



Non-steroidal anti-inflammatory drugs: Overview

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ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are used as common medicine to treat various symptoms of arthritis. NSAIDs stop cells making prostaglandins and provide relief from pain and stiffness. They work quickly, usually within a few hours and are usually taken by mouth in tablet or capsule form. NSAIDs are also available as liquids, injections, creams, sprays and suppositories. These NSAIDs are structurally diverse and differ in pharmacokinetic and pharmacodynamic properties, but ultimately they share the same mode of action and cause serious cardiovascular side effects and complications. In this review, our group focused on various NSAIDs including its medicinal use and also showed the regulation of nitric oxide including its therapeutic effect in human and diminishes gastrointestinal toxicity. The nitric oxide releasing NSAIDs may be useful to treat wide variety of diseases like colitis, thrombosis, restenosis and bronchial asthma.

Key words: non-steroidal anti-inflammatory; nitric oxide; human

INTRODUCTION

NSAIDs are used commonly prescribed drugs that are popular and provide effective treatment which is used for the treatment of various inflammatory diseases. More than 50% of patients taking NSAIDs have some damage associated with the upper gastrointestinal tract and showed adverse reactions include discomfort, ulcers, and bleeding [1, 2, 3]. The adverse effect of these NSAIDs is due to prostaglandins, potent mediator of inflammation i.e. edema, pain, and vasodilation. The inhibition of these mediators which directly correlates with analgesic and anti-inflammatory properties [4, 5]. However, long term use of these NSAIDs is associated with severe gastropathy that may arise from induction of gastric mucosal cell apoptosis and also induce apoptosis *in vitro* in case of cancer cells i.e. esophageal and gastric adenocarcinoma cells, lung carcinoma cells, myeloid leukemia cells, and prostate carcinoma cells. NSAIDs also induce apoptosis in normal gastric mucosal cells, hepatocytes and chondrocytes [6].

Generally, NSAIDs block mucosal prostaglandin synthesis by inhibiting cyclooxygenase (COX) activity i.e. COX1 and 2. Normally, COX-1-

derived prostaglandins are responsible for maintaining homeostasis e.g. intestinal mucosa. Number of studies related to COX1 under consideration and claimed that decline in the level or amount of COX1 alone was thought to cause an inhibition of blood flow in the intestinal mucosa and increased mucosal permeability, resulting in mucosal injury [7, 8]. So, COX 1 is important to generated prostaglandins required to maintain normal physiological functions such as gastric mucosa protection, platelet aggregation whereas COX 2 generated pro-inflammatory mediators and served as the most relevant as well as important target in inflammation.

ASPIRIN

One of the most active constituents i.e. salicylic acid found in the bark of trees including willow tree and also reported in number of fruits and vegetables. In this regard, salicylic acid and its structurally modified components are also reported in normal human diet and perform specific function against number of diseases [9, 10]. One of the important features comes from the father of modern medicine (Hippocrates; 460–370 BCE) for the use of salicylic acid and recommended for chewing bark tree in order to relief from fever and pain and

also given in the form of tea obtained from willow bark to pregnant women during childbirth [11, 12].

After the consumption of these salicylic acid entities obtained from chewing bark tree showed some serious health issues because of prolonged use of drug and leads to gastrointestinal irritation, which includes vomiting, bleeding and ulcers [13, 14]. In an effort to search for those compounds or chemicals that inhibits gastrointestinal irritation after the consumption of salicylic acid entities. One of the scientists, Hoffmann's modified the structure of salicylic acid chemically through modification of the hydroxyl group on the benzene ring. Due to chemical transformation, new molecule was synthesized i.e. aspirin and showed that body could absorb without showed any significant gastrointestinal distress. Once the patient will ingested, the new molecule (aspirin) was converted back to salicylic acid in the digestive system i.e. stomach followed by liver and then finally excreted in blood and providing the desired therapeutic benefits. So, modern based synthetic drug i.e. aspirin can be considered a drug-delivery system for a natural product that has been in medical use for literally thousands of years [15, 16].

Aspirin, one of well-known non-steroidal anti-inflammatory drug and is generally used for the prevention of cardiovascular disease and also provides protection against different types of cancer particularly colorectal cancer including reduction in the burden of cancers of oesophagus, stomach, pancreas, lung, prostate etc. In general, aspirin provide some benefits (prevention all cancer types) and also showed some drawbacks (gastrointestinal and intracranial bleeding) [14, 15, 16]. Specifically, number of researchers worked on aspirin related to different aspects of immunopharmacological applications and found that aspirin substantially decline in the level of 2-hydroxyglutarate which is present abundantly in the blood of healthy volunteers and considered as a driver of cancer development (known as an oncometabolite) because increased levels have been reported in cancers of the blood and brain and several groups are currently studying as a molecule/drug that promotes tumor formation [17].

Aspirin is commonly used for treatment of inflammation and showed enormous range of effects including reducing pain or fever, inhibiting blood clotting etc and most probably inhibits the prostaglandin biosynthesis (COX-1 and COX-2) because of acetylation and consumed higher doses of aspirin that are needed to recover from chronic inflammatory diseases [18, 19]. In spite of this disease in order to reduce its aspirin burden, salicylate that are available used for the treatment

of inflammation and ineffective as a cyclooxygenase inhibitor. This is due to lacking of acetyl group but showed some additional effects e.g. salicylate or aspirin has been shown to inhibit the activation of the transcription factor NF- κ B in human monocytes via a cyclooxygenase-independent mechanism [20].

Acetaminophen: Also called as paracetamol (synthesized in 1878, Morse) one of the most popular and commonly used drugs for the treatment of pain and fever and occupied an important position under the category of analgesic drugs [21, 22]. In addition, this drug does not show any side effects related to gastrointestinal or cardiovascular tract where as other NSAIDs showed. In fact, this is probably the most commonly prescribed drug for children [23].

According to two independent groups (i.e. Zygmunt and colleagues; Bertolini and colleagues) claimed through experimental data and demonstrated that the analgesic effect of acetaminophen is due to the indirect stimulation of specialized receptors (cannabinoid CB1) that are present in central nervous system where as cannabinoid CB2 presently in peripheral tissues [24, 25]. The main feature of the cannabinoid receptors i.e. CB1 and CB2, both of them are coupled to G proteins. So, acetaminophen undergoes deacetylation to its primary amine (p-aminophenol) which is bound with arachidonic acid to form N-arachidonoylphenolamine (also called as AM404, endogenous cannabinoid) in presence of enzyme fatty acid amide hydrolase. AM404 is an agonist at vanilloid subtype 1 receptors (TRPV1); inhibitor of cellular anandamide uptake, which leads to increased levels of endogenous cannabinoids. Moreover, decline in the level of cyclooxygenases (COX1 and 2) in the central nervous system (CNS) at concentrations that are probably not attainable with analgesic doses of acetaminophen [26, 27]. CB1 receptor antagonist, at a dose level that completely prevents or maintained the analgesic activity of a selective CB1 receptor agonist or acetaminophen. Thus, acetaminophen behaves like a pro-drug, the active one being a cannabinoid [26, 27].

Indomethacin (1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid): One of the most commonly used NSAIDs i.e. Indomethacin (potent inhibitor of prostaglandin synthesis i.e. cyclooxygenases (COX) 1 and 2) that exhibits various properties i.e. antipyretic and analgesic [28]. Since first time it's introduced in the year 1962 and used as a pharmaceutical agent where the drug is formulated as capsules and is used extensively in the treatment of rheumatic (acute

and chronic) arthritis and other inflammatory disorders [29, 30]. In addition, indomethacin consumed through mouth and may cause serious health problems related to cardiovascular and gastrointestinal tract. In addition, Indomethacin including other NSAIDs may cause ulcers, perforations and sudden bleeding in stomach or intestine [29].

Recently, scientists focused on new oral delivery systems for existing drugs in order to improve therapeutic efficacy and reduce its various side effects. Some studies have already being reported which involved the synthesis of indomethacin derivatives and showed less side effects as compared to parent compound [30, 31]. In order to reduce its burden, several researchers working on indomethacin derivatives including gastroprotective prophylaxis with inhibitors of proton pump including gastric antiseecretory drugs, or analogs of prostagandin i.e. misoprostol which protects the gastrointestinal tract against mucosal damage caused by conventional NSAIDs.

Only minimal amounts of free drug i.e. indomethacin are found in the urine. No evidence was obtained for accumulation of the drug in man. Little passage into cerebrospinal fluid takes place and concentrated in various tissues of the guinea pig, but not of the rat [32]. The drug is highly bound to plasma protein. No evidence for accumulation of the drug in the intestinal wall of either rats or guinea pigs was seen. The dog was shown to convert indomethacin to indomethacin glucuronide, which is excreted in the bile [32].

In addition, there are number of NSAIDs that are shown in **Table 1** along with structure and medicinal uses.

Immune system: The immune system is frequently targeted by many pathogenic micro-organisms and responsible for causing disease in humans. Number of research studies has already been done in rodents including humans have shown several mechanisms related to NSAIDs that can be used to explain its side effects and also responsible for causing disease [3, 4]. Infact, these NSAIDs affects general immunity, causing intestinal barrier dysfunction, systemic inflammation and immunodeficiency that contribute to the morbidity and mortality of patients with disease.

Cyclooxygenases (COX1 and COX2): Cyclooxygenase (COX), key enzyme responsible for the synthesis of prostaglandins from arachidonic acid. Firstly, it was purified in the year 1976 (cloned in 1988) and then identified its product in the year 1991 from second gene with

COX activity i.e. COX2. The main function of both cyclooxygenases (COX1 and COX2) as shown in **Fig.2**. Both isoforms of COX are almost identical in structure but showed some variations in case of substrate, inhibitor selectivity and their intracellular locations [33, 34]. In case of inflammatory diseases, COX2 induce pro-inflammatory cytokines (IFN-gamma and TNF alpha) including growth factors and is present constitutively in the central nervous system (brain and spinal cord), where it may be involved in nerve transmission (pain and fever). Prostaglandins made by COX2 are also important in ovulation and also involved in birth process. The discovery of COX2 has made possible the design of drugs that reduce inflammation without removing the protective prostaglandins in the stomach and kidney made by COX-1 [33, 34]. These highly selective COX-2 inhibitors may not only be anti-inflammatory but may also be active in colon cancer and Alzheimer's disease.

COX1 Continuously stimulated by the body and its concentration in the human body always remain stable and creates prostaglandins used for basic housekeeping throughout body and stimulate normal body functions such as stomach mucous production, regulation of gastric acid and kidney water excretion where as **COX2** Induced (normally not present in cells) but only present in specialized cells (EX a549 lung cells) and is generally used for signaling pain and inflammation and produces prostaglandins for inflammatory response and its production is stimulated by inflammatory cytokines and growth factors.

Nitric oxide and Th1 helper cells versus inflammation: Nitric oxide is associated with some of the most important inflammatory diseases including rheumatoid arthritis, diabetes, systemic lupus erythematosus, and septic shock and played a effector molecule for the defense against intracellular as well as extracellular pathogens including virus, bacteria, and parasites. During inflammation, the most prominent role of CD4+ helper T cells 1 (Th 1), characteristically produce IFN-gamma and TNF alpha which activates the macrophages and responsible for induction of nitric oxide through inducible nitric oxide synthase (iNOS) as shown in **Fig.1**. In contrast, Th2 helper cells produce IL-4, IL-5 and IL-10 which can inhibit the nitric oxide production. The main function of Th 1 helper cells are directly associated with inflammatory diseases and eliminates the intracellular pathogens, whereas Th 2 cells are closely involved in allergy and expulsion of extracellular parasites [35, 36]. So, there is a close correlation between T helper 1 cells and nitric oxide in case of inflammatory diseases and also exists a reciprocal regulatory mechanism between

them. In case of inflammatory diseases, high production of nitric oxide from activated macrophages or antigen presenting cells (activated through IL-12) or due to the induction of T helper 1 cells showed cytotoxic effect whereas low production of nitric oxide had a selective enhancing effect on the induction and differentiation of T helper1 (up-regulating cGMP and induces the expression of IL-12 receptor β 2) but not T helper 2 cells [37]. So, nitric oxide directly acts on T cells but in synergy with IL-12 produced by antigen-presenting cells (APCs). The selectivity and the participation of cGMP in case of T helper 1 cells would open a venue of investigation into the role of nitric oxide [38, 39] in immunomodulation (stimulatory, proinflammatory or suppressive, anti-inflammatory). Apart from this, nitric oxide has a diversity of physiological functions, but its excess production has been implicated in the inflammatory process [38, 39]. Several known immunopharmacological agents used in the treatment of various inflammatory diseases e.g. arthritis have been shown to inhibit nitric oxide production in antigen presenting cells i.e. macrophages and dendritic cells i.e.

- Aspirin may stimulate nitric oxide release from vascular endothelium, a pivotal factor for maintenance of vascular homeostasis [40].
- Clinical evidence suggests that low-dose aspirin may improve vascular endothelial function. Since other cyclooxygenase (COX) inhibitors showed no beneficial vascular effects, aspirin may exhibit a vasculoprotective, COX-independent mechanism.
- Acetaminophen inhibits nitric oxide production from murine spinal cord and correlates with analgesic effects [39, 40].

Recently, several natural plant based anti-inflammatory drugs have been identified as well as reported in various medicinal plants and used in traditional medicine for various purposes e.g. pain, fever and inflammation. In the past few years, the mechanisms of some of these natural compounds have been partly elucidated, and they are now being reconsidered for treatment of chronic inflammatory and neurodegenerative diseases. In most cases these drugs work by inhibiting the transcription of COX-2 rather than its activity. In addition they prevent the expression of several pro-inflammatory genes.

CONCLUSION

In spite of this, non-steroidal anti-inflammatory drugs showed significant adverse effects especially on human gastrointestinal tract and kidneys, which pose critical limits to their clinical use in chronic conditions. For the last so many years, researchers focused on those non-steroidal anti-inflammatory drugs which are safe and showed no adverse effect. Few years back, celecoxib (COX2 inhibitor) have been introduced in the market and claimed that it is present in several tissues including gastric epithelium and maintaining its COX1 activity and showed less adverse effect in comparison to those non-steroidal anti-inflammatory drugs that are available in the market. Later shown that this drug withdrawn from the market because of cardiovascular adverse effect. Another strategy i.e. nitric oxide production were followed with respect to classical non-steroidal anti-inflammatory drugs, release of nitric oxide from these compounds occurs through slow kinetics, mimicking the physiological levels of NO produced by constitutive NOS, and thus protecting the gastrointestinal mucosa.

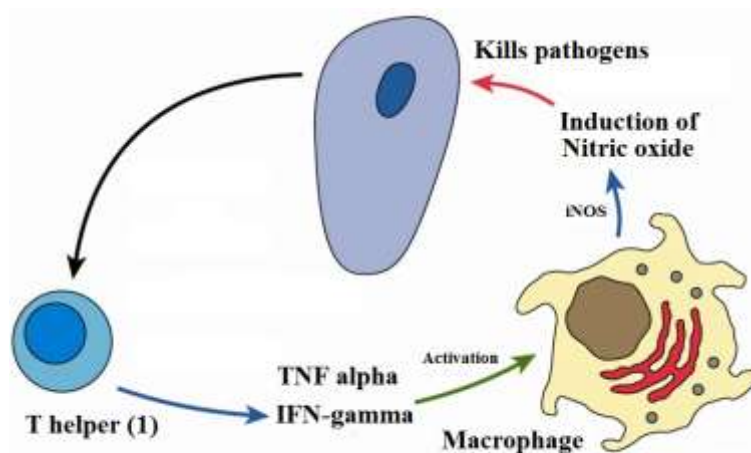


Fig.1. Direct killing by cytokine-activated macrophages.

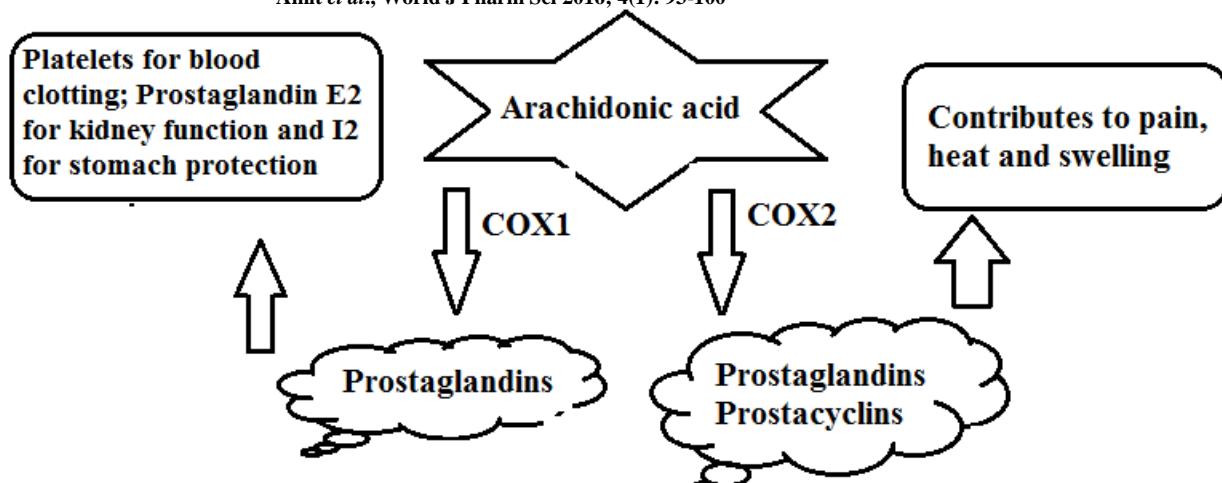
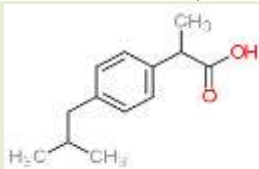


Fig.2. Pathways for cyclooxygenases (COX1 and COX2).

| Name | Structure | IUPAC Name | Medicinal properties |
|-------------------|-----------|---|---|
| Aspirin | | 2-acetoxy benzoic acid | Adjuvant Therapy, Coronary Artery Disease, Myocardial Infarction, Venous Leg Ulcer, Diabetes Type 2, cerebral Infarction, Cardiovascular Diseases, Atherosclerosis etc. |
| Celecoxib | | 4-[5-(4-Methylphenyl)-3-(trifluoromethyl)pyrazol-1-yl] benzenesulfonamide | Ankylosing Spondylitis, Osteoarthritis, Hypertension, Nasopharyngeal Carcinoma, Tonsillitis, Adenotonsillectomy etc |
| Diclofenac sodium | | 2-[2-(2,6-dichloroanilino)phenyl]acetic acid | Primary Dysmenorrhea, Ankle Sprain, Anemia, Actinic Keratosis, Diabetic Oculopathy, Osteoarthritis, |
| Diflunisal | | 2',4'-difluoro-4-hydroxybiphenyl-3-carboxylic acid | Type 2 Diabetes, Amyloid Polyneuropathy, |

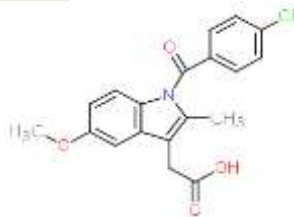
Ibuprofen



(*RS*)-2-(4-(2-Methylpropyl)phenyl)propanoic acid

Spinal Cord Injury, Fever, Chronic Pelvic Pain, Periodontitis,

Indomethacin



2-{1-[(4-Chlorophenyl)carbonyl]-5-methoxy-2-methyl-1*H*-indol-3-yl}acetic acid

Hemorrhage, Post-ERCP Acute Pancreatitis, Intraventricular Hemorrhage, Patent Ductus Arteriosus, Preterm Labor, Obstetric Labor etc

Mefenamic acid



2-(2,3-dimethylphenyl)aminobenzoic acid

Infertility, Dysmenorrhea, Uterine Hemorrhage, Endometrioma, Irreversible Pulpitis, Contraception etc

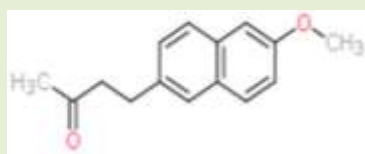
Meloxicam



4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide-1,1-dioxide

Osteoarthritis of the Knee, Arthritis, Rupture; Graafian Follicle, Contraception, Dysmenorrhea etc

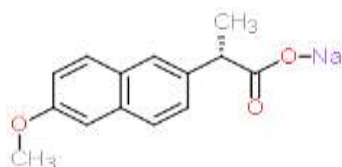
Nabumetone



4-(6-methoxy-2-naphthyl)-2-butanone

Osteoarthritis of the Knee, Migraine Without Aura etc

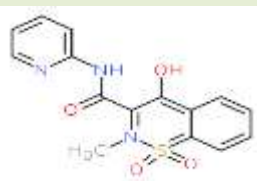
Naproxen sodium



sodium;(2*S*)-2-(6-methoxynaphthalen-2-yl)propanoate

Migraine Disorders, Heterotopic Ossification, Arthritis, Osteoarthritis, Gastric Ulcer, Contraception; Bleeding, Hypertension etc

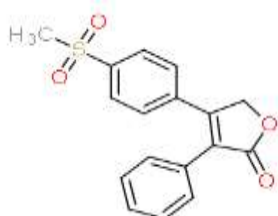
Piroxicam



4-hydroxy-2-methyl-1,1-dioxo-N-pyridin-2-yl-1H-2-benzothiazine-3-carboxamide

Primary Dysmenorrhea, Contraception, Renal Colic, Hypertension, Dermatitis, Inguinal Hernia; Postoperative Pain, Headache (Migraine), Bunionectomy etc

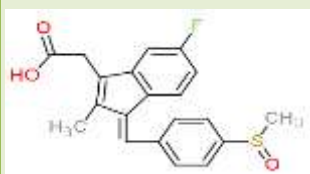
Rofecoxib



4-(4-methylsulfonylphenyl)-3-phenyl-5H-furan-2-one

Glioma, Osteoarthritis, Antiphospholipid Antibody Syndrome, Pain, Alzheimer Disease, Colorectal Adenoma, Barrett's Esophagus, Rheumatoid Arthritis, Headache etc

Sulindac



2-[(3Z)-6-fluoro-2-methyl-3-[(4-methylsulfinylphenyl)methylidene]inden-1-yl]acetic acid

Familial Adenomatous Polyposis, Liver Metastasis, Colorectal Neoplasms, Melanoma etc

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