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REVIEW

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New insights into the role of COX 2 in inflammation

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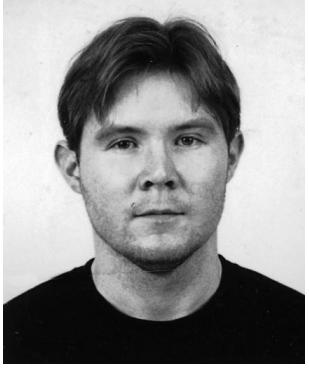

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Abstract Cyclo-oxygenase (COX) is responsible for the synthesis of bioactive prostanoids, the inhibition of which serves as the basis for the mode of action of clinically used nonsteroidal anti-inflammatory drugs. While there were suggestions as early as the 1970s that an inducible isoform of COX exists, it was only in the early 1990s that COX 2 was identified, cloned and sequenced. Not surprisingly, this new isoform was expressed at sites of inflammation and reported to contribute to the inflammatory response. Recently, however, evidence is emerging to suggest that COX 2 also has anti-inflammatory properties. In this review, the two faces of COX 2 are examined, with emphasis on its role in regulating inflammatory resolution, including possible mechanisms of action

Key words Cyclo-oxygenase · Nonsteroidal anti-inflammatory drugs · Inflammation · Peroxisome proliferator-activated receptor · Cyclopentenone prostaglandins · Stress proteins

Abbreviations *COX:* Cyclo-oxygenase · *HSP:* Heat shock protein · *NSAID:* Nonsteroidal anti-inflammatory drug · *PG:* Prostaglandin · *PPAR:* Peroxisome proliferator-activated receptor

Introduction

Two isoforms of cyclo-oxygenase (COX) have been identified and characterised [1]. COX 1 is constitutively expressed in most tissues, where it synthesises prostaglandins (PGs) at low levels to maintain physiological functions [1, 2]. COX 2, on the other hand, is highly inducible in response to proinflammatory stimuli, cytokines and mitogens resulting in exaggerated PG release [3, 4, 5]. While this was the belief upon the initial discovery of COX 2, it is now known that there are exceptions to this generalisation. Phorbol ester induced differentiation of the THP-1 human monocytic leukemia cell line is associated with COX 1 induction [6], while stem

cell factor and dexamethasone induce COX 1 expression in mouse bone marrow-derived mast cells [7]. Further, COX 2 is expressed constitutively throughout the forebrain in discrete populations of neurons and in the cortex and hippocampus [8], in human tracheal epithelial cells [9] and in the macula densa of the juxtaglomerular apparatus and adjacent epithelial cells of the cortical thick ascending limb [10]. The two COX isoforms are distributed equally on the lumenal surfaces of the endoplasmic reticulum and nuclear envelope of human monocytes, murine NIH 3T3 cells and human umbilical vein endothelial cells [11]. Within the nuclear envelope COX 1 and COX 2 are present in equal amounts on both the inner and outer nuclear membranes. The COX-1 gene lacks a TATA box which is typical of a "house-keeping" gene [12]. COX 2 contains a TATA box and several inducible enhancer elements including CEBP/NF-IL6, CRE and NFB [13]. Both COX isoforms have about 60% sequence identity at the protein level and catalyze the conversion of arachidonic acid to PGH_2 [14]. The kinetic constants of the two isoforms for this reaction are similar, and the PGH_2 produced is metabolised further to PGE_2, PGI_2, PGF_2, PGD_2, and thromboxane A_2 [15].

COX 2 specific NSAIDs

It is the inhibition of PG synthesis that accounts, at least in part, for the anti-inflammatory properties of non-steroidal anti-inflammatory drugs (NSAIDs) [16]. While NSAIDs are widely used for the treatment of inflammatory joint diseases, they are associated with renal and gastrointestinal toxicity [17]. It has been suggested that these side effects result from the inhibition of cytoprotective COX 1. Thus the discovery of COX 2 and the original belief that it is expressed only at sites of inflammation raised the possibility that the beneficial effects of NSAIDs maybe separated from their side effects by the selective inhibition of COX 2 [18]. This provided the impetus for the development of highly selective COX 2 inhibitors.

Consequent to the discovery of COX 2 were studies that classified existing NSAIDs according to their ability to inhibit either COX 1 or COX 2. Depending on the in vitro assay system used for screening, NSAIDs were shown to be either COX 1 or dual COX isoform inhibitors [19, 20, 21, 22, 23, 24, 25]. This property of dual inhibition certainly seemed to explain the unwanted side effects of NSAIDs. The emergence of COX 2 inhibitors with up to 1000-fold selectivity for the inhibition of the inducible isoform over COX 1 in various in vitro assays systems [20], importantly, showed little or no renal or gastric toxicity [26]. Thus research into the development of selective COX 2 inhibitors has resulted in the availability of various structurally diverse pharmacological tools crucial for experimental research such as NS-398 [27], DUP 697 [28] and L-745,337 [29]. Moreover, the concerted effort in COX 2 research has resulted in the recent licensing of the first two highly selective clinically used COX 2 inhibitors, Celebrex [30] and Vioxx [31].

A proinflammatory role for COX 2

With the availability of effective inhibitors of COX 2, various studies reported a proinflammatory role for this inducible enzyme, suggesting that its inhibition would be a major target for the future treatment of inflammatory arthropathies. One of the first series of studies to implicate a role for COX 2 in inflammation described the spatial and temporal distribution of COX 2 in both acute [32] and chronic inflammatory models [33, 34]. Injecting croton oil, in Freund's complete adjuvant, into an established subcutaneous air pouch on the dorsum of mice results in the formation of granulomatous tissue which lines the air pouch cavity. This is a highly vascularised tissue that bears histological similarities to the destructive pannus tissue typical of a chronic rheumatoid arthritic joint [34]. In this model, COX activity increased progressively as the granulomatous tissue developed. Of the COX metabolites measured, PGE_2 was most abundant. Immunohistochemistry demonstrated that fibroblasts and macrophage-like cells were labeled positively for COX 2. In addition, capillaries in the granuloma and endothelial cells of venules in the loose connective tissue of the dermis also showed immunoreactivity for the inducible isoform [33].

The distribution of COX 2 and COX activity was also described in an acute carrageenin-induced pleurisy [32]. In this model, COX 2 protein is present throughout this lesion with an initial peak in expression and PGE_2 synthesis 2 h after carrageenin injection. This peak was concomitant with polymorphonuclear leucocyte influx. Both polymorphonuclear leucocyte and phagocytosing mononuclear cells labelled positively for COX 2. In contrast, COX 1 protein expression was unaltered. Collectively, these studies suggested that COX 2 is the predominant isoform expressed in both acute and chronic inflammation.

While COX 2 expression in inflammatory tissue may implicate a causative role for this isoform in inflammation, it was necessary to examine the effects of various selective COX 2 inhibitors on established inflammatory parameters. Administration of carrageenin into a preformed air pouch induces a rapid inflammatory response in the rat. This is characterised by exudate formation and PG synthesis. In this model, COX 2 mRNA and protein expression mirrored the development of inflammation and PG synthesis. Immunohistochemistry showed that macrophages and fibroblasts present in the pouch lining were the cells responsible for COX 2 expression [35]. It was demonstrated that the nonselective COX isoform inhibitor, indomethacin, blocked PG production in both the pouch fluid and the stomach in addition to producing stomach lesions. In contrast, the COX 2 inhibitor NS-398, while completely suppressing PGs in the pouch, failed to inhibit stomach PGs or to cause gastric lesions

[35]. In a carrageenin-induced paw swelling model, SC-58125 inhibited the edema formation that was associated with the induction of COX 2 mRNA, protein and PG synthesis in the paw tissue [36]. The analgesic activity of this selective COX 2 inhibitor was also evaluated in this model. SC-58125 blocked the carrageenin-induced hyperalgesia with efficacy equal to that obtained by the dual inhibitor, indomethacin.

The role of COX 2 in adjuvant-induced arthritis was also assessed [37]. In this chronic inflammatory model, the induction of COX 2 mRNA and protein was correlated with the synthesis of PGs in the inflamed joint and edema formation. Administration of SC-58125 inhibited paw swelling and reduced PGE_2 in the paw tissue to control levels.

A role for COX 1 in inflammation

Thus evidence was emerging to strongly suggest a proinflammatory role for COX 2. Did this imply that COX 2 is solely responsible for propagating the inflammatory response and synthesising proinflammatory PGs? A closer analysis of some reports revealed that the COX 2 inhibitors used were administered at doses that were in excess of that required to inhibit COX 2. For instance, in the carrageenin air pouch study mentioned above [36], the concentration of the highly selective COX 2 inhibitor, SC-58125, required to inhibit exudate formation was a 100-fold greater than that necessary to inhibit exudate PG levels. This compound is 100-fold more potent as an inhibitor of COX 2 than COX 1 in vitro [38]. It is therefore possible that, at the doses used in this model, SC-58125 also inhibited COX 1.

We have reported the involvement of COX 1 in a carrageenin-induced pleurisy [39]. In this model, the selective COX 2 inhibitors NS-398 and nimesulide inhibited inflammation only within the first 2 h after carrageenin injection and were without effect by 6 h, despite the presence of COX 2 protein and COX activity at this time point. In contrast, the more selective COX 1 inhibitors, aspirin and piroxcam, had greater anti-inflammatory effects than the COX 2 inhibitors at both 2 and 6 h after carrageenin injection. Since COX 1 is also expressed in the inflammatory cells of this model, we suggested that COX 1 plays a more important role in driving the inflammation in this model than does COX 2.

Studies with COX deficient mice further questioned whether COX 2 is exclusively responsible for synthesising PG and driving the inflammatory response. For instance, in COX 1 deficient mice, edema induced by arachidonic acid was inhibited by 70% compared with wild-type mice [40], suggesting a role for COX 1 and not COX 2 in arachidonic acid induced ear inflammation. Additionally, triphorbol myristate acetate was equally inflammatory in both the COX deficient and wild-type mice [40]. In these studies ear swelling was monitored for 48 h after triphorbol myristate acetate treatment, and no difference between genotypes was observed at any time point. It has also been reported that COX 2 deficient and wild-type mice show similar triphorbol myristate acetate responses in the ear swelling [41] as well as the carrageenin-induced paw edema model [42]. Since COX 2 null mice still express COX 1, it is conceivable that this constitutive isoform is synthesising sufficient bioactive PGs for inflammation to proceed. These studies with arachidonic acid, triphorbol myristate acetate and carrageenin in the COX deficient mice indicate that both COX 1 and COX 2 can contribute PGs to the inflammatory response.

Wild-type and COX 2 deficient mice also responded similarly in their initial inflammatory response in the carrageenin-induced paw inflammation model [43]. However, indomethacin, but not the COX 2 selective inhibitor NS-398, significantly inhibited carrageenin-induced edema in both wild-type and COX 2 deficient mice. This observation contributed to the conclusions that significant inhibition of PG synthesis occurs only at NSAID doses that also inhibit COX 1, therefore suggesting that COX 1 contributes to the inflammatory response. Furthermore, the data implicated a role for COX 2 in the resolution phase of the inflammatory response, as lymphocyte infiltration and swelling persisted in the COX 2 deficient mice compared with wild-type mice.

A protective role for COX 2

Consistent with these findings are studies that have evaluated the effects of COX 1 and COX 2 inhibitors in an animal model of colitis. In this model, the majority of PGs synthesised by the colonic mucosa are from COX 2 [44]. Treatment with a selective COX 2 inhibitor (L-745,337) at doses that did not inhibit COX 1 caused significant inhibition of mucosal PG synthesis and a marked exacerbation of colonic damage. Continuous treatment for up to 1 week resulted in perforation of the bowel wall and 100% mortality in treated animals. It has also been shown that COX 2 mRNA and protein were strongly induced in the mouse stomach in which an ulcer had been induced concomitant with increased mucosal PG synthesis [45]. Treatment of the mice with the selective COX 2 inhibitor, NS-398, resulted in a reduction in mucosal PG synthesis and significant inhibition of ulcer healing. These results have recently been extended by others who also showed marked inhibition of gastric ulcer healing in rats by the COX 2 inhibitor L-745,337 [46] at doses that selectively inhibit this inducible isoform [29]. Therefore these studies question whether gastroprotective PGs are synthesised by COX 1 only and suggest that COX 2 plays a significant role in protecting the gastrointestinal tract from injury. Indeed, it has been shown recently that COX 2 plays an important role in the protection conferred on rat stomach following mild irritation [47]. In this experimental model, instillation of 20% ethanol protects against the subsequent instillation of 70% or 96% ethanol. Administration of the selective COX 2 inhibitors NS-398, DFU or

L-745,337 effectively blocked the protection conferred by 20% ethanol.

Recently COX 2 has been shown to modulate the intestinal immune response to dietary antigen [48]. Feeding hen egg-white lysozyme to mice along with COX 2 inhibitors results in increased proliferation of lamina propria mononuclear cells and crypt epithelial cells, crypt expansion and villus blunting. Lamina propria mononuclear cells produce high levels of COX 2 dependent arachidonic acid metabolites, which act as immunomodulators in the immune response to dietary antigen. These findings establish that COX 2 dependent arachidonic acid metabolites are essential in the development and maintenance of intestinal immune homeostasis.

Also, both COX isoforms protect against allergic lung inflammation [49]. After ovalbumin sensitisation and challenge, lung inflammation, airway hyperresponsiveness, and indices of lung injury were significantly greater in COX 1 deficient mice than in wild-type mice. Thus COX 1 products have mainly protective effects in the allergic mouse lung. Similarly, ovalbumin sensitised COX 2 deficient mice also showed greater lung inflammation after allergen exposure, suggesting that COX 2 derived eicosanoids are also protective.

An anti-inflammatory role for COX 2

These findings with NSAIDs and COX null mice indicate that both COX 1 and COX 2 contribute to PG production at the site of inflammation, and also that COX 2 derived PGs have roles in the resolution or healing phase as well as in the early stages of the inflammatory response. As part of our ongoing COX 2 research program, we have established that COX 2 is directly involved in regulating inflammatory resolution in a carrageenin-induced pleurisy [50]. In this study we showed that there were two phases of COX 2 expression, an early peak that was associated with the onset of inflammation, polymorphonuclear leucocyte influx, PGE_2 production and COX activity. As previously reported [39], both the dual and the COX 2 selective inhibitors inhibited this phase of the inflammatory response. However, 48 h after irritant injection we saw a second, much greater peak in COX 2 protein expression, associated with the resolution of the inflammation, late influx of mononuclear cells but without the presence of detectable PGE_2 in the exudate or PGE_2 production ex vivo (Fig. 1). Treatment with COX 2 selective inhibitors from 24 h to 48 h post-injection of irritant, i.e. during the resolving phase, resulted in a prolongation of the inflammatory response.

We suggested that a prostanoid product of COX 2 would be the most likely candidate to be responsible for the effects seen. Paradoxically, this implied that in a carrageenin-induced pleurisy, mononuclear cell COX 2 regulates inflammatory resolution. Although this concept may appear implausible, the following mechanism may make an anti-inflammatory role for mononuclear cell COX 2 more likely. In stimulated macrophages cyclopentenone PGs of the J-series (PGD_2 metabolites) bind to and activate the nuclear receptor peroxisome proliferator activated receptor (PPAR)-γ, resulting in various anti-inflammatory events [51, 52]. Thus, during the later phase of a carrageenin-induced pleurisy when the predominant cell type is the mononuclear cell, one possibility is that nuclear membrane associated COX 2 synthesises anti-inflammatory cyclopentenone PGs which bind to and activate PPARs resulting in the activation of mechanisms which leads to inflammatory resolution. To investigate this further we assayed the levels of PGD_2 and its metabolite $15deoxy\Delta^{12-14}PGJ_2$ in the exudates. We found that whilst the levels of these were initially raised, their levels fell during the onset and development of the inflammatory response but rose again during the phase of resolution (Fig. 2). Furthermore, upon treatment with the COX 2 inhibitors, their levels fell dramatically,

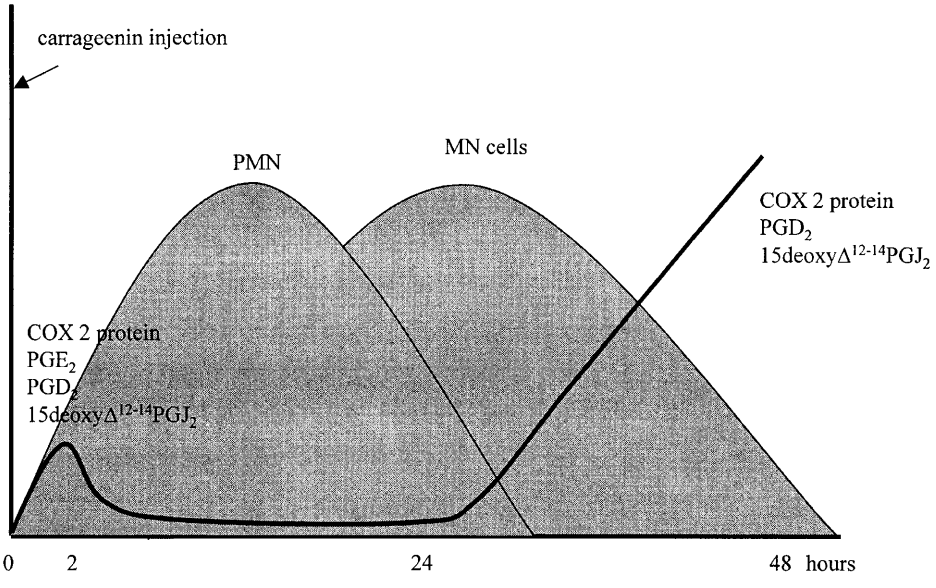

Fig. 1 The profile of polymorphonuclear neutrophil (*PMN*) and mononuclear (*MN*) cell influx into the carrageenin-induced pleurisy. Superimposed is the profile of COX 2 expression and eicosanoids synthesis. It is clear that PMNs do express COX 2 and are most probably responsible for the early expression of COX 2 and cypPG release. However, the wave of MN cells are most probably responsible for the second peak of COX 2 expression at resolution

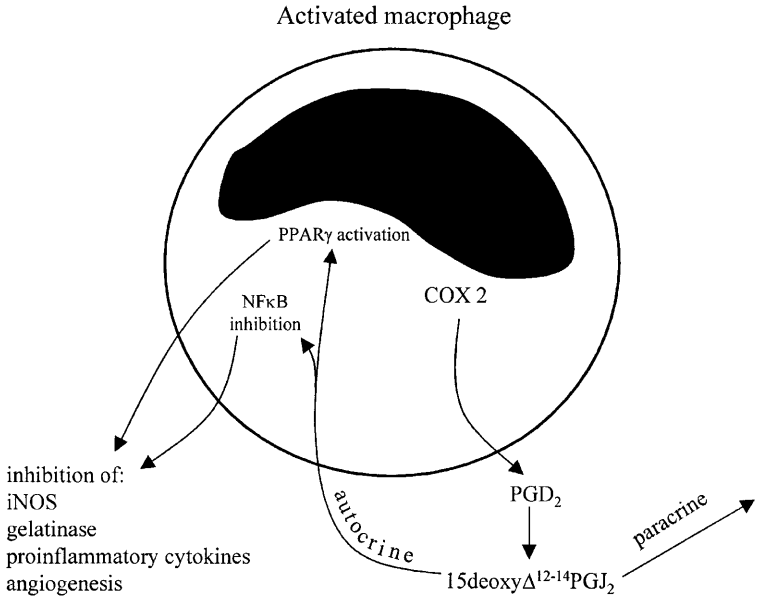

Fig. 2 A mechanism by which COX 2 may control resolution. Here, activated macrophages are depicted as expressing COX 2 which in turn preferentially releases PGD_2 and its anti-inflammatory metabolites $15deoxy\Delta^{12-14}PGJ_2$. The cyclopentenone PGs then exerts their anti-inflammatory effects by the activation of PPARγ and/or the inhibition of nuclear factor κB (*NFκB*) activation in either an autocrine or paracrine fashion. *iNOS* Inducible nitric oxide synthase

and finally, replacement with exogenous PGD_2 and $15deoxy\Delta^{12-14}PGJ_2$ reversed the exacerbation of inflammation caused by these NSAIDs. These results suggest that COX 2 aids resolution at the later mononuclear cell dominated phase by generating an alternate set of anti-inflammatory PGs. We suspect that these anti-inflammatory PGs are in fact the cyclopentenone PGs.

Cyclopenetenone PGs and their anti-inflammatory properties

Without going any further, a brief description of PPARs and their ligands is necessary. PPARs are a group of nuclear receptors that are key regulators of glucose and energy homeostasis and influence body weight. Interest in PPARs was heightened with the discovery that clinically important compounds such as the fibrate class of hypolipidemic drugs, the thiazolidinediones which are orally active antidiabetic agents, some NSAIDs and fatty acids activate them. To date there are three PPARs: PPARα, PPARδ (also called NUC1, FAAR or β) and γ (for review see [53, 54, 55]).

PPARα is expressed mainly in the liver, kidney, heart and mouse brown adipose tissue, where its activation induces the enzyme systems of the β and ω oxidation pathways [56]. These are responsible for the destruction of long-chain fatty acids and xenobiotics, and it is thought that the hypolipidemic activity of the fibrate-based drugs is mediated by the induction of these metabolic pathways. In contrast, PPARδ is less well described but is widely expressed, raising the suggestion that it has a broader housekeeping function in lipid homeostasis [57]. However, PPARγ has been shown to be a key receptor for controlling adipocyte differentiation [58, 59] and exists as three species, termed γ1, γ2 and γ3. The first is ubiquitously expressed, whereas PPARγ2 is expressed mainly in adipose tissue and is probably the most important for adipocyte function [60, 61]. The third receptor PPARγ3 is the main receptor subtype in macrophages [62].

The oxidation pathways induced by peroxisome proliferators metabolise PGs and leukotrienes as well as other long chain fatty acids. Indeed, it has been shown that mice deficient in PPARα have a prolonged response to arachidonic acid induced ear inflammation. While this is predominantly a leukotriene-mediated reaction, these authors went on to show that leukotriene B_4 itself is a ligand for PPARα [63]. It was proposed that production of leukotriene B_4 would induce inflammation but also lead to activation of PPARα and induce the systems required for its oxidation and elimination, thus terminating the inflammatory response. We have extended these findings by demonstrating that activation of PPARα by the selective agonist Wy14,643 reduces inflammation in the arachidonic acid ear model in mice, and that this is associated with the induction of peroxisomal enzyme pathways both in the livers of treated mice and also locally in the ear tissue at the site of inflammation [64].

However, it is PPARγ and its endogenous ligand $15deoxy\Delta^{12-14}PGJ_2$ [65, 66] that has attracted most of the attention in terms of its ability to resolve inflammation. In addition to endogenous ligands, certain NSAIDs such as indomethacin and ibuprofen have also been shown to bind to and activate PPARγ [67]. Interestingly, this may help explain the additional anti-inflammatory properties of these NSAIDs seen at concentrations higher than that necessary to inhibit PGs synthesis. The mechanisms by which PPAR activation exerts their anti-inflammatory effects are not well defined, simply because this is a relatively new field of inflammatory research. However, what is compelling is that PPAR activation seems to regulate such a diverse class of so-called proinflammatory mediators.

It has been shown that the synthetic PPARγ ligand BRL49563 and the endogenous ligand 15deoxy$\Delta^{12-14}PGJ_2$ can inhibit the synthesis of tumor necrosis factor α and interleukins 1β and 6 in monocytes at physiologically relevant concentrations [51]. Similarly, we have shown that treatment with the natural PPARγ agonist $\Delta^{12}PGJ_2$ can inhibit expression of the proinflammatory inducible nitric oxide synthase enzyme system in macrophages [64]. This has been extended to include gelatinase b and scavenger receptor A genes [52], important in the generation of atherosclerotic lesions. This was shown to be mediated in part by interference with nuclear factor κB, activator protein 1 and the signal transducer and activator of transcription protein that are important transcription factors responsible for the activation of various proinflammatory pathways in macrophages [52].

In addition to regulating proinflammatory mediator release, PPAR activation also has profound effects on angiogenesis [68, 69]. This is the process of blood vessel formation crucial for the growth and survival of such destructive tissues as solid tumors and pannus tissue, the latter widely held to be responsible for cartilage and joint destruction in arthritis (for review see [70, 71]). It has been shown that the PPARγ activators such as BRL49563 and 15deoxy$\Delta^{12-14}PGJ_2$ are potent angiostatic agents in vitro, inhibiting endothelial tube formation in collagen gels, vascular endothelial cell growth factor receptor and urokinase plasminogen activator expression as well as inhibiting corneal angiogenesis in response to vascular endothelial cell growth factor in vivo [68]. 15deoxy$\Delta^{12-14}PGJ_2$ is also able to induce apoptosis in human umbilical vein and bovine brain microvascular endothelial cells [69] which contributes to their angiostatic activity.

COX 2 activation of the stress response

PPARγ agonist concentrations used in these studies are often much higher than that required for receptor activation. This suggests that additional pathways mediate the beneficial effects of these agonists. One possibility of interest as an additional anti-inflammatory mode of action of the PGs is the induction of the stress response.

In response to a wide variety of stressful stimuli, cells rapidly induce a set of gene products that have become known as the heat shock proteins (HSPs) or stress proteins. This confers protection against the cytotoxic effects of cytokines and results in the amelioration of a variety of inflammatory conditions (reviewed in [72, 73]). In vivo, exposure of animals to prior heat shock is highly protective against the damaging effects of intratracheal administration of phospholipase A_2, associated with a huge increase in the expression of HSP70 in the lung tissue [74], reduces mortality associated with endotoxic shock and sepsis [75] and transgenic animals which overexpress HSP70 are protected against the effects of ischaemic/reperfusion injury in the myocardium [76]. Heme oxygenase 1, also known as HSP32, is expressed during the inflammatory response in many experimental models. In the rat carrageenin pleurisy model for instance, enhancing heme oxygenase 1 expression pharmacologically, substantially diminishes the severity of the inflammatory response while inhibiting its expression exacerbates edema formation and cellular influx [77].

Therefore, what is the evidence to connect products of COX enzyme activity to the stress response? The link between COX and the stress proteins comes from work primarily in the field of virology and cell replication. It has been shown that cyclopentenone PGs of the A and J series inhibit virus replication [78, 79]. A common characteristic of these PGs is the ability to induce HSP synthesis, via activation of heat shock factor [80, 81]. These PGs also inhibit tumor cell proliferation (reviewed in [82, 83]). Indeed, the inhibition of inducible nitric oxide synthase expression and activity in murine macrophages described above [64] was shown at least in part to be dependent on the induction of heme oxygenase 1 activity, a feature which has also been subsequently confirmed in the inhibition of nitric oxide production by microglial cells in response to PPARγ agonists such as 15deoxy$\Delta^{12-14}PGJ_2$ [84].

Although there is evidence to suggest that the cyclopentenone PGs particularly of the J series are activators of PPARγ, and this assumption is often made. As mentioned above, the concentrations of PGS required for these effects are in excess of the concentrations considered necessary for PPAR activation. Further evidence of a PPAR-independent mechanism comes from promoter studies on the heme oxygenase 1 regulatory elements in which the stimulation of heme oxygenase 1 expression by $\Delta^{12}PGJ_2$ is via a novel regulatory element controlled by a dimeric complex which does not appear to be PPAR related [85].

Conclusion

It now appears that COX 2 plays an important role in the maintenance of normal renal function [41], the female reproductive system [86] and the regulation of bone resorption [87] as well as playing a reparative role in the gastrointestinal system after injury. Moreover, Celebrex but not ibuprofen was shown recently to inhibit urinary excretion of prostacyclin by healthy human volunteers, whereas only ibuprofen inhibited thromboxane. The authors speculated that individuals with a high risk of cardiovascular diseases receiving long-term dosing with COX 2 inhibitors may have an increased incidence of thrombosis [88]. In 1999 two selective COX 2 inhibitors were licensed, Celebrex and Vioxx. Whether these clinically available inhibitors affect COX 2 dependent physiological systems, facilitate the development of cardiovascular diseases or induce asthmatic attacks in aspirin-sensitive asthmatics will require post-marketing studies. While conventional NSAIDs also inhibit COX 2 to varying degrees, it is the ratio of COX 1 to COX 2 inhibition that may be an important factor. In the meantime, its cru-

cial to highlight the potential problems that may be associated with selective COX 2 inhibitors.

This review documents the evolution of COX 2 research in terms of its initial discovery and the original hypothesis that its inhibition would provide a valuable therapeutic target for the treatment of inflammatory diseases, to the current research findings and emerging hypotheses. To this end we report its proinflammatory properties and also the recent and provocative notion that COX 2 may also regulate inflammatory resolution. In terms of the latter concept, we attempt to explain these mechanisms of action in light of our own findings and the current literature suggesting that COX 2 has indeed anti-inflammatory properties which may be mediated at least in part by PPAR activation and/or stress protein expression. In science we are constantly reminded never to ignore the unusual. In the field of COX 2 research, this is certainly no exception.

References

1. Smith WL, Garavito RM, DeWitt DL (1996) Prostaglandin endoperoxide H synthases cyclooxygenases)-1 and -2. J Biol Chem 271:33157–33160
2. Wu KK (1995) Inducible cyclooxygenase and nitric oxide synthase. Adv Pharmacol 33:179–207
3. Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR (1991) TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J Biol Chem 266:12866–12872
4. Hla T, Neilson K (1992) Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci USA 89:7384–7388
5. Jones DA, Carlton DP, McIntyre TM, Zimmerman GA, Prescott SM (1993) Molecular cloning of human prostaglandin endoperoxide synthase type II and demonstration of expression in response to cytokines. J Biol Chem 268:9049–9054
6. Smith CJ, Morrow JD, Roberts LJ 2d, Marnett LJ (1993) Differentiation of monocytoid THP-1 cells with phorbol ester induces expression of prostaglandin endoperoxide synthase-1 (COX-1). Biochem Biophys Res Commun 192:787–793
7. Samet JM, Fasano MB, Fonteh AN, Chilton FH (1995) Selective induction of prostaglandin G/H synthase I by stem cell factor and dexamethasone in mast cells. J Biol Chem 270: 8044–8049
8. Kaufmann WE, Worley PF, Pegg J, Bremer M, Isakson P (1996) COX-2, a synaptically induced enzyme, is expressed by excitatory neurons at postsynaptic sites in rat cerebral cortex. Proc Natl Acad Sci USA 93:2317–2321
9. Walenga RW, Kester M, Coroneos E, Butcher S, Dwivedi R, Statt C (1996) Constitutive expression of prostaglandin endoperoxide G/H synthetase (PGHS)-2 but not PGHS-1 in human tracheal epithelial cells in vitro. Prostaglandins 52:341–359
10. Harris RC, McKanna JA, Akai Y, Jacobson HR, Dubois RN, Breyer MD (1994) Cyclooxygenase-2 is associated with the macula densa of rat kidney and increases with salt restriction. J Clin Invest 94:2504–2510
11. Spencer AG, Woods JW, Arakawa T, Singer II, Smith WL (1998) Subcellular localization of prostaglandin endoperoxide H synthases-1 and -2 by immunoelectron microscopy. J Biol Chem 273:9886–9893
12. Kraemer SA, Meade EA, DeWitt DL (1992) Prostaglandin endoperoxide synthase gene structure: identification of the transcriptional start site and 5'-flanking regulatory sequences. Arch Biochem Biophys 293:391–400
13. Appleby SB, Ristimaki A, Neilson K, Narko K, Hla T (1994) Structure of the human cyclo-oxygenase-2 gene. Biochem J 302:723–727
14. Smith WL, Dewitt DL (1996) Prostaglandin endoperoxide H synthases-1 and -2. Adv Immunol 62:167–215
15. Samuelsson B (1987) An elucidation of the arachidonic acid cascade. Discovery of prostaglandins, thromboxane and leukotrienes. Drugs 33:2–9
16. Vane JR (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nat New Biol 231:232–235
17. Armstrong CP, Blower AL (1987) Non-steroidal anti-inflammatory drugs and life threatening complications of peptic ulceration. Gut 28:527–532
18. Vane J (1994) Towards a better aspirin. Nature 367:215–216
19. Barnett J, Chow J, Ives D, Chiou M, Mackenzie R, Osen E, Nguyen B, Tsing S, Bach C, Freire J, et al (1994) Purification, characterization and selective inhibition of human prostaglandin G/H synthase 1 and 2 expressed in the baculovirus system. Biochim Biophys Acta 1209:130–139
20. Gierse JK, Hauser SD, Creely DP, Koboldt C, Rangwala SH, Isakson PC, Seibert K (1995) Expression and selective inhibition of the constitutive and inducible forms of human cyclooxygenase. Biochem J 305:479–484
21. Kargman S, Wong E, Greig GM, Falgueyret JP, Cromlish W, Ethier D, Yergey JA, Riendeau D, Evans JF, Kennedy B, Tagari P, Francis DA, O'Neill GP (1996) Mechanism of selective inhibition of human prostaglandin G/H synthase-1 and -2 in intact cells. Biochem Pharmacol 52:1113–1125
22. Riendeau D, Percival MD, Boyce S, Brideau C, Charleson S, Cromlish W, Ethier D, Evans J, Falgueyret JP, Ford-Hutchinson AW, Gordon R, Greig G, Gresser M, Guay J, Kargman S, Leger S, Mancini JA, O'Neill G, Ouellet M, Rodger IW, Therien M, Wang Z, Webb JK, Wong E, Chan CC, et al (1997) Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. Br J Pharmacol 121:105–117
23. O'Neill GP, Mancini JA, Kargman S, Yergey J, Kwan MY, Falgueyret JP, Abramovitz M, Kennedy BP, Ouellet M, Cromlish W, et al (1994) Overexpression of human prostaglandin G/H synthase-1 and -2 by recombinant vaccinia virus: inhibition by nonsteroidal anti-inflammatory drugs and biosynthesis of 15-hydroxyeicosatetraenoic acid. Mol Pharmacol 45:245–254
24. Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR (1993) Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proc Natl Acad Sci USA 90:11693–11697
25. Meade EA, Smith WL, DeWitt DL (1993) Differential inhibition of prostaglandin endoperoxide synthase (cyclooxygenase) isozymes by aspirin and other non-steroidal anti-inflammatory drugs. J Biol Chem 268:6610–6614
26. Futaki N, Yoshikawa K, Hamasaka Y, Arai I, Higuchi S, Iizuka H, Otomo S (1993) NS-398, a novel non-steroidal anti-inflammatory drug with potent analgesic and antipyretic effects, which causes minimal stomach lesions. Gen Pharmacol 24: 105–110
27. Futaki N, Takahashi S, Yokoyama M, Arai I, Higuchi S, Otomo S (1994) NS-398, a new anti-inflammatory agent, selectively inhibits prostaglandin G/H synthase/cyclooxygenase (COX-2) activity in vitro. Prostaglandins 47:55–59
28. Gans KR, Galbraith W, Roman RJ, Haber SB, Kerr JS, Schmidt WK, Smith C, Hewes WE, Ackerman NR (1990) Anti-inflammatory and safety profile of DuP 697, a novel orally effective prostaglandin synthesis inhibitor. J Pharmacol Exp Ther 254:180–187
29. Chan CC, Boyce S, Brideau C, Ford-Hutchinson AW, Gordon R, Guay D, Hill RG, Li CS, Mancini J, Penneton M, et al (1995) Pharmacology of a selective cyclooxygenase-2 inhibitor, L-745:337: a novel nonsteroidal anti-inflammatory agent with an ulcerogenic sparing effect in rat and nonhuman primate stomach. J Pharmacol Exp Ther 274:1531–1537

30. Penning TD, Talley JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, Graneto MJ, Lee LF, Malecha JW, Miyashiro JM, Rogers RS, Rogier DJ, Yu SS, AndersonGD, Burton EG, Cogburn JN, Gregory SA, Koboldt CM, Perkins WE, Seibert K, Veenhuizen, AW, Zhang YY, Isakson PC (1997) Synthesis and biological evaluation of the 1:5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). J Med Chem 40:1347–1365
31. Prasit P, Wang Z, Brideau C, Chan CC, Charleson S, Cromlish W, Ethier D, Evans JF, Ford-Hutchinson AW, Gauthier JY, Gordon R, Guay J, Gresser M, Kargman S, Kennedy B, Leblanc Y, Leger S, Mancini J, O'Neill GP, Ouellet M, Percival MD, Perrier H, Riendeau D, Rodger I, Zamboni R, et al (1999) The discovery of rofecoxib, [MK 966, Vioxx, 4-(4' methylsulfonylphenyl)-3-phenyl-2 (5H)-furanone], an orally active cyclooxygenase-2-inhibitor. Bioorg Med Chem Lett 9:1773–1778
32. Tomlinson A, Appleton I, Moore AR, Gilroy DW, Willis D, Mitchell JA, Willoughby DA (1994) Cyclo-oxygenase and nitric oxide synthase isoforms in rat carrageenin-induced pleurisy. Br J Pharmacol 113:693–698
33. Appleton I, Tomlinson A, Mitchell JA, Willoughby DA (1995) Distribution of cyclooxygenase isoforms in murine chronic granulomatous inflammation. Implications for future anti-inflammatory therapy. J Pathol 176:413–420
34. Vane JR, Mitchell JA, Appleton I, Tomlinson A, Bishop-Bailey D, Croxtall J, Willoughby DA (1994) Inducible isoforms of cyclooxygenase and nitric-oxide synthase in inflammation. Proc Natl Acad Sci USA 91:2046–2050
35. Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG, Isakson PC, Seibert K (1994) Selective inhibition of inducible cyclooxygenase 2 in vivo is antiinflammatory and nonulcerogenic. Proc Natl Acad Sci USA 91: 3228–3232
36. Seibert K, Zhang Y, Leahy K, Hauser S, Masferrer J, Perkins W, Lee L, Isakson P (1994) Pharmacological and biochemical demonstration of the role of cyclooxygenase 2 in inflammation and pain. Proc Natl Acad Sci USA 91:12013–12017
37. Anderson GD, Hauser SD, McGarity KL, Bremer ME, Isakson PC, Gregory SA (1996) Selective inhibition of cyclooxygenase (COX)-2 reverses inflammation and expression of COX-2 and interleukin 6 in rat adjuvant arthritis. J Clin Invest 97:2672–2679
38. Reddy BS, Rao CV, Seibert K (1996) Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. Cancer Res 56:4566–4569
39. Gilroy DW, Tomlinson A, Willoughby DA (1998) Differential effects of inhibitors of cyclooxygenase (cyclooxygenase 1 and cyclooxygenase 2) in acute inflammation. Eur J Pharmacol 355:211–217
40. Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD, et al (1995) Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell 83:483–492
41. Morham SG, Langenbach R, Loftin CD, Tiano HF, Vouloumanos N, Jennette JC, Mahler JF, Kluckman KD, Ledford A, Lee CA, et al (1995) Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. Cell 83:473–482
42. Dinchuk JE, Car BD, Focht RJ, Johnston JJ, Jaffee BD, Covington MB, Contel NR, Eng VM, Collins RJ, Czerniak PM, et al (1995) Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. Nature 378:406–409
43. Wallace JL, Bak A, McKnight W, Asfaha S, Sharkey KA, MacNaughton WK (1998) Cyclooxygenase 1 contributes to inflammatory responses in rats and mice: implications for gastrointestinal toxicity. Gastroenterology 115:101–109
44. Reuter BK, Asfaha S, Buret A, Sharkey KA, Wallace JL (1996) Exacerbation of inflammation-associated colonic injury in rat through inhibition of cyclooxygenase-2. J Clin Invest 98:2076–2085
45. Mizuno H, Sakamoto C, Matsuda K, Wada K, Uchida T, Noguchi H, Akamatsu T, Kasuga M (1997) Induction of cyclooxygenase 2 in gastric mucosal lesions and its inhibition by the specific antagonist delays healing in mice. Gastroenterology 112:387–397
46. Schmassmann A, Peskar BM, Stettler C, Netzer P, Stroff T, Flogerzi B, Halter F (1998) Effects of inhibition of prostaglandin endoperoxide synthase-2 in chronic gastro-intestinal ulcer models in rats. Br J Pharmacol 123:795–804
47. Gretzer B, Ehrlich K, Maricic N, Lambrecht N, Respondek M, Peskar BM (1998) Selective cyclo-oxygenase-2 inhibitors and their influence on the protective effect of a mild irritant in the rat stomach. Br J Pharmacol 123:927–935
48. Newberry RD, Stenson WF, Lorenz RG (1999) Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen. Nat Med 5:900–906
49. Gavett SH, Madison SL, Chulada PC Scarborough PE, Qu W, Boyle JE, Tiano HF, Lee CA, Langenbach R, Roggli VL, Zeldin DC (1999) Allergic lung responses are increased in prostaglandin H synthase-deficient mice. J Clin Invest 104:721–732
50. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA (1999) Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med 5:698–701
51. Jiang C, Ting AT, Seed B (1998) PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. Nature 391:82–86
52. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK (1998) The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. Nature 391: 79–82
53. Gelman L, Fruchart JC, Auwerx J (1999) An update on the mechanisms of action of the peroxisome proliferator-activated receptors (PPARs) and their roles in inflammation and cancer. Cell Mol Life Sci 55:932–943
54. Vamecq J, Latruffe N (1999) Medical significance of peroxisome proliferator-activated receptors. Lancet 354:141–148
55. Fruchart JC, Duriez P, Staels B (1999) Peroxisome proliferator-activated receptor-alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. Curr Opin Lipidol 10:245–257
56. Braissant O, Wahli W (1998) Differential expression of peroxisome proliferator-activated receptor-alpha, -beta, and -gamma during rat embryonic development. Endocrinology 139:2748–2754
57. Xing G, Zhang L, Zhang L, Heynen T, Yoshikawa T, Smith M, Weiss S, Detera-Wadleigh S (1995) Rat PPAR delta contains a CGG triplet repeat and is prominently expressed in the thalamic nuclei. Biochem Biophys Res Commun 217:1015–1025
58. Tontonoz P, Hu E, Spiegelman BM (1994) Stimulation of adipogenesis in fibroblasts by PPAR gamma 2, a lipid-activated transcription factor. Cell 79:1147–1156
59. Tontonoz P, Hu E, Graves RA, Budavari AI, Spiegelman BM (1994) mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 8:1224–1234
60. Fajas L, Auboeuf D, Raspe E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, Vidal-Puig A, Flier J, Briggs MR, Staels B, Vidal H, Auwerx J (1997) The organization, promoter analysis, and expression of the human PPARgamma gene. J Biol Chem 272:18779–18789
61. Zhu Y, Qi C, Korenberg JR, Chen XN, Noya D, Rao MS, Reddy JK (1995) Structural organization of mouse peroxisome proliferator-activated receptor gamma (mPPAR gamma) gene: alternative promoter use and different splicing yield two mPPAR gamma isoforms. Proc Natl Acad Sci USA 92: 7921–7925
62. Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, Witztum JL, Auwerx J, Palinski W, Glass CK (1998) Expression of the peroxisome proliferator-activated receptor gamma (PPARgamma) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein. Proc Natl Acad Sci USA 95:7614–7619

63. Devchand PR, Keller H, Peters JM, Vazquez M, Gonzalez FJ, Wahli W (1996) The PPARalpha-leukotriene B4 pathway to inflammation control. Nature 384:39–43
64. Colville-Nash PR, Qureshi SS, Willis D, Willoughby DA (1998) Inhibition of inducible nitric oxide synthase by peroxisome proliferator-activated receptor agonists: correlation with induction of heme oxygenase 1. J Immunol 161:978–984
65. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM (1995) 15-Deoxy-delta 12:14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR gamma. Cell 83:803–812
66. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM (1995) A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. Cell 83:813–819
67. Lehmann JM, Lenhard JM, Oliver BB, Ringold GM, Kliewer SA (1997) Peroxisome proliferator-activated receptors alpha and gamma are activated by indomethacin and other non-steroidal anti-inflammatory drugs. J Biol Chem 272:3406–3410
68. Xin X, Yang S, Kowalski J, Gerritsen ME (1999) Peroxisome proliferator-activated receptor gamma ligands are potent inhibitors of angiogenesis in vitro and in vivo. J Biol Chem 274: 9116–9121
69. Bishop-Bailey D, Hla T (1999) Endothelial cell apoptosis induced by the peroxisome proliferator-activated receptor (PPAR) ligand 15-deoxy-Delta12:14-prostaglandin J2 J Biol Chem 274:17042–17048
70. Colville-Nash PR, Scott DL (1992) Angiogenesis and rheumatoid arthritis: pathogenic and therapeutic implications. Ann Rheum Dis 51:919–925
71. Colville-Nash PR, Willoughby DA (1997) Growth factors in angiogenesis: current interest and therapeutic potential. Mol Med Today 3:14–23
72. Moseley PL (1998) Heat shock proteins and the inflammatory response. Ann N Y Acad Sci 856:206–213
73. Gaston JS (1998) Heat shock proteins as potential targets in the therapy of inflammatory arthritis. Biotherapy 10:197–203
74. Villar J, Edelson JD, Post M, Mullen JB, Slutsky AS (1993) Induction of heat stress proteins is associated with decreased mortality in an animal model of acute lung injury. Am Rev Respir Dis 147:177–181
75. Villar J, Ribeiro SP, Mullen JB, Kuliszewski M, Post M, Slutsky AS (1994) Induction of the heat shock response reduces mortality rate and organ damage in a sepsis-induced acute lung injury model. Crit Care Med 22:914–921
76. Mestril R, Giordano FJ, Conde AG, Dillmann WH (1996) Adenovirus-mediated gene transfer of a heat shock protein 70 (hsp 70i) protects against simulated ischemia. J Mol Cell Cardiol 28:2351–2358
77. Willis D, Moore AR, Frederick R, Willoughby DA (1996) Heme oxygenase: a novel target for the modulation of the inflammatory response. Nat Med 2:87–90
78. Mastromarino P, Conti C, Petruzziello R, De Marco A, Pica F, Santoro MG (1993) Inhibition of Sindbis virus replication by cyclopentenone prostaglandins: a cell-mediated event associated with heat-shock protein synthesis. Antiviral Res 20:209–222
79. Conti C, Mastromarino P, Tomao P, De Marco A, Pica F, Santoro MG (1996) Inhibition of poliovirus replication by prostaglandins A and J in human cells. Antimicrob Agents Chemother 40:367–372
80. Elia G, Polla B, Rossi A, Santoro MG (1999) Induction of ferritin and heat shock proteins by prostaglandin A1 in human monocytes. Evidence for transcriptional and post-transcriptional regulation. Eur J Biochem 264:736–745
81. Rossi A, Elia G, Santoro MG (1997) Inhibition of nuclear factor kappa B by prostaglandin A1: an effect associated with heat shock transcription factor activation. Proc Natl Acad Sci USA 94:746–750
82. Kikuchi Y, Kita T, Hirata J, Fukushima M (1994) Preclinical studies of antitumor prostaglandins by using human ovarian cancer cells. Cancer Metastasis Rev 13:309–315
83. Sasaki H, Fukushima M (1994) Prostaglandins in the treatment of cancer. Anticancer Drugs 5:131–138
84. Petrova TV, Akama KT, Van Eldik LJ (1999) Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric-oxide synthase by 15-deoxy-Delta12:14-prostaglandin J2. Proc Natl Acad Sci USA 96:4668–4673
85. Koizumi T, Odani N, Okuyama T, Ichikawa A, Negishi M (1995) Identification of a cis-regulatory element for delta 12-prostaglandin J2-induced expression of the rat heme oxygenase gene. J Biol Chem 270:21779–21784
86. Lim H, Paria BC, Das SK, Dinchuk JE, Langenbach R, Trzaskos JM, Dey SK (1997) Multiple female reproductive failures in cyclooxygenase 2-deficient mice. Cell 91:197–208
87. Wolfe MM, Lichtenstein DR, Singh G (1999) Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. N Engl J Med 340:1888–1899
88. McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA (1999) Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. Proc Natl Acad Sci USA 96:272–277