complex than previously appreciated. It is therefore likely that the therapeutic use of selective COX-2 inhibitors, hence their impact on the clinical management of important human disease, may be limited to a few specific pathologies. In fact, it is evident that COX-2 inhibitors should be administered when the clinical scenario demonstrates the certain absence of any pre-existent damage within the gastrointestinal compartment as well as the absence of any risk of thrombosis. We propose that the complexity of the situation in the field of COX-derived mediators should be borne in mind when anti-inflammatory therapy is required. In fact, it is possible that alternative strategies may be pursued to achieve an effective anti-inflammatory therapy minimising undesired side-effects, for instance the concomitant blockade of 5-LOX and COX. It has been shown that the dual inhibition of 5-LOX and COX leads to reduced synthesis of both prostaglandins and leukotrienes, affecting vascular alterations and cellular infiltration that occur in inflammation. Thus, dual 5-LOX/COX inhibitors may prove to be effective in chronic inflammatory processes as well as in allergic inflammatory diseases such as bronchial asthma [53,54]. Moreover, there are indications that dual 5-LOX/COX inhibition does not cause gastrointestinal damage; rather, the inhibition of leukotriene synthesis may lead to protective effects on the gastrointestinal mucosa [55].

Acknowledgments

L.P. is supported by grants from the University of Salerno (60% 2001, 2002). M.P. is supported by a fellowship from the Arthritis Research Campaign, UK.

References

[1] Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem 1996;271:33157–60.
[2] Marnett LJ, Rowlinson SW, Goodwin DC, Kalgutkar AS, Lanzo CA. Arachidonate acid oxygenation by COX-1 and COX-2. Mechanisms of catalysis and inhibition. J Biol Chem 1999;274:22903–6.
[3] Katori M, Majima M. Cyclooxygenase-2: its rich diversity of roles and possible application of its selective inhibitors. Inflamm Res 2000;49:367–92.
[4] FitzGerald GA, Patrono C. The coxibs, selective inhibitors of cyclooxygenase-2. N Engl J Med 2001;345:433–42.
[5] Hawkey CJ. COX-2 inhibitors. Lancet 1999;353:307–14.
[6] Talley JJ, Brown DL, Carter JS, Graneto MJ, Koboldt CM, Masferrer JL, Perkins WE, Rogers RS, Shaffer AF, Zhang YY, Zweifel BS, Seibert K. 4-[5-Methyl-3-phenylisoxazol-4-yl]-benzenesulfonamide, valdecoxib: a potent and selective inhibitor of COX-2. J Med Chem 2000;43:775–7.
[7] Riendeau D, Percival MD, Brideau C, Charleson S, Dube D, Ethier D, Falgueyret J-P, Friesen RW, Gordon R, Greig G, Guay J, Mancini J, Ouellet M, Wong E, Xu L, Boyce S, Visco D, Girard Y, Prasit P, Zamboni R, Rodger IW, Gresser M, Ford-Hutchinson AW, Young RN, Chan C-C. Eterocoxib (MK-0663): preclinical profile and comparison with other agents that selectively inhibit cyclooxygenase-2. J Pharmacol Exp Ther 2001;296:558–66.
[8] Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol 1998;38:97–120.
[9] Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 2001;294:1871–5.
[10] Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, Lee L, Isakson P. Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proc Natl Acad Sci USA 1994;91:12013–7.
[11] Crofford LJ, Wilder RL, Ristimaki AP, Sano H, Remmers EF, Epps HR, Hla T. Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues. Effects of interleukin-1β, phorbol ester, and corticosteroids. J Clin Invest 1994;93:1095–101.
[12] Nantel F, Denis D, Gordon R, Northey A, Cirino M, Metters KM, Chan CC. Distribution and regulation of cyclooxygenase-2 in carrageenan-induced inflammation. Br J Pharmacol 1999;128:853–9.
[13] Anderson GD, Hauser SD, McGarity KL, Bremer ME, Isakson PC, Gregory SA. Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. J Clin Invest 1996;97:2672–9.
[14] 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 antiinflammatory and nonulcerogenic. Proc Natl Acad Sci USA 1994;91:3228–32.
[15] Smith CJ, Zhang Y, Koboldt CM, Muhammad J, Zweifel BS, Shaffer A, Talley JJ, Masferrer JL, Seibert K, Isakson PC. Pharmacological analysis of cyclooxygenase-1 in inflammation. Proc Natl Acad Sci USA 1998;95:13313–8.
[16] Wallace JL, Chapman K, McKnight W. Limited anti-inflammatory efficacy of cyclo-oxygenase-2 inhibition in carrageenan-airpouch inflammation. Br J Pharmacol 1999;126:1200–4.
[17] Wallace JL, Bak A, McNight W, Asfaha S, Sharkey KA, MacNaughton WK. Cyclooxygenase 1 contributes to inflammatory responses in rats and mice: implications for gastrointestinal toxicity. Gastroenterology 1998;115:101–9.

[18] Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD, Kim HS, Smithies O. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell 1995;83:483–92.
[19] Morham SG, Langenbach R, Loftin CD, Tiano HF, Vouloumanos N, Jennette JC, Mahler JF, Kluckman KD, Ledford A, Lee CA, Smithies O. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. Cell 1995;83:473–82.
[20] Ochi T, Goto T. Differential effect of FR122047, a selective cyclooxygenase-1 inhibitor, in rat chronic models of arthritis. Br J Pharmacol 2002;135:782–8.
[21] 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.
[22] Ferreira SH. Prostaglandins, aspirin-like drugs and analgesia. Nature 1972;240:200–3.
[23] Ferreira SH, Nakamura M, Abreu Castro MS. The hyperalgesic effects of prostacyclin and PGE_2. Prostaglandins 1978;16:31–7.
[24] Ballou LR, Botting RM, Goorha S, Zhang J, Vane JR. Nociception in cyclooxygenase isozyme-deficient mice. Proc Natl Acad Sci USA 2000;97:10272–6.
[25] Ochi T, Motoyama Y, Goto T. The analgesic effect profile of FR122047, a selective cyclooxygenase-1 inhibitor, in chemical nociceptive models. Eur J Pharmacol 2000;391:49–54.
[26] 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;111:445–54.
[27] Vane JR, Botting RM. Formation and actions of prostaglandins and inhibition of their synthesis. In: Vane JR, Botting RM, editors. Therapeutic roles of selective COX-2 inhibitors. London: William Harvey Press; 2001, p. 1–47.
[28] Bombardier C, Laine L, Reicin A, Shapiro D, Burgos-Vargas R, Davis B, Day R, Bosi Ferraz M, Hawkey CJ, Hochberg MC, Kvien TK, Schnitzer TJ. For the VIGOR Study Group. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. N Engl J Med 2000;343:1520–8.
[29] Silverstein FE, Faich G, Goldstein JL, Simon LS, Pincus T, Whelton A, Makuch R, Eisen G, Agrawal NM, Stenson WF, Burr AM, Zhao WW, Kent JD, Lefkowith JB, Verburg KM, Geis GS. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis. The CLASS study: a randomized controlled trial. JAMA 2000;284:1247–55.
[30] Zimmermann KC, Sarbia M, Schrör K, Weber A-A. Constitutive cyclooxygenase-2 expression in healthy human and rabbit gastric mucosa. Mol Pharmacol 1998;54:536–40.
[31] Wallace JL, McNight W, Reuter BK, Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. Gastroenterology 2000;119:706–14.
[32] Tanaka A, Hase S, Miyazawa T, Takeuchi K. Up-regulation of cyclooxygenase-2 by inhibition of cyclooxygenase-1: a key to nonsteroidal anti-inflammatory drug-induced intestinal damage. J Pharmacol Exp Ther 2002;300:754–61.
[33] Wallace JL. Selective COX-2 inhibitors: is the water becoming muddy? Trends Pharmacol Sci 1999;20:4–6.
[34] Mizuno H, Sakamoto C, Matsuda K, Wada K, Uchida T, Noguchi H, Akamatsu T, Kasuga M. Induction of cyclooxygenase 2 in gastric mucosal lesions and its inhibition by the specific antagonist delays healing in mice. Gastroenterology 1997;112:387–97.
[35] Schmassmann A, Peskar BM, Stettler C, Netzer P, Stroff T, Flogerzi B, Halter F. Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastro-intestinal ulcer models in rats. Br J Pharmacol 1998;123:795–804.
[36] Maricic N, Ehrlich K, Gretzer B, Schuligoi R, Respondek M, Peskar BM. Selective cyclo-oxygenase-2 inhibitors aggravate ischaemia–reperfusion injury in the rat stomach. Br J Pharmacol 1999;128:1659–66.
[37] Reuter BK, Ashafa S, Buret A, Sharkey KA, Wallace JL. Exacerbation of inflammation-associated colonic injury in rat through inhibition of cyclooxygenase-2. J Clin Invest 1996;98:2076–86.
[38] Jones MK, Wang H, Peskar BM, Levin E, Itani RM, Sarfeh IJ, Tarnawski AS. Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. Nat Med 1999;5:1418–23.
[39] Paleolog EM, Fava RA. Angiogenesis in rheumatoid arthritis: implications for future therapeutics. Springer Semin Immunopathol 1998;20:73–94.
[40] Harris RC, McKanna JA, Akai Y, Jacobson HR, Dubois RN, Breyer MD. Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. J Clin Invest 1994;94:2504–10.
[41] Nantel F, Meadows E, Denis D, Connolly B, Metters KM, Giaid A. Immunolocalization of cyclooxygenase-2 in the macula densa of human elderly. FEBS Lett 1999;457:475–7.
[42] Zhang M-Z, Wang J-L, Cheng H-F, Harris RC, McKanna JA. Cyclooxygenase-2 in rat nephron development. Am J Physiol 1997;273:F994–1002.
[43] Dinchuk JE, Car BD, Focht RJ, Johnston JJ, Jaffee BD, Covington MB, Contel NR, Eng VM, Collins RJ, Czerniak PM, Gorry SA, Trzaskos JM. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. Nature 1995;378:406–9.
[44] Catella-Lawson F, McAdam B, Morrison BW, Kapoor S, Kujubu D, Antes L, Lasseter KC, Quan H, Gertz BJ, FitzGerald GA. Effects of specific inhibition of cyclooxygenase-2 on sodium balance, hemodynamics and vasoactive eicosanoids. J Pharmacol Exp Ther 1999;289:735–41.
[45] Rocha JL, Fernandez-Alonso J. Acute tubulointerstitial nephritis associated with the selective COX-2 enzyme inhibitor, rofecoxib. Lancet 2001;357:1946–7.
[46] Patrono C. Aspirin as an anti-platelet drug. N Engl J Med 1994;330:1287–94.
[47] McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad Sci USA 1999;96:272–7.
[48] Praticò D, Tillmann C, Zhang Z-B, Li H, FitzGerald GA. Acceleration of atherogenesis by COX-1-dependent prostanoid formation in low density lipoprotein receptor knockout mice. Proc Natl Acad Sci USA 2001;98:3358–63.
[49] Crofford LJ, Oates JC, McCune WJ, Gupta S, Kaplan MJ, Catella-Lawson F, Morrow JD, McDonagh KT, Schmaier AH. Thrombosis in patients with connective tissue diseases treated with specific cyclooxygenase 2 inhibitors. A report of four cases. Arthritis Rheum 2000;43:1891–6.
[50] Adderley SR, Fitzgerald DJ. Oxidative damage of cardiomyocytes is limited by extracellular regulated kinases 1/2-mediated induction of cyclooxygenase-2. J Biol Chem 1999;274:5038–46.
[51] Shinmura K, Tang X-L, Wang Y, Xuan Y-T, Liu S-Q, Takano H, Bhatnagar A, Bolli R. Cyclooxygenase-2 mediates the cardioprotective effects of the late phase of ischemic preconditioning in conscious rabbits. Proc Natl Acad Sci USA 2000;97:10197–202.
[52] Boers M. NSAIDs and selective COX-2 inhibitors: competition between gastroprotection and cardioprotection. Lancet 2001;357:1222–3.
[53] Parente L. Pros and cons of selective inhibition of cyclooxygenase-2 versus dual lipoxygenase/cyclooxygenase inhibition: is two better than one? J Rheumatol 2001;28:2375–82.
[54] Fiorucci S, Meli R, Bucci M, Cirino G. Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy? Biochem Pharmacol 2001;62:1433–8.
[55] Wallace JL, Carter L, McNight W, Tries S, Laufer S. ML3000 reduces gastric prostaglandin synthesis without causing mucosal injury. Eur J Pharmacol 1994;271:525–31.