Role of Cyclooxygenase Pathway and Risk Associated with Non-Steroidal Anti-inflammatory Drugs Therapy in Cardiovascular Diseases

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ABSTRACT
Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the cyclooxygenase enzyme activity through different mechanisms and prevent inflammation. But they all have different risks associated with them. Some are associated with gastrointestinal bleeding and some are strongly allied with the cardiovascular risks. Cyclooxygenase enzyme regulates prostaglandin synthesis by converting arachidonic acid present at the sn-2 position of membrane phospholipids to prostaglandin H2. Prostaglandin H2 is the precursor of all prostaglandins. There are two isoforms of cyclooxygenase enzyme, cyclooxygenase-1 and cyclooxygenase-2 which differ in their active site due to an isoleucine to valine substitution at amino acid 523 in cyclooxygenase-2. Cyclooxygenase-1 is constitutively expressed in platelets where it helps in the formation of thromboxane whereas cyclooxygenase-2 is inductive form and is expressed in the endothelial cells due to shear stress and forms prostacyclins. Both thromboxanes and prostacyclins maintain the homeostasis of the vascular wall. During vascular injury prostacyclin production decreases as a result of which thromboxane synthesis increases in the platelets which leads to platelet aggregation. Although, being strongly associated with cardiovascular risks, NSAIDs are still prescribed to the patients to prevent pain according to their condition. So this review aims to summarise the mechanism of cyclooxygenase pathway, possible mechanism of action of NSAIDs and the risks of cardiovascular events associated with the use of NSAIDs.

Keywords: Cyclooxygenase; Prostacyclins; Thromboxane; Prostaglandins; Cardiovascular Disease; NSAIDs.

1. INTRODUCTION
Non-Steroidal Anti-inflammatory Drugs (NSAIDs) are the most prescribed drugs over years in various conditions like pain, arthritis, osteoarthritis and other medical conditions. They show anti-inflammatory, antipyretic and analgesic properties by inhibiting the synthesis of prostaglandins via cyclooxygenase pathway. Yet there are some side effects related to the use of NSAIDs like gastric ulcers, gastrointestinal bleeding and recently it has also been found that continuous use of NSAIDs is also linked to cardiovascular diseases. Despite many advances in the prevention and management, cardiovascular diseases still remain the leading cause of morbidity and mortality in individuals. The main target for the action of NSAIDs is cyclooxygenase (COX) enzyme, which is the key enzyme for the synthesis of prostaglandins (PGs). Cyclooxygenase enzyme acts on arachidonic acid (C-20:4) released from sn-2 position of the phospholipids present on cell membrane through the action of phospholipase A2 during inflammation. Cyclooxygenase enzyme catalyzes the double dioxygenation of arachidonic acid via two reactions, first a cyclooxygenation reaction in which an oxygen molecule binds to arachidonic acid to form prostaglandin G2 and second a peroxidation reaction where prostaglandin G2 is reduced to a more stable prostaglandin H2. There are two isoforms of cyclooxygenase enzyme (cyclooxygenase-1 and cyclooxygenase-2) which differ in their active site due to an isoleucine to valine substitution at amino acid 523 in cyclooxygenase-2. This substitution makes cyclooxygenase-2 more permissive to utilize other fatty acids in addition to arachidonic acid. Cyclooxygenase enzymes are homodimeric in nature and each dimer subunit consists of three domains, namely the membrane binding domain, the epidermal growth factor binding domain, and the catalytic domain. Cyclooxygenase-1 is constitutively expressed in various cells like platelets, endothelial lining of stomach, kidneys, etc. but expression of cyclooxygenase-2 depends on other factors like shear stress, inflammatory mediators, etc.

2. SYNTHESIS OF PROSTAGLANDINS
The prostanoids belong to the oxylipin family of lipids synthesised by specific prostaglandin synthase enzyme which confer stereo specificity and chirality on every functional group. The naming of each prostaglandin is done by prefixing 'PG' followed by a letter A to K that depends on position and nature of substituents on their ring as shown in Fig. 2. The total numbers of double bonds in the alkyl substituents are denoted by a numerical suffix (1 to 3), where the numbering depends on the nature of the precursor fatty acid. Prostaglandins are produced by cyclooxygenase enzyme inside the cell as the signal passes from the membrane receptor causing an increase in intracellular concentration of arachidonic acid via calcium-dependent phospholipase A2. First molecule...
as an agonist for platelet aggregation\textsuperscript{22}. It also mobilizes calcium ions from intracellular storage sites and triggers platelet granules to release active substances like thromboglobulin, coagulation factor VIII, von Willebrand factor, fibrinogen, and ADP\textsuperscript{23}. Thromboxane directs different biological processes like vasoconstriction, endothelial adhesion molecules expression and proliferation via its cell surface receptor TP\textsuperscript{24}. On the other hand Prostacyclins exert multiple vasoprotective effects in the cardiovascular system by vasodilation and inhibition of platelet aggregation similar to the action of endothelium-derived nitric oxide (NO)\textsuperscript{25}. Enzymes required for the production of prostacyclins are localised around the nuclear membrane and on the endoplasmic reticulum of the vascular endothelial cells\textsuperscript{26}. An increase in intracellular calcium is required for activation of cytoplasmic PLA2. Shear stress in the endothelium activates the cytoplasmic PLA2 which removes arachidonic acid from the membrane, which is acted upon by cyclooxygenase-2 and thus gets converted into prostacyclins with the help of prostaglandin I synthase\textsuperscript{27}. Prostacyclins increase the concentration of cAMP which is the inhibitor of platelet aggregation\textsuperscript{28}. They also inhibit the expression of platelet activation markers like platelet fibrinogen receptor, P-selectin, platelet-leukocyte aggregation, and granular release of serotonin\textsuperscript{29}. Shear stress also leads to generation of nitric oxide via endothelial nitric oxide synthase, which in turn inhibits platelet aggregation by increasing the production of cGMP and suppressing the action of thromboxane synthase\textsuperscript{30}. This action by endothelial cells masks the action of thromboxane formed in the platelets and maintains the integrity of the vascular wall\textsuperscript{31}. As shown in Fig. 4, in normal homeostasis prostacyclins are continuously formed by the cyclooxygenase-2 action in the

3. MECHANISM OF ACTION OF THROMBOXANE AND PROSTACYCLINS

Thromboxane is predominantly synthesised in the platelets with the help of thromboxane synthase\textsuperscript{31}. Thromboxane increases the G-protein activation which releases ADP from dense granules, which acts

Figure 1. Glycosylation sites in cyclooxygenase-1 and cyclooxygenase-2. (Sp- signal peptide, Dm- dimerization domain, EG- epidermal growth factor domain, Mb- membrane binding domain, Cat- catalytic domain, and MTS- membrane targeting sequence).

Figure 2. Structure of prostaglandins, prostacyclins (PGI) and thromboxane (TXA, TXB).

Figure 3. Generation of prostaglandins from arachidonic acid via action of cyclooxygenase enzyme.
vascular endothelial cells and bind to the IP receptor on platelets which causes an increase in the intracellular concentration of cAMP that suppresses the cytosolic concentration of Ca²⁺ and hence blocks the generation of thromboxane. In case of vascular injury, platelets bind to the exposed collagen with the help of vWF, which activates phospholipase. Phospholipase increases the cytosolic concentration of Ca²⁺ which binds to PLA2 and releases arachidonic acid on which cyclooxygenase enzyme acts and thromboxane is generated. As thromboxane is generated, it comes out of the platelets and binds to other platelet membrane receptors and leads to the production of more thromboxane and thus causes platelet aggregation.

4. BALANCE BETWEEN PROSTACYCLIN AND THROMBOXANE IN CARDIOVASCULAR DISEASES

During vascular injury, ADP and thrombin are released from adherent platelets which results in activation of cytoplasmic PLA2 and generation of thromboxanes. Many studies have established thromboxane as a mediator in cardiovascular diseases. Aspirin irreversibly inhibits platelet cyclooxygenase, even when given in low doses, thus being used as an antiplatelet therapy for the preventing vascular thrombotic events and indicating the role of thromboxane as a platelet agonist in cardiovascular diseases. Prostacyclins have vasodilatory as well as antithrombotic effects which are opposed by thromboxanes. Knockout mice for prostacyclin receptor showed propensity towards atherosclerosis, increased thrombosis, and proliferative response to carotid vascular injury. Mice knocked out for both, prostacyclins and thromboxanes receptors, had similar vascular remodeling as wild-type control mice. Deletion of prostacyclin receptors led to aggregation of platelets and did not inhibit thrombus formation. Prostacyclin concentration remains higher in normal homeostasis so that it can prevent platelet aggregation but in case of vascular injury, endothelial cells are no longer able to produce prostacyclin due to which thromboxane production from platelets increases platelet aggregation.

5. NSAIDS MECHANISM OF ACTION AND RISKS ASSOCIATED WITH THEM

NSAIDs are having anti-inflammatory, analgesic and antipyretic activity and they achieve their effect by inhibiting prostaglandin synthesis. NSAIDs vary in their selectivity for the cyclooxygenase-1 and 2, hence are categorised as nonselective, semi selective and selective cyclooxygenase inhibitors. Non selective NSAIDs like aspirin, ibuprofen and naproxen, inhibit both isoforms of cyclooxygenase enzyme and among them, aspirin irreversibly inhibits the cyclooxygenase activity. At low therapeutic doses, aspirin is up to 100-fold more potent in inhibiting platelet cyclooxygenase-1 than cyclooxygenase-2 by covalent acetylation of serine residues in their
active sites that leads to permanent inhibition of cyclooxygenase enzyme activity\textsuperscript{43}. Aspirin exhibits cardio protective effects by inhibiting platelet aggregation in the arteries of the heart, but administration of non-selective NSAIDs are associated with serious conditions like peptic ulcers and gastrointestinal bleeding because blocking of cyclooxygenase enzyme in stomach leads to disruption of the integrity of the stomach lining and blocking the same in kidneys has a decisive role in the regulation of glomerular filtration\textsuperscript{44}.

In a cohort study in a group of 52,293 patients taking NSAIDs, the relative risk of hospitalisation for upper GI problems was 3.9 (2.93-4.78)\textsuperscript{57}. Naproxen mimics the activity of acetylsalicylic acid (aspirin) by suppressing platelet cyclooxygenase, but the role of naproxen in the development of CVD is controversial\textsuperscript{46}. It has been suggested that ibuprofen is strongly associated with the cardiovascular risks similar to that of celecoxib (a selective NSAID)\textsuperscript{47}. A second class of NSAIDs (diclofenac, indomethacin, meloxicam) inhibits cyclooxygenase-2 up to a greater extent as compared to cyclooxygenase-1 hence called semi-selective NSAIDs, but any evidence of it being associated with cardiovascular diseases is not known\textsuperscript{48}. Diclofenac has been shown to increase the occurrences of myocardial infarction and stroke even at lower doses than naproxen at a higher dose\textsuperscript{49}. Cardiovascular risks associated with the use of meloxicam are still unknown, as the number of trials assessing meloxicam are very limited\textsuperscript{50}. Another class of NSAIDs (celecoxib and rofecoxib) selectively inhibit cyclooxygenase-2, hence are termed as selective NSAIDs\textsuperscript{51}. Selective NSAIDs are recommended for the patients who have painful intestinal bleeding problems\textsuperscript{52}. The VIGOR study for assessing the risk of gastrointestinal events on administration of selective NSAIDs showed that the risk of development of gastrointestinal events was lower in the case of rofecoxib as compared to naproxen\textsuperscript{53}. However, prolonged exposure to any class of NSAIDs, (irrespective of their mechanism of action) has unveiled potential adverse effects on cardiovascular events, depending on the dosage and duration of these drugs, in patients having or not having preexisting cardiovascular conditions\textsuperscript{44}.

Rofecoxib contains a methylsulfonyl group in place of carboxyl group of NSAIDs which binds to the hydrophobic region in the active binding site of cyclooxygenase-2\textsuperscript{55}. Even in very small doses, Rofecoxib inhibits the synthesis of prostacyclins by selectively inhibiting cyclooxygenase-2 and allowing the formation of thromboxane in platelets, which increases the risk of cardiovascular diseases\textsuperscript{56}. The VIGOR study was performed to evaluate the cardiovascular safety of rofecoxib as compared to naproxen in which 8076 people volunteered and calculated relative risk was 2.37 (1.39-4.06) with rofecoxib\textsuperscript{57}. Similarly, in the Adenomatous Polyp Prevention on Vioxx (APPROVe) study which was done to analogize rofecoxib and placebo, an increased episode of thrombotic events explicitly myocardial infarction and stroke, with rofecoxib therapy was observed\textsuperscript{58}.

Another selective NSAID, celecoxib contains a sulfonamide group in place of a carboxyl group but risks associated with smaller doses of celecoxib are not clear so far\textsuperscript{59}. A meta-analysis of randomised control trials comparing the risks involved in taking selective NSAIDs with those in other non-selective NSAIDs and placebo was performed on a total of 116,429 patients. In this study, acute myocardial infarction (AMI) was considered as the primary outcome and the results for three non-selective NSAIDs (naproxen, ibuprofen, diclofenac) and two coxibs (rofecoxib, celecoxib) was statistically evaluated in which the highest risk of AMI as compared to placebo was associated with ibuprofen (RR = 1.61; 0.50-5.77) and rofecoxib (RR = 2.12; 1.26-3.56)\textsuperscript{60}. Recently, in a meta-analysis of 23 controlled observational studies (six cohorts and 17 case-controls), 75,520 coxibs users and 375,619 users of nonselective NSAIDs were compared to 594,720 patients with absolutely no exposure to NSAIDs and it was analysed that out of all non-selective NSAIDs, the maximum risk for cardiovascular diseases was contingent on diclofenac (RR = 1.40; 1.16-1.70) and the safest was naproxen (RR = 0.9; 0.87-1.07) and in case of rofecoxib (RR = 1.35; 1.15-1.59), the risk was significantly influenced by the dose\textsuperscript{61}.

6. CONCLUSION

Cardiovascular diseases remain the pivotal topic of research as it is one of the leading causes of mortality all over the world. There are different mechanisms by which it occurs but alteration of cyclooxygenase pathway can be regarded as an important one. In normal homeostasis, endothelial cells produce prostacyclins by the action of cyclooxygenase-2 enzyme, which maintains the integrity of endothelial cells through inhibition of platelet aggregation and vasodilation. During vascular injury, platelets bind to collagen with the help of vWF and increase cytosolic calcium ion concentrations, activating cytoplasmic PLA2 which ultimately leads to the formation of thromboxane. NSAIDs are the drugs which are used worldwide by patients for cardiovascular diseases. These drugs inhibit the action of cyclooxygenase enzyme directly and further block the formation of thromboxane and prostacyclins. Among the NSAIDs, non-selective NSAIDs inhibit both the isoforms of cyclooxygenase hence they are associated with the greater risk of peptic ulcers and gastrointestinal bleeding. Naproxen also mimics the action of aspirin by blocking cyclooxygenase-1 enzyme but its association with cardiovascular risks is controversial. Ibuprofen is strongly associated with the cardiovascular risks similar to other selective NSAIDS. Diclofenac is associated with myocardial infarction even at very small doses and risk associated with meloxicam is not known due to limited number of trials. Selective NSAIDs (like rofecoxib and celecoxibs), are used for those patients who have intestinal bleeding problems but their use is strongly associated with the cardiovascular risks. Both selectively inhibit the COX-2 enzyme and the formation of prostacyclins in the endothelial cells. From the above data it can be concluded that selective COX-2 inhibitors are strongly associated with the cardiovascular risks as compared to the non-selective NSAIDs.

7. FUTURE PROSPECTS

NSAIDs have various side effects in patients with cardiovascular conditions because they may cause hemorrhagic events. The chances of these associated side effects are more in those patients taking NSAID monotherapy and also in those taking concomitant antiplatelet and/or anticoagulant therapies,
target-specific oral anticoagulants and vitamin K antagonists. Hence, the understanding of probable danger associated with the use of NSAIDs in patients who have cardiovascular risk factors is crucial. It has been stated that risk of cardiovascular diseases with NSAIDs appears to augment as the dosage and period of therapy increases. For NSAID therapy, a careful assessment of the individual risks and benefits should be conducted before prescribing the lowest effective dose for the shortest duration of time. Although patients should also be educated on the risks of continued NSAID therapy and the importance of reporting all the NSAIDs used during medical history. So, it can be concluded that there is no such COX inhibitor present so far that has minimum or no side effects and can be used widely. As such, various aspects of NSAID induced cardiotoxicity still need to be investigated; including the significance of NSAID induced cardiovascular risks, so that an accurate and competent drug can be formulated in near future that could reduce the side effects of NSAIDs.

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