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A review on formulation and evaluation of Cox2 inhibitor

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Abstract
Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used therapeutic agents that exhibit frequent and sometimes severe adverse effects, including gastrointestinal ulcerations and cardiovascular disorders. In an effort to obtain safer NSAIDs we assessed the direct cyclooxygenase (COX) inhibition activity and we investigated the potential COX binding mode of some previously reported 2-(trimethoxyphenyl)-thiazoles. The *In vitro* COX inhibition assays were performed against ovine COX-1 and human recombinant COX-2. Molecular docking studies were performed to explain the possible interactions between the inhibitors and both COX isoforms binding pockets Inflammation is a complex phenomenon necessary in human defense mechanisms but also involved in the development of some human diseases. The discovery of cyclooxygenase-2 (COX2) improved the pharmacology of no steroidal anti-inflammatory drugs (NSAID) giving a clear mechanism for prostaglandin regulation *In vivo* and providing a new target for the development of COX-2-selective drugs without gastrointestinal side-effects. Keeping in view the importance of this pharmacological class, several literature reports have underlined the impact of these anti-inflammatory compounds in therapeutics. The present review considers the most recently published literature concerning COX-2 inhibitors until 2016. Through a wide chemical classification, the last developments concerning this therapeutic family by highlighting structure-activity relationships insights and mechanisms are presented. A summary of the principal adverse effects observed and an overview of the new potential therapeutic indications for COX-2 inhibitors are also reported.

Keywords: COX-2 inhibitors, Inflammation, Non-steroidal anti-inflammatory Drugs, Cyclooxygenase, COX-1/COX-2 inhibition, Structure–activity relationship

Introduction

It is well established that NSAIDs act by blocking the production of pro-inflammatory prostaglandins through the inhibition of cyclooxygenase (COX). At least two isoforms of COX are known COX-1 and COX-2. COX-1 is mainly considered a "housekeeping enzyme". It is widely distributed in most tissues where it performs mainly physiological roles like: protecting the gastric mucosa, kidney function maintenance and protection or regulating platelet aggregation stimulating thromboxane A2 (TXA2). By contrast COX-2 is viewed primarily as responsible for the initiation and maintenance of the inflammation process with only minor physiological roles like stimulating prostacyclin (PGI2) production and thus preventing platelet aggregation.

Inflammation is a complex phenomenon essential in human defense mechanisms but is also involved in the development of some human diseases. The inflammation process is characterized by four cardinal signs redness, heat, pain and swelling. Among all the mediators participating in the inflammation process the Prostaglandins (PG) remain the major target of anti-inflammatory therapy since non-steroidal anti-inflammatory drugs (NSAIDS) mechanism of action lies in the inhibition of PG biosynthesis. The story started in 1971 when Vane demonstrated that the blockade of prostaglandin synthesis by aspirin was due to the inhibition of a Prostaglandin G/H Synthase (PGHS) enzyme that he proposed to name Cyclooxygenase (COX). Twenty years later, a second COX isoform was discovered. The first COX isoform, identified by Vane and isolated in 1976 by Hemler was renamed COX-1 whereas the second one unknown until 1991 was named COX-2. These two isoforms catalyze the same biotransformation of arachidonic acid but present some differences, notably in terms of expression, function and structure COX-1 is constitutively expressed in most tissues and exerts housekeeping functions such as maintaining the homeostasis or gastric cytoprotection. On the opposite, COX-2 is inductible, usually undetectable under physiological conditions in most tissues except prostate, kidney, brain and smooth muscle. The COX-2 isoform is notably activated by pro-inflammatory stimuli and presents mainly a pro-inflammatory function. However, evidences for COX-2 involvement in gastric mucosal defense, renal homeostasis and vascular systems have been made. COX-1 and COX-2 share a sequence identity of 60%, present closely similar three-dimensional structure but their active sites.Non-Steroidal Anti-inflammatory drugs (NSAIDs), including selective cyclooxygenase (COX)-2 inhibitors, have come to play an important role in the pharmacologic management of musculoskeletal disorders. Clinical trials have established the efficacy of COX-2 inhibitor like Etoricoxib in

Osteoarthritis Rheumatoid Arthritis Acute Gouty Arthritis, Ankylosing Spondylitis, Low back pain, acute postoperative pain and primary dysmenorrheal. The present research has been undertaken with the aim to develop a novel semisolid dosage form of etoricoxib, which would attenuate the gastrointestinal relater toxicities associated with oral administration. Etoricoxib is a highly selective cyclooxygenase-2 (cox-2) inhibitor. In the present study, a fixed concentration of Etoricoxib cream (2%) was prepared by using a different combination of active ingredient and excipients Coxibs are NSAIDs that are highly selective for the COX2 enzyme. Because the COX2 enzyme mediates prostaglandin production responsible for inflammation and pain, coxibs are analgesic and antiinflammatory, but they lack the side effects related to inhibiting the COX1 enzyme (e.g., bleeding and gastrointestinal irritation). Like nonselective NSAIDs, which affect both COX1 and COX2 enzymes, coxibs are used in both OA and RA. The three currently approved coxibs are celecoxib, rofecoxib, and valdecoxib. Examples of nonselective NSAIDs are indomethacin, ibuprofen, and diclofenac. Some patients respond to one NSAID or coxib but not to another; the reasons for this remain unclear. It is recommended that more than one of these drugs be tried before considering this type of therapy unsuccessful. The use of some coxibs have been restricted because of their propensity to cause increases in blood pressure and because their use is associated with a higher incidence of myocardial infarction.

Pharmacosomes are novel vesicular drug delivery system. They are colloidal dispersion of drug covalently bound to lipids. They provide an efficient method for the delivery of drug to the target site. The physicochemical properties depend on drug as well as the lipid. Pharmacosomes may be hexagonal aggregates, ultrafine vesicular and micellar form. Both synthetic and natural drugs which are facing difficulties like low solubility and low permeability can be effectively formulated. Pharmacosomes have been prepared for various NSAIDs, proteins, cardiovascular and antineoplastic drugs. Developing the Pharmacosomes of the drugs has been found to improve the absorption and minimize the gastrointesinal toxicity. Pharmacosomes are amphiphilic complexes of drug with lipids. The amiphiphilic character help to reduce interfacial tension leading to increase in contact area and increase bioavailability of drugs. Inflammation is the part of the body defense mechanism. It is the process by which the immune system recognizes and removes harmful stimuli and begins healing process. There are two types of inflammation: acute and chronic inflammation. The symptoms of acute inflammation include pain, redness, swelling and heat. Chronic inflammation includes fatigue, chest pain, abdominal pain, rash, fever and joint pain. There are more than 100 different types of arthritis and related conditions. Arthritic joint symptoms include swelling, pain, stiffness and finally decreased range of motion. The most common treatment for rheumatoid arthritis or arthritis include non-steroidal anti-inflammatory drugs (NSAIDs) corticosteroids, disease modifying anti- rheumatic drugs (DMARDS), and some biological agent. Oral route is the most preferable route of drug delivery. Treatment with NSAID through oral route is associated with side effects like ulceration and GI bleeding. It's poor water solubility which affect it's dissolution in GI fluid, lead to poor bioavailability. Selective COX- 2 inhibitors should be used in patients at higher risk of peptic ulcer, perforation or bleeds. If selected, they should be administered in the lowest dose for the shortest period of time. Moreover, it should be avoided in patients with history of ischemic heart disease/hypertension/cardiac failure/cerebrovascular disease etc. In order to avoid this toxicity, cardiovascular risk and for better therapeutic effect, is to be delivered through skin. Pharmacosomes are novel drug delivery system, resolving so many related problems and issues to the conventional dosage form such as the drug release at the specific site of desired rate for achieving controlled or targeted drug delivery. When handling, there are possibility of leaching as the drug is bounded to the lipid by covalent bonding and also the entrapment efficiency is high. Pharmacosome drug delivery system suitable for both hydrophilic and lipophilic drugs. Pharmacosomes reduce the cost of therapy, adverse effects and toxicity. They improve in the bioavailability of poorly soluble drugs.

Discuss some cardiovascular issues associated with selective COX2 inhibitors

NSAIDs and coxibs do not provide the same protective effects as low-dose aspirin. Coxibs (selective COX2 inhibitors) decrease vascular prostacyclin (PGI2) production and may affect the balance between prothrombotic and antithrombotic eicosanoids. However, the available studies can suggest only that there is a potential increase in cardiovascular events compared to the traditional NSAIDs. In patients taking a coxib agent, the recommendation is to maintain low-dose daily aspirin in patients who are at significant risk of a cardiovascular event. However, the use of low-dose acetyl salicylic acid (ASA) does not consistently negate the potential cardiovascular risk of COX2 inhibitors.

COX-2 Inhibition as an Example of an Anti-Inflammatory Therapeutic Approach in MD

COX-2 inhibitors influence the CNS serotonergic system, either directly or via CNS immune mechanisms. In a rat model, treatment with rofecoxib was followed by an increase of serotonin in the frontal and the temporoparietal cortex (Sandrini, Vitale, & Pini, 2002). Therefore COX-2 inhibitors would be expected to show a clinical antidepressant effect. In the depression animal model of the bulbectomized rat, a decrease in hypothalamic cytokine levels and a change in behavior have been observed after chronic celecoxib treatment (Myint, Steinbusch, *et al.*, 2007). In another animal model of depression, however, the mixed COX-1/COX-2 inhibitor acetylsalicylic acid showed an additional antidepressant effect by accelerating the antidepressant effect of fluoxetine (Brunello *et al*., 2006). A significant therapeutic effect of the COX-2 inhibitor celecoxib in MD was also found in a randomized, double-blind pilot add-on study of reboxetine and celecoxib versus reboxetine and placebo (Müller *et al*., 2006). Interestingly, the ratio of kynurenine to tryptophan, which represents the activity of the proinflammatory cytokine-driven enzyme IDO, predicted the antidepressant response to the celecoxib therapy. Patients with a high activity of IDO, i.e., a high proinflammatory activity, responded better to celecoxib. Another randomized, double-blind study in 50 depressed patients suffering from MD also showed a significantly better outcome of the COX-2 inhibitor celecoxib plus fluoxetine than with fluoxetine alone (Akhondzadeh *et al*.,

2009). This finding was replicated using the combination of sertraline and celecoxib in 40 depressed patients (Abbasi, Hosseini, Modabbernia, Ashrafi, & Akhondzadeh, 2012). Interestingly, the blood levels of IL-6 predicted the antidepressant response both in the sertraline (plus placebo) and in the celecoxib (plus sertraline) groups.

Evaluation methods of COX-2 Inhibitors

Various methods have been developed to evaluate drugs inhibitory activity against COX-1 and COX-2. *In vitro* assays use both enzymes and cells. The most frequently used enzymatic methods are based on purified or recombinant enzymes or microsomal preparation of cell line U937 Cellular methods include human whole blood insect cells various mammalian cells and platelets. However, the use of different and non-standardized methods for the evaluation of COX-1 and COX-2 IC50 jeopardizes the comparison of these data tween studies. To ensure a solid evaluation of COX-2 potency at least one enzymatic and one cellular experiment using as reference known COX-2 inhibitors should be combined. Four main *In vivo* assays are used: carrageenan-induced paw edema assay carrageenan induced analgesia models in rat's 3adjuvantinduced arthritis model and endotoxin induced pyretic response in rats these assays enable to quantify respectively the anti-inflammatory, the analgesic, the chronic anti-inflammatory and the antipyretic properties of compound.

COX-2 Selective Agents

Analogues of Classical NSAIDs Numerous analogues of classical NSAIDs were synthesized with the aim to preserve or enhance their potency and to improve their selectivity. Salicylates Derivatives Numerous analogues of aspirin have been developed; among them the substitution of the acetate group by a sulfonamide moiety increased the COX-2 selectivity by 1000- to 10000-fold. Meclofenamic acid modified compounds the replacement of meclofenamic acid carboxylate group with amides enabled to increase the COX-2 selectivity by 900 to 1400-fold other compounds were complexed with metals and demonstrated potent and selective COX-2. Inhibition. Aryl acetic acids several analogues of indomethacin displayed great potency and selectivity against COX-2. The indomethacin structure modifications included the synthesis of ortho-carbaborane derivatives replacing the Me (R1) of the parent drug with a CF3 group the acid carboxylic moiety by large and complex substituents (SI up to 333000 and IC50 = 0.3 nM for compounds 36r and 36s). SAR studies on the diclofenac scaffold indicate that the introduction of halogen atoms (Cl or F) enhance the selectivity. The same effect was observed with an alkyl group in meta-position on the phenyl bearing the COOH moiety. Particularly lumiracoxib (38c) exhibited a high COX-2 potency (IC50 = 7 nM) and selectivity (SI> 1428) explained by its methyl group that enabled a better insertion in the COX-2 active site. However, lumiracoxib was withdrawn from the market in several countries, according to severe liver side effects. Concerning etodolac derived compounds they have shown poor COX2 selectivity (40b, SI>45) compared to his molecule parent (40a, SI = 142). Indeed, replacing the oxygen atom of etodolac by a methyl moiety drives to a decrease of COX-2 selectivit. Arylpropionic acids Flurbiprofen derivatives modified on the phenyl ring attached to the arylpropionic acid presented enhanced COX-2 selectivity and inhibition potency. Some ketoprofen analogues (42b-c) displayed a strong enhancement of selectivity (COX-2 SI >1100) by replacing the R3 substituent (N3 >> SO2Me > NHCOMe). This can be explained by the insertion and stabilization of this 4-N3Ph group into the COX-2 side pocket. Oxicams modified analogues enhance COX-2 selectivity by >200-fold with IC50 = 0.06 µM.

COX Inhibitors: FUTURE THERAPIES FOR CANCERS AND NEURONAL DISEASES COX

Inhibitors were developed to treat inflammation and can thus be used in a large number of inflammatory diseases. However, for more than twenty years, the use of COX inhibitors to treat cancers and neuronal diseases such as Alzheimer's and Parkinson's is investigated.

Cancer

The involvement and the over-expression of COX-2 in cancers have been reported. Evidence suggests that NSAIDS tumor inhibition could be mediated by their ability to inhibit angiogenesis and to restore apoptosis in APC-deficient cells. However, the knowledge of the anti-oncogenic mechanism remains uncompleted and the involvement of LOX and COX- independent pathways were also highlighted. NSAIDs could be used to prevent and to participate in tumor decrease in several cancers The COX-2 overexpression in colorectal cancer reaches 80%. Several clinical studies revealed that NSAIDS could be used for the prevention (long-term use of aspirin and treatment of colon cancer celecoxib and rofecoxib in animal models, other NSAIDs like piroxicam, indomethacin, sulindac, ibuprofen or ketoprofen decrease 40-50% the risk to develop the colon cancer. Similarly, in familial adenomatous polyposis (FAP), celecoxib or the combination of aspirin and sulindac inhibits significantly the growth of adenomatous polyps and leads to the regression of existing polyps. Several studies described the potency of aspirin and NSAIDs in the prevention of esophageal cancer Coxibs and particularly celecoxib are used to prevent tobacco-related cancers or to reduce tumoral growth Celecoxib and its derivatives are efficient anti proliferative agents in prostate and breast cancer Nimesulide is used in the treatment of breast cancer where COX-2 is overexpressed up to 40%.

Alzheimer's disease (AD)

Several epidemiological studies suggest an association between the long term use of NSAIDS and a reduced risk of AD However, this protection differs according to the NSAIDS used. Some NSAIDS such as ibuprofen and naproxen demonstrated significantly reduced AD risk However, rofecoxib in a 12-18 month clinical trial of in patients with mild cognitive impairment showed on the contrary no protective effect on AD development and was even suspected to increase the rate of conversion to AD. In fact, several factors seem to be essential for NSAIDS protection effect against AD early NSAIDS chronic exposition a COX-1 inhibition potency. The initial hypothesis that the COX-2 inhibition could be responsible for a reduced neuro inflammation and thus the protective effect is now refuted but the complete mechanisms of amyloid accumulation reduction are still unclear.

Parkinson's disease (PD)

Patients suffering from PD present an over-expression of COX-2 in the brain. Several evidences indicate that COX-2

is involved in the pathogenesis of PD and could be an interesting target to delay the apparition of PD or to stop its progression. Rofecoxib and Parecoxib are notably considered as neuroprotective agents.

Conclusion

Since its discovery in 1990's, COX-2 enzyme has aroused the development of a great number of selective inhibitors with chemical diversity, different in inhibitory potency, contrasting in their ability to reduce side effects and enhancing tolerability compared to classical NSAIDs. Recently, an important effort has been focused on COX-2 selective drugs as future therapies for a wide kind of cancers and neuronal diseases. Currently, new researches have turned toward nanotechnology where selective drugs will be associated with nanoparticles

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