

Prostanoids actions in cardiovascular physiopathology

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ABSTRACT

Prostaglandins (PGs) and thromboxanes (TXs) play a pivotal role in cardiovascular physiopathology. They are synthesized from arachidonic acid by the enzymatic action of cyclooxygenases (COXs), leading to the production of an unstable intermediate, PGH₂ that is subsequently converted to the different prostaglandins and thromboxanes (PGE₂, PGD₂, PGI₂, PGF_{2α} and TXA₂) by the action of different synthases and isomerases. There are two well characterized COX enzymes, termed COX-1 and COX-2, with different properties. While COX-1 is expressed constitutively in most tissues and is thought to be involved in homeostatic prostanoid biosynthesis, COX-2 is

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Abbreviations: COX, Cyclooxygenase; DP, PGD receptor; EP, PGE receptor; FP, PGF receptor; IL, Interleukin; IP, PGI receptor; NSAIDs, non-steroidal anti-inflammatory drugs; PG, prostaglandin; PGES, PGE synthase; PGDS, PGD synthase; PGFS, PGF synthase; PGIS, PGI synthase; TP, Thromboxane receptor; TX, Thromboxane; TXS, Thromboxane synthase.

transcriptionally up-regulated in response to mitogens and pro-inflammatory stimuli being the predominant isoform involved in the inflammatory response.

In the cardiovascular system, prostanoids have been shown to modulate the pathogenesis of vascular diseases as thrombosis and atherosclerosis through a variety of processes, including platelet aggregation, vasorelaxation and vasoconstriction, local inflammatory response and leukocyte-endothelial cell adhesion. Multiple studies using pharmacological inhibitors and genetically deficient mice have demonstrated the importance of prostanoid-mediated actions on cardiovascular physiology. However, recent withdrawal of COX-2 selective inhibitors from the clinic because of their adverse effects in patients with potential cardiovascular risk has opened a debate about the role of COX-derived prostanoids in vascular pathologies and the benefits and risks for the use of COX inhibitors in cardiovascular diseases.

Key words: Prostaglandins.- Thromboxanes.- Cyclooxygenases.- Prostanoid receptors.- NSAIDs.- Cardiovascular physiopathology.

RESUMEN

Acciones de los prostanoides en la fisiopatología cardiovascular

Las prostaglandinas (PGs) y los tromboxanos (TXs) juegan un papel esencial en la fisiopatología cardiovascular. Estos prostanoides son sintetizados a través de la acción enzimática de las ciclooxygenasas (COXs) sobre el ácido araquidónico, lo que lleva a la producción de un intermediario inestable, la PGH_2 , a partir de la cual diversas sintetasas e isomerasas generarán las diferentes prostaglandinas y tromboxanos (PGE_2 , PGD_2 , PGI_2 , $PGF_{2\alpha}$ and TXA_2). Existen dos ciclooxygenasas bien caracterizadas denominadas COX-1 y COX-2, con diferentes propiedades. COX-1 se expresa constitutivamente en la mayoría de los tejidos, estando implicada en la biosíntesis de prostanoides con funciones homeostáticas. Por otro lado, la expresión de COX-2 se induce en respuesta a mitógenos y estímulos pro-inflamatorios, constituyendo la isoforma predominantemente implicada en la respuesta inflamatoria.

Los prostanoides modulan la patogénesis de enfermedades vasculares como la trombosis y la aterosclerosis a través de una serie de procesos como: la agregación plaquetaria, la vasodilatación y vasoconstricción, y la respuesta inflamatoria local. Múltiples estudios han demostrado la importancia de las acciones mediadas por los prostanoides en la fisiopatología cardiovascular, bien mediante el uso de inhibidores farmacológicos o a través del análisis de ratones genéticamente deficientes. Sin embargo, la reciente retirada del mercado de inhibidores selectivos de COX-2 a causa de sus efectos adversos en pacientes con riesgo cardiovascular, ha abierto el debate sobre el papel de los prostanoides en la patología vascular y sobre las ventajas o inconvenientes del uso de inhibidores de COXs en las enfermedades cardiovasculares.

Palabras clave: Prostaglandinas.- Tromboxanos.- Ciclooxigenasas.- Receptores de prostanoides.- AINEs.- Fisiopatología cardiovascular.

INTRODUCTION

Prostanoids, including prostaglandins (PGs) and thromboxanes (TXs) are a group of bioactive lipids that play a very important role in many physiological and pathological processes, including inflammation (1), cancer (2), angiogenesis (3) and cardiovascular diseases (4-7). Arachidonic acid liberated from membrane phospholipids by several phospholipases, is metabolized by the sequential action of cyclooxygenases (COX) and prostaglandin or thromboxane synthases to produce the diverse classes of prostanoids (8, 9). COX enzymes catalyze the formation of an unstable endoperoxide intermediate, PGH_2 , which in turn can be metabolized by cell-specific isomerases and synthases to a range of eicosanoids with potent and diverse biological effects, as PGD_2 , PGE_2 , $\text{PGF}_{2\alpha}$, PGI_2 , and TXA_2 (Figure 1). Finally after their synthesis, prostanoids are quickly released to the extracellular medium exerting multiple effects upon interaction with prostanoid receptors present in the neighbouring cells.

CYCLOOXYGENASES

There are two main COX isoenzymes named COX-1 and COX-2, encoded by two separate genes (8-10). A COX-3 enzyme derived from alternative splicing of the COX-1 gene has been also described, although its role is still unclear (11). COX-1 is constitutively expressed in most, but not all, cell types and tissues and is responsible for vascular, renal and gastric homeostasis (12). In contrast, COX-2 expression is generally induced at sites of inflammation by many stimuli that includes among others, proinflammatory cytokines as interleukin (IL)- 1β , tumor necrosis factor (TNF)- α , growth factors, mechanical stress, oxidized lipids, free radicals and bacterial products (9-13). Those stimuli activate many

transcription factors such as nuclear factor (NF)- κ B, NF-IL6 (C/EBP), cAMP response element-binding protein (CREBP), Activator protein (AP) -1, interferon regulatory factors (IRFs) and nuclear factor of activated T cells (NFAT) (13-15) that induce COX-2 transcription in different cell types. COX-2 transcriptional induction can be inhibited by anti-inflammatory and immunosuppressive drugs as glucocorticoids and Cyclosporin A (16, 17) as well as by anti-inflammatory cytokines such as IL-4, IL-10 and IL-13 (18, 19).

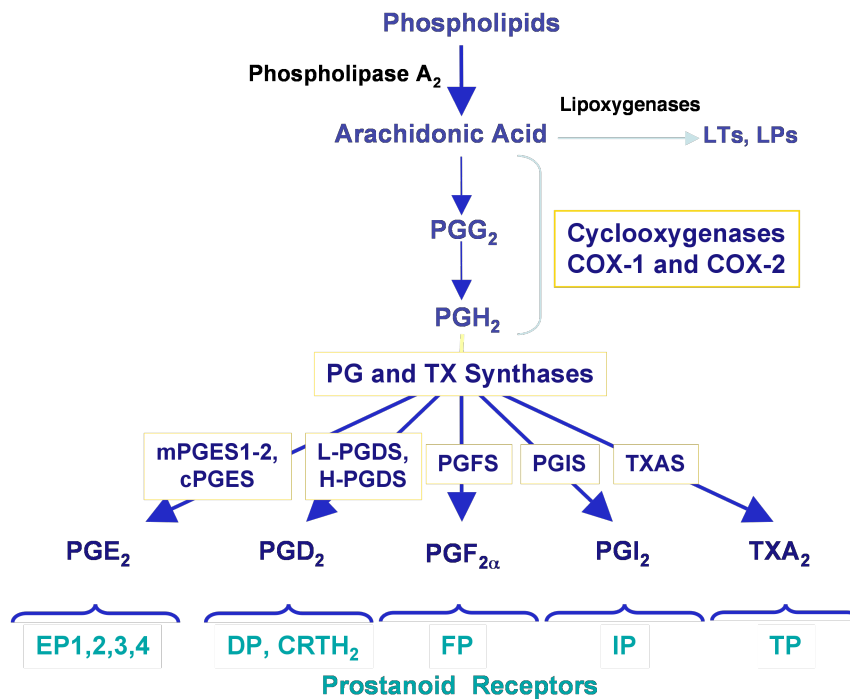


FIGURE 1.- **Biosynthesis of prostanoinds.** Arachidonic acid is liberated from the membrane phospholipids by phospholipase A_2 (PLA_2) and converted to PGH_2 and then to PGG_2 by cyclooxygenases ($COX-1$ or $COX-2$). Subsequent conversion of PGH_2 to different prostanoinds (PGs, prostaglandins and TXs, thromboxanes) is catalyzed by the respective synthases and isomerases. Prostanoinds signal through G protein-coupled receptors with seven trans-membrane domains. PGE_2 signals via 4 different receptors

(EP1, EP2, EP3 and EP4). PGD_2 , $PGF_{2\alpha}$, PGI and TXA_2 bind to the DP and $CRTH_2$, FP, IP and TP receptors respectively.

Cyclooxygenases are the target of non-steroidal anti-inflammatory drugs (NSAIDs). It is generally accepted that these drugs exert their anti-inflammatory and analgesic actions through inhibition of COX enzymatic activity. Classic NSAIDs, such as aspirin, inhibit both isoforms at standard doses. Inhibition of COX-1 may account for some of the unwanted side effects of these drugs such as gastrointestinal and renal toxicity. On the other hand, as COX-2 is thought to be the predominant isoform involved in the inflammatory response, the ability of NSAIDs to inhibit COX-2 activity may explain their therapeutic effects as anti-inflammatory drugs (Figure 2). Therefore, most of the new research on anti-inflammatory drugs has been aimed at targeting the COX-2 inducible production of PGs (8, 20). Newly developed drugs with high selectivity against COX-2 such as Celecoxib and Rofecoxib have been proved to be potent anti-inflammatory compounds without causing gastric toxicity (21).

Many of the functions associated with each COX isoform have been defined by the therapeutic or adverse effects resulting from pharmacological inhibition by NSAIDs selective for each isoform. However, increasing evidence has shown that certain actions of NSAIDs could be mediated through mechanisms independent of cyclooxygenase activity and prostaglandin production that may be relevant to their effects *in vivo* (22). Thus, extrapolating the role of COX-derived PGs by the use of COX inhibitors may lead to confusing conclusions. In this sense, information provided by the use of mice genetically deficient in COX-1 or COX-2 has provided valuable insight into the roles played by those enzymes *in vivo* (12, 23, 24). These studies have shown that these two closely related enzymes have a non-redundant role. Overall, they have indicated that, contrary to expectations, both, COX-2 and COX-1 participate in the maintenance of normal physiology. In this sense, despite being mostly inducible, COX-2 deficiency in mice produces dramatic phenotypic changes related to normal development and in the maintenance of homeostasis. Thus, COX-2 deficiency results in a severe defect in renal development and in reproduction in females (25, 26).

Surprisingly, COX-1 KO mice show no gastric pathology and are resistant to classical NSAIDs-induced gastric ulceration. COX-1 deficient mice show reduced platelet aggregation that is consistent with the fact that platelets express only COX-1. However, those mice also have a decreased arachidonic acid-induced inflammation indicating a role of this enzyme in inflammatory responses (27).

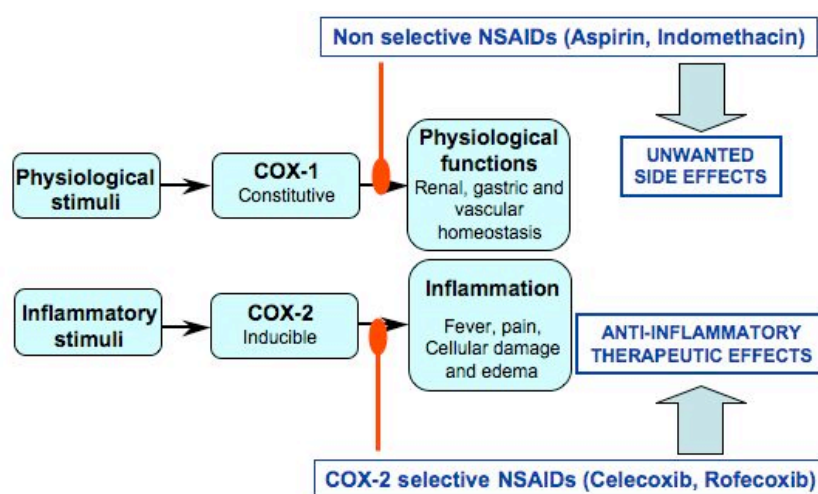


FIGURE 2.- *Cyclooxygenases are the target of non-steroidal anti-inflammatory drugs (NSAIDs). NSAIDs exert their anti-inflammatory and analgesic actions through inhibition of COX enzymatic activity. Classical non selective NSAIDs, such as Aspirin and Indomethacin inhibit COX-1 and COX-2 isoforms at standard doses. Unwanted side effects of these drugs such as gastrointestinal and renal toxicity occurs through the inhibition of the COX-1 –dependent production of prostanoids involved in physiological functions. The ability of NSAIDs to inhibit COX-2 activity explains their therapeutic effects as anti-inflammatory drugs. Newer NSAIDs belonging to the family of Coxibs, such as Celecoxib and Rofecoxib, with high selectivity against COX-2, have been shown to exhibit potent anti-inflammatory properties without causing gastric toxicity.*

Subcellular location of COX isoenzymes may also be important in determining their distinct functions. Thus, COX-1 has been mainly

located in the endoplasmic reticulum and nuclear envelope whereas COX-2 is preferentially expressed around the nuclear membrane (9, 12).

PROSTAGLANDIN AND THROMBOXANE SYNTHASES

Despite the importance of COX isoenzymes in prostanoid generation, the actual prostanoid profile synthesized by a particular cell type is determined by the presence of different downstream PG and TX synthases and isomerases (28) (Figure 1). A particular cell type may predominantly express a particular isomerase, which will largely determine which prostanoid is generated. Moreover, some cell types can express more than one isomerase and a particular COX isoenzyme can couple to different isomerases in the same cell (29). Thus, COX-1 preferentially couples with Thromboxane synthase (TXS) and PGF_{2 α} synthase (PGFS), while COX-2 is associated to Prostacyclin synthase (PGIS) and microsomal PGE₂ synthase -1 (mPGES-1) (29, 30). Evenmore, PGH₂ and other endoperoxides generated in one cell type may be secreted and metabolized by isomerases present in surrounding cells by the so-called “transcellular metabolism” (31).

PGE₂ is synthesized by the action of PGE synthases (PGES) of which there are 3 types: one cytosolic (cPGES) and two membrane-associated PGE synthases (mPGES)-1 and -2. The (cPGES) belongs to the glutathione transferase family, is ubiquitously expressed and is preferentially functionally coupled to COX-1, thus, involved in housekeeping production of PGE₂ (32, 33). In contrast, mPGES-1, which belongs to the Membrane-Associated Proteins involved in Eicosanoid and Glutathione metabolism (MAPEG) superfamily, is inducible by similar stimuli that induce COX-2, being its induction also suppressed by glucocorticoids. Moreover, mPGES-1 appears functionally coupled with COX-2 and its induction is usually coordinated with COX-2 (34). A second type of membrane-bound microsomal PGES is the mPGES-2, able to couple with both COX isoenzymes, although its functional significance is still unclear (35).

PGD₂ is synthesized from PGH₂ by the action of PGD synthases (PGDS) of which there are two isoforms. The haematopoietic PGDS is

present in mast cells, basophils, and in a subset of T helper cells (Th2) and may play a role in allergy (36). The lipocalin-type PGD synthase is mostly expressed in brain and it is especially abundant in the cerebrospinal fluid (37). Interestingly, PGD₂ can be further metabolized by dehydration to PGJ₂, delta12-PGJ₂, and 15- deoxy-delta(12,14)-PGJ₂, which is a ligand for the nuclear transcription factor peroxisomal proliferator activated receptor (PPAR)-gamma having anti-inflammatory activity (38).

PGF_{2α} can be synthesized from PGH₂ by PGH 9-11-endoperoxide reductase or PGF synthase (PGFS). In addition it can be generated from PGE₂ by PGE 9-ketoreductase or from PGD₂ by PGD-11-ketoreductase (39).

Prostacyclin, PGI₂, is produced by the action of PGI synthase (PGIS) which can be induced by mechanical stress, via activation of AP-1 (40).

TXA₂ is synthesized from PGH₂ by thromboxane synthase (TXS), which is constitutively expressed in platelets as well as other blood cells and various tissues as kidney, lung, liver and placenta. Its regulation takes place mainly at the transcriptional level (41).

PROSTANOID RECEPTORS

Prostanoids actions, with the possible exception of cyclopentenone PGs, are mediated through cell surface receptors. These are cell seven membrane spanning G-protein coupled receptors that are classified into five basic types, named according to their ligands: DP for PGD₂, EP for PGE₂, FP for PGF₂, IP for PGI₂, and TP for TXA₂ (6, 42).

DP (also named DP1) is weakly expressed in brain despite the important action of PGD₂ in this organ. It is moderately expressed in the ileum with very weak expression in the lung. DP2, also called CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells), is expressed on Th2 immune cells being a chemoattractant receptor for Th2 lymphocyte subset in allergic inflammation (43).

FP is most abundantly expressed in the corpus luteum according to the role played by PGF_{2α} in menstrual cycle. It is also expressed in the

heart, lung, kidney, and stomach, although its expression in these tissues does not vary during the estrous cycle as does in the corpus luteum (44). There are several splice variants of FP (FP_A and FP_B) but its particular role is unclear.

IP is expressed in larger quantities by dorsal root ganglion neurons but also by platelet precursors (megakaryocytes) as well as in the smooth muscle cells of arteries consistent with the important action of PGI₂ in the cardiovascular system (45).

PGE₂ has four receptors, EP1 to 4. In addition, there are several splice variants of EP3. More importantly, each one is linked to a different transduction pathway that may even give rise to opposite effects (activation or inhibition) on cellular responses (6). Thus, EP1 induces an inhibition of adenylate cyclase leading to a decrease in cAMP whereas EP2 and EP4 receptors activate this enzyme. On the other hand, EP3 is coupled to G α q and its activation results in intracellular calcium increase. EP3 and EP4 receptors have a wide distribution throughout the body, being expressed in almost all tissues examined. In contrast, EP1 is restricted to the lung, kidney and stomach, and EP2 is scarcely expressed.

In the cardiovascular system, IP, DP, EP4 and EP2 receptors that mediate cAMP increases are termed “relaxant” receptors, whereas TP, FP, and EP1 receptors, which induce calcium mobilization, represent the “contractile” receptor group. EP3, which reduces cAMP levels, has been named the “inhibitory” receptor (6).

Nuclear actions of prostanoids have also been reported. Thus, PGJ₂ derivatives can bind and activate peroxisome proliferator-activated receptors (PPARs) nuclear transcription factors (46). There are four types of PPARs (alpha, delta, gamma-1, and gamma-2), which may bind various PGs with different sensitivity (47). In this way, PGs may also act as intracellular signalling molecules and regulate gene expression (48). Generally PPARs induces transcription of anti-inflammatory genes as well as inhibits activation of pro-inflammatory ones (46). Besides, cyclopentenone PGs (that includes PGA1) have a reactive ring which may lead to have receptor-independent actions through redox alterations (49).

PROSTANOIDS IN CARDIOVASCULAR HOMEOSTASIS

Among eicosanoids, TXA₂ and PGI₂ are thought to be the most important prostanoids in controlling the homeostasis of the cardiovascular system, as proposed more than 20 years ago by Bunting, Moncada and Vane (5, 50, 51). They are synthesized by blood platelets and vascular endothelium, respectively, and have opposed biological activities. TXA₂ is potent vasoconstrictor as well as a potent inducer of platelet adhesion and regulates renal hemodynamics and sodium handling by the kidney (4). On the other hand, PGI₂ is the predominant prostanoid produced by cells of the vasculature, having an important vasodilator effect by promoting renal sodium excretion and regulating the growth of vascular smooth muscle cells. Moreover, it opposes TXA₂ effects on platelets inhibiting their aggregation and action (28, 52).

Due to this, many aspects of cardiovascular disease have traditionally been explained by alterations in the balance between PGI₂ and TXA₂ during the interactions between platelets and vessel wall (53) although recent studies argue about this theory (54). Since platelets express COX-1 but not COX-2, it is inferred that TXA₂ production by TXAS uses PGH₂ derived from COX-1. In this regard, platelet TXA₂ is blunted in COX-1 deficient mice, which have decreased platelet aggregation and thrombosis (55). Mice deficient in the TP (51, 56) or human patients with a genetic disorder in the TP receptor (57, 58) exhibit an increase in bleeding tendency and resistance to platelet aggregation, confirming the role of TXA₂ in those activities and consistent with the effects of TP antagonists in humans. Conversely, transgenic mice specifically overexpressing TP in the vasculature results in placental ischemia during pregnancy and suppression of TXA₂ formation rescue the phenotype (59). Since TXA₂ is a mitogen of vascular smooth muscle cells, TP KO mice have decreased vascular proliferation and platelet response after artery injury to the carotid although they are normotensive (51).

Whether PGI₂ is synthesized through COX-1 or COX-2 has been of paramount importance in classical cardiovascular research. Initial reports indicated that vascular endothelial cells and smooth muscle cells have COX-1, and that PGI₂ can be formed through COX-1 which was supported by the fact that in endothelial cells, PGIS co-localizes with

COX-1 but not with COX-2 (10, 54). However, PGIS expression can be induced together with COX-2 in endothelial cells by hemodynamic shear stress or by oxidized low density lipoproteins (oxLDLs) leading to PGI₂ production (60, 61). Moreover, in healthy individuals COX-2 specific inhibitors reduce PGI₂ metabolites in urine without affecting TXA₂ metabolites (62). This suggests that COX-2 is a major source of PGI₂ in the human cardiovascular system and plays a role under physiological conditions. The predominant coupling of PGI₂ to COX-2 suggests that COX-2 specific inhibitor could be prothrombotic, since anti-aggregatory PGI₂ circulating levels within would be reduced. This has been proposed to explain the putative associations between COX-2 inhibitors and the development of cardiovascular complications (63, 64). In contrast to TP deficient mice, IP knockout mice have normal blood pressure and develop normally without suffering from spontaneous thrombosis. However, after endothelial damage they show an enhanced thrombotic response compared to control littermates (65), indicating that PGI₂ does play a major role only in response to stress and not in the basal systemic circulation. Mice lacking IP have enhanced vascular smooth cell proliferation and platelet activation in response to vascular injury likely caused by enhanced TXA₂/PGI₂ ratio (51). Simultaneous deletion of the TP and the IP abrogated both increased responses (51). Selective inhibition, knockout, mutation or deletion of COX-2 or IP has been shown to accelerate thrombogenesis. These responses were attenuated by COX-1 knock down (66). IP KO mice also show vascular hyperplasia and increased vascular remodeling (67) as well as cardiac hypertrophy and fibrosis (68). Taken together those results indicate that PGI₂ regulates the cardiovascular activity of TXA₂ and further support the hypothesis that cardiovascular homeostasis is resulting from the balance between these two eicosanoids.

ROLE OF PROSTANOIDS IN ATHEROSCLEROSIS

Atherosclerosis is one of the most important diseases of the developed countries and can be considered as a multifactorial inflammatory disease triggered by high levels of cholesterol in serum

leading to an inflammation in the intima of large arteries and involving several cell types including immune cells as T lymphocytes and monocytes/macrophages as well as endothelial and smooth muscle cells and platelets (69, 70). Among those, monocytes/macrophages play an important key role in many phases of atherogenic process. Thus, after an atherogenic stimulus, monocyte-macrophages reversibly adhere to the endothelium and migrate across it, leading to a prolonged retention of those cells in the intima which is central in atherogenesis. A variety of substances including prostanoids have been implicated in the pathogenesis of atherosclerosis.

Prostanoids may be involved in atherosclerosis through their ability to regulate a variety of mechanisms potentially involved in the pathogenesis such as inflammation, vasodilatation, vasoconstriction, platelet aggregation and leukocyte-endothelial cell adhesion, and leukocyte migration among others (71, 72). In this regard, COX-2 expression has been observed in symptomatic atherosclerotic lesions (73). This enzyme may play a dual role in the pathogenesis of the atherosclerosis. Initially, COX-2 expression is induced in monocytes by pro-inflammatory cytokines and several growth factors. Then, COX-2-mediated PG production by activated macrophages may promote atherosclerosis in the artery wall through several mechanisms, as induction of other proinflammatory mediators or by favouring migration of macrophages and other immune cells or by induction of adhesion molecules. Later on, COX-2-derived PGI₂, likely from the endothelial cells, may have a protective role in atherogenesis by favouring vasodilatation. Moreover, in atherosclerotic patients, PGI₂ can be formed through the action of both COX-1 and COX-2 (54). COX-1 but not COX-2 is expressed in normal arteries, whereas both isoforms are expressed in atherosclerotic lesions. In those lesions COX-2 is expressed not only by monocyte/macrophages, but also by endothelial and proliferating smooth muscle cells (7). Atherogenic lipoproteins, such as oxidized low density lipoproteins (oxLDL) may promote atherosclerosis first by contributing to inflammation by activating monocytes/macrophages and later by stimulating lipid uptake by macrophages, leading to foam cell formation. Interestingly, oxLDL has apparently contradictory effect on macrophages, since they can activate the expression of some pro-inflammatory genes

whereas reduce COX-2 expression (74). In agreement with this is the fact that macrophage-derived foam cells from the atherosclerotic lesions in mice, did not express COX-2. Thus induction COX-2 expression in macrophages takes place before their transformation into foam cells in the plaques.

In spite of the clear involvement of COX-2 derived prostanoids in vascular atherosclerosis, results on the effect of COX-2 selective inhibitors on the formation and progression of atherosclerotic plaques are controversial. Results from different studies have shown increased, reduced as well as unaltered atherogenesis (7, 62, 75-78). Those discrepant effects of COX-2 may reflect the dual role of COX-2 in promoting (early) but protecting later atherosclerotic lesions, mentioned above. Results about the influence of COXs in atherosclerosis in animal models, apart from those using pharmacological inhibitors, have been obtained by means of cell transplantation from fetal liver or bone marrow from COX-1 or COX-2 deficient mice into ApoE or LDLR KO mice (75, 79, 80). The size of the atherosclerotic lesions was significantly reduced when cells deficient in COX-2 were transplanted compared to the mice transplanted with wild type fetal liver cells (75, 80). Those results implicate COX-2 expression in the macrophage and not in other cells as endothelial cells, smooth muscle cells, or T-cells in promoting atherosclerotic lesion formation. Efforts to obtain information from mice deficient in both COX-2 and ApoE or LDLR genes have been unsuccessful due to the severe renal defects of COX-2 mice. Regarding to COX-1, both proatherosclerotic and anti-atherosclerotic roles also have been reported (79, 81).

Various prostanoids may play a significant role in the atherogenic process (71, 82). Patients with extensive disease as well as murine models of atherosclerosis have enhanced formation of TXA₂. Even more, TP antagonists decrease atherogenesis in mice. TP deficiency in atherosclerotic mice models induced a significant delay in atherogenesis, compared with mice deficient in apoE alone (72).

In contrast, PGI₂ may theoretically have a beneficial effect in the atherogenic process by limiting platelet adhesion to the endothelium and activation in the plaques. In accordance with this, local delivery of PIGS gene through an adenoviral vector reduces the platelet deposition seen

following vascular injury (83). Genetic deletion of the IP receptor, both in the LDLR and in the ApoE KO mice models, aggravated atherogenesis (72, 84). As platelets are thought to contribute to the development and progression of atherosclerosis in the late phase, PGI₂ may likely suppress lesion formation by limiting platelet deposition. Those mice exhibited a significant acceleration in atherogenesis with enhanced platelet activation and increased rolling of leukocytes on the vessel walls. Those results indicate that TXA₂ promotes whereas PGI₂ prevents the initiation and progression of atherogenesis by modulating platelet activation and leukocyte-endothelial cell interaction.

In addition to PGI₂ and TXA₂, other PGs, such as PGE₂ and PGD₂, could also play an important role in the pathophysiology of atherosclerosis. In this regard, vasoconstrictor responses to PGE₂ are greatly increased in atherosclerosis (85) and PGE₂ may also promote angiogenesis (3). Moreover, inducible mPGES-1 has been detected in activated macrophages in atherosclerotic lesions where it colocalizes with COX-2 both in mice and humans (73). Disruption of this enzyme in mice reduced foam cell formation and atherosclerosis in fat-fed LDLR^{-/-} mice. mPGES-1 deletion augmented both PGIS and TXS expression in endothelial cells (86). On the other hand, COX-2 derived PGs as PGD₂ may possess anti-inflammatory and anti-atherosclerotic properties in such a way that the balance between PGDS and PGES has been shown to be a major determinant of atherosclerotic plaque instability (87).

PHARMACOLOGICAL IMPLICATIONS

Based on the hypothesis that atherosclerosis is an inflammatory disease, it was proposed that COX inhibition by NSAIDs, and in particular selective inhibition of COX-2 by the Coxibs family of NSAIDs, might have protective and even anti-atherogenic effects. However, clinical studies have indicated that there is an increased risk of atherothrombosis in individuals taking these drugs in such a way that some of them as Rofecoxib have been recently withdrawn from the market (88-91). These undesirable effects of COX-2 selective inhibitors have been explained by the TXA₂/PGI₂ balance theory by which selective

inhibition of COX-2 lead to a reduction in the production of the vasodilator PGI₂ whereas production of the vasoconstrictor and pro-aggregatory TXA₂, mostly COX-1–dependent, remains unaffected. Thus, disruption of the physiological balance between TXA₂ and PGI₂ accelerates atherosclerosis and increases the risk of thrombosis and other cardiovascular complications (Figure 3).

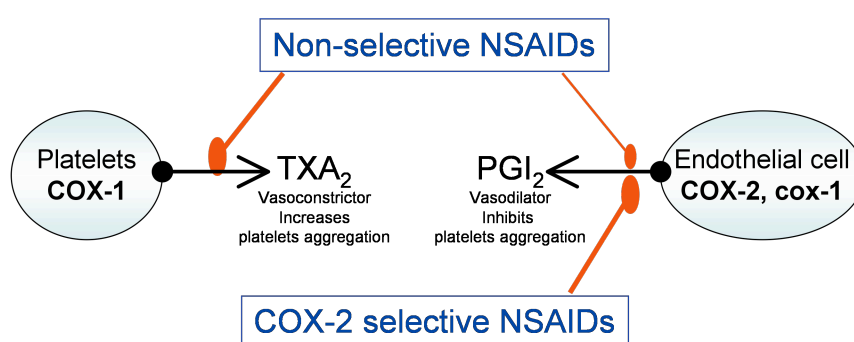


FIGURE 3.- *The balance hypothesis for the cardiovascular effects of NSAIDs.* Cardiovascular homeostasis is controlled by the balance between COX-1 –dependent production of TXA₂ by platelets and COX-2 –mediated PGI₂ (prostacyclin) production by endothelial cells. Whereas TXA₂ function as a potent platelet activator and vasoconstrictor, PGI₂ inhibits platelet aggregation and thrombosis. Classical non-selective NSAIDs reduce the production of TXA₂ by platelets through their ability to inhibit COX-1 activity thus displaying anti-thrombotic properties. In contrast, COX-2 selective NSAIDs reduce PGI₂ formation by endothelial cells consequently disturbing the equilibrium between TXA₂ and PGI₂ and potentially favouring pro-thrombotic cardiovascular events.

Nevertheless, the mechanisms underlying the pathogenesis of cardiovascular complications upon NSAIDs administration remain to be clarified as the TXA₂/PGI₂ balance theory is somewhat simple and it does not consider some important clinical and experimental evidences as the increased risk of cardiovascular events by some non-selective NSAIDs, the contribution of other prostanoids to the overall effect in cardiovascular physiopathology, and the COX-2 independent effects of NSAIDs. In this sense, a more profound knowledge of the complex

relations between prostanoids-mediated actions and function of cells in the cardiovascular system is required to clearly understand the benefits and risks of NSAIDs on cardiovascular diseases.

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