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Review on Screening and Analysis of Anti-Inflammatory Drugs through Biochemical Techniques

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Abstract: *Non-steroidal anti-inflammatory drugs (NSAIDs) are competitive inhibitors of cyclooxygenase (COX), the enzyme that promotes the bioconversion of arachidonic acid to inflammatory prostaglandins (PGs). Their use is associated with adverse consequences, including as gastrointestinal and renal damage. The inhibition of COX-2 provides the therapeutic anti-inflammatory action, but the decrease of COX-1 activity causes the unwanted side effects of NSAIDs. Therefore, it was thought that more targeted COX-2 inhibitors might have fewer negative effects. Several selective COX-2 inhibitors (rofecoxib, celecoxib, valdecoxib, etc.) were created as safer NSAIDs with an improved stomach safety profile. However, the recent removal of several COXIBs from the market, such as rofecoxib, due to their negative effects on the cardiovascular system clearly encourages researchers to look for and evaluate other templates that have COX-2-inhibiting properties. The identification of new uses for selective COX-2 inhibitors in the treatment of cancer and neurological disorders like Parkinson's disease continues to pique interest in the development of these medications*

Keywords: Selective COX-2 Inhibitors, Cyclooxygenase, SAR, NSAIDs, and Coxibs

I. INTRODUCTION

The word "inflammation" is derived from the word "flame," which denotes warmth or redness. Aulus Cornelius (c. 25 BC to c. 50 AD) had noted the four hallmarks of inflammation: pain (dolor), redness (rubor), warmth (calor), and swelling (tumor). Anti-inflammatory drugs: A medication or chemical that lessens the body's inflammation (pain, swelling, and redness). Anti-inflammatory drugs prevent the body from producing some of the chemicals that lead to inflammation. Anti-inflammatory medication classification:

I. Non selective COX inhibitorsA Salicylates e.g. Aspirin

B. Propionic acid derivatives e.g. Ibuprofen, Naproxen, Ketoprofen, Flubiprofen

C. Fenamates e.g. Mephenamic acid

D. Enolic acid derivatives

e.g. Piroxicam, Tenoxicam

E. Acetic acid derivatives e.g. Ketorolac, Indomethacin, Nabumetone

F. Pyrazolone derivatives e.g. Phenylbutazone, Oxyphenbutazone, Propyphenazone II. Preferencial COX 2 inhibitors e.g. Nimesulide, Diclofenac, Aceclofenac, Meloxicam, Etorolac

III. Selective COX 2 inhibitors e.g. Celecoxib, Etoricoxib, Parecoxib

IV. Analgesic antipyretic activity with poor anti-inflammatory action

A. Para aminophenol derivative e.g. Paracetamol (Acetaminofen)

B. Nefopam is an example of a benzoxazocine derivative. Since the discovery of a second isoenzyme of cyclooxygenase (COX), COX-2, researchers have hypothesized that the anti-inflammatory benefits of non-steroidal anti-inflammatory drugs (NSAIDs) are generated by a different mechanism than the often reported side effects of these therapies. These include impaired stomach cytoprotection, renal function, and inhibition of platelet aggregation. While COX-2 synthesis is increased, particularly during inflammatory processes, constitutive COX-1 isoenzyme, often known

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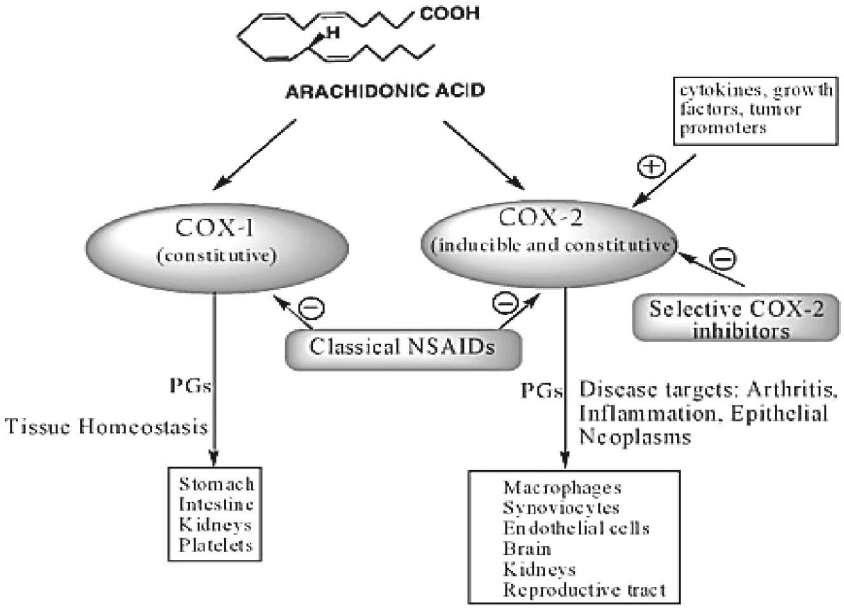
as a "housekeeping" enzyme, is found in most tissues under normal conditions. It has been proposed that COX-2 inhibition is a key target for the anti-inflammatory benefits of non-steroidal anti-inflammatory medications, whereas COX-1 inhibition is the source of these medications' adverse effects on the kidneys and stomach. Numerous in vitro experiments have been developed to evaluate and describe the relative inhibitory effects of non-steroidal anti-inflammatory drugs against COX-1 and COX-2. While the inhibitory activities are given as IC50 values, or the dosages that inhibit activity by 50%, the ratios of the IC50 values for COX-1 and COX-2 are utilized to show the indices of selectivity. A large variety of IC50 values and ratios, as well as sometimes ambiguous comparisons, have resulted from the creation of several systems. By providing a critical analysis of the current in vitro assays, this review aims to draw attention to test interpretation challenges. In addition, each molecule's pharmacokinetic properties will be considered, and the in vitro findings' in vivo relevance will be examined. Results related to human pharmacy The results of COX-2 selectivity studies conducted in vitro will be compared with studies that examined the differentiating inhibition of prostanoid synthesis in vivo in various tissues. The isozymes of COX: The precise mechanism of action of NSAIDs, despite their widespread usage over the previous century, remained unclear until Vane identified the COX enzyme in 1971. This enzyme is the molecular target of NSAIDs. The early 1990s saw the discovery of a second isoform (COX-2) that differed from the first and was later named COX-1. The COX-1 and COX-2 are two isoenzymes. The human genes that correspond to the two enzymes are located on different chromosomes and have different properties because isoenzymes are genetically different proteins. Numerous organs constitutively express COX-1, and the PGs it generates mediate "housekeeping" functions include renal blood flow regulation, gastric mucosa cytoprotection, and platelet aggregation. Conversely, COX-2 expression is not present in most normal tissues but is rapidly triggered. Proinflammatory cytokines (IL-1b, TNFα), lipopolysaccharides, mitogens and oncogenes (phorbol esters), growth factors (fibroblast growth factor, FGF; platelet-derived growth factor, PDGF; epidermal growth factor, EGF), hormones (luteinizing hormone, LH), and anomalies in water-electrolyte hemostasis are some of the stimuli that cause an increase in prostaglandin (PG) synthesis in inflammatory and malignant tissues. Thus, the inducible isozyme has been connected to pathogenic processes including inflammation and several types of cancer.The link between the two isoforms is more complicated, according to recent study. While COX-2 is constitutively generated in several tissues and organs, such as the kidneys, brain, and reproductive tract, COX-1 may in fact play a part in inflammatory processes.



Structure of Enzymes:

The COX isoenzymes are membrane-bound enzymes found in the endoplasmic reticulum (ER). The three-dimensional structure of the ovine COX-1 was first published in 1994, followed shortly after by the crystal structures of human and

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murine COX-2. COX attempts to create monomeric species as a homodimer, but all it has created are inactive enzymes. The crystal structures of the COX isoforms are highly structurally homologous and consistent with a high sequence similarity (around 60%), and the overall structures of COX-1 and COX-2 are highly conserved. The COX monomer is composed of three structural domains: an N-terminal domain that resembles epidermal growth factor (EGF); a membrane binding domain (MBD) that attaches the protein to one leaflet of the lipid bilayer and is about 48 amino acids long; and a large C-terminal globular catalytic domain that includes the COX active site, which can hold the substrate or inhibitors, and the peroxidase active site, which has the heme cofactor.

Evaluation of COX-2 inhibitory efficacy in vitro: COX-2 inhibition was evaluated in vitro using enzyme immunoassay. The test compound's capacity to inhibit COX-2 (human recombinant) was investigated using an enzyme immunoassay (EIA) kit (Catalogue No. 560131, Cayman Chemical, Ann Arbor, MI, USA) and the manufacturer's instructions. The test compound was dissolved in DMSO to produce a solution with a final concentration of 10 µM. A reaction buffer solution (960µl, 0.1M Tris-HCL, pH-8, including 5 mM EDTA and 2 mM phenol) containing COX-2 enzymes (10 µl) and heme (10 µl) was mixed with 10 microliters of the 10-µM test drug solution. After that, these solutions were incubated for ten minutes at 37°C. After that, 10 µl of Following the addition of the AA solution, 50 µl of 1 M HCL was added to terminate the COX reaction. Arachidonic acid (AA) is converted to PGH2 by cyclooxygenase, and stannous chloride subsequently reduces it to PGF2α. PGF2α was produced by reducing PGH2 with 100 µl of stannous chloride, and its concentration was measured by an enzyme immunoassay method. This was caused by the competition between PGs and PG-acetyl cholinesterase conjugation (PG tracer) for the limited amount of PG antiserum. The amount of PG tracer that can bind to the PG antiserum is inversely proportional to the concentration of PGs in the well since the tracer concentration of PGs is kept constant while the PG concentration varies. Monoclonal antibodies against mice that have already been attached to the well are bound by this antibody and PG combination. The acetylcholine esterase substrate and other Ellman's reagents are added to the well after the plate has been cleaned to remove any remaining chemicals. The product of this enzymatic reaction produced a distinct yellow color, which was measured spectrophotometrically (using a Micro titre Plate reader) at 412 nm: Absorbance α [PG tracer bound] α 1/PG units. The amount of PG tracer bound to the well and the amount of free PGs present in the well during the incubation are inversely proportional. The % inhibition was calculated by comparing the chemical treated by the control incubations.

In vitro human whole blood assay:

This assay uses whole blood that has been clot to measure COX-1 activity and whole blood that has been stimulated with lipopolysaccharide to measure COX-2 activity. There are several benefits to the human wholeblood assay. The target cells for the anti-inflammatory effects (monocytes) and side effects (platelets) of nonsteroidal anti-inflammatory medications (NSAIDs) are intact human cells. Additionally, the complete blood's plasma proteins provide a more accurate depiction of interactions that occur in vivo when nonsteroidal anti- inflammatory medications are present. To enable a direct comparison of the outcomes from each assay, the same volunteer (or patient) provides the entire blood required for both tests at the same time. Lastly, blood from volunteers (or patients) who have been given nonsteroidal anti-inflammatory medication treatment in the past (ex vivo assay), enabling a comparison of the applicability of in vitro findings in vivo. The primary disadvantage is that distinct incubation periods are required for COX-1 and COX-2 since COX-2 must be stimulated. Furthermore, target cells for the therapeutic or deleterious effects of nonsteroidal antiinflammatory medicines would be more typical of cell types other than platelets and monocytes, such as stomach mucosal cells and synoviocytes. Table below presents a summary of the findings from various laboratories that used the human whole blood assay. With a few notable exceptions, the COX-2 selectivity rank order is consistent across laboratories. On both isoenzymes, standard non-steroidal anti-inflammatory medications work about equally well. Diclofenac has the best profile among non-steroidal anti-inflammatory medications that are commonly used. Etodolac, nimesulide, and meloxicam are examples of compounds that selectively (ratio 3 to 30) inhibit COX-2; in contrast, flosulide, DuP-697, NS-398, L-745, 337, and SC 58125 exhibit COX-2 selectivity. This overall pattern is consistent with the outcomes of utilizing human recombinant enzymes. The COX-1/COX2 selectivity ratios achieved with various non-steroidal anti-inflammatory medicines utilizing human whole-blood test or human recombinant enzymes are shown in Fig. 2. The bars show the range of ratios that various laboratories were able to generate using the same model. This illustration clearly shows a parallel pattern. Compared to the whole-blood experiment, the selectivity ratio range is

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larger when whole cell recombinant enzymes are utilized. Furthermore, compared to recombinant enzymes, the variations in COX-2 selectivity amongst the drugs are less pronounced in the whole blood experiment. Variations in the concentrations of proteins could be connected to this. As of right now, new in vitro assays are being created. Human cells used in these test systems include synoviocytes, chondrocytes, and gastric mucosa cells, which are targets for the anti-inflammatory or side effects of nonsteroidal anti-inflammatory medications. Complete validation of these models still requires the establishment of standardized circumstances. Nevertheless, the drugs evaluated showed a similar trend for ranking COX-2 selectivity, as seen with human recombinant enzymes and the human whole blood testing [30–32]. It should be emphasized, nonetheless, that it will be challenging to Figure 2 shows selectivity ratios for a number of non-steroidal anti-inflammatory medications achieved with human recombinant enzymes in a whole cell assay (dark gray bars) [19, 20, 22], in a microsomal assay (light gray bars) [16, 17, 19], or in a black bar assay for human whole blood [24–27, 36]. Only estimates were used to determine the comparatively low values for L745,337 and SC58125 (hatched bars) in the whole blood assay. Because of issues with solubility at high doses, IC50 values for cyclooxygenase-1 inhibition could not be obtained, making exact ratios impossible to calculate. Use these models to simulate drug binding to proteins as nearly as possible to a human whole-blood experiment.

In vivo assay: Carrageenan-induced rat paw edema assay: Using carrageenan-induced rat paw edema, the selected samples demonstrating promising average (activity in all solvents) COX-2 selective activities were assessed for in vivo anti-inflammatory research. animal model of edema. The assay ran according to the preceding description.45 In short, 20 µl of carrageenan (1 % w/v) in 0.9% saline was subplantarly injected to cause edema on the right hind paw. One hour prior to carrageenan injection, the extract of the chosen samples was made in 1% w/v gum acacia and given orally at doses of 100 mg/kg and 250 mg/kg. A standard group received indomethacin (20 mg/kg, p.o.) and a control group was given a vehicle alone. After induction, the volume of the injected and contralateral paws was measured one, three, and five hours later. A plethysmometer (Orchid Scientific Laboratory) to measure the degree of inflammation. The value was given as the percentage of volume reduction at various time intervals relative to the control group.

In Vivo Wistar rat model study:

Live an overabundance of anesthetic was administered intraperitoneally to induce unconsciousness in male Wistar rats (220 ± 250 g; Tuck, Rayleigh, U.K.) using thiobutabartibal sodium (Inactin; 120 mg kg71, i.p.; RBI, Natick, U.S.A.). Rats were given specific medications on a minimum of four separate research days. A homoeothermic blanket that was attached to a rectal probe was used to keep the patient's body temperature at 37 °C. Ventilation was facilitated by cannulating the trachea. A pressure transducer (type 4-422-0001, Transamerica Instruments) was cannulated into the right carotid artery and attached to monitor systemic blood pressure, which was displayed on a Graphtec Linearcorder. In order to facilitate medication injections and saline infusions, the jugular vein was also cannulated. After surgery, the animals were kept for 30 minutes to stabilize before time (t=760) at which a control plasma sample was extracted. An intravenous bolus of aspirin (20 mg kg71; n = 4), diclofenac (3 mg kg71; n = 4), L-745,337 (30 mg kg71; n = 5), nimesulide (15 mg kg71; n = 4), sodium salicylate (20 mg kg71; n = 4), or sulindac (10 mg kg71; n = 5) was given to the rats one hour later (t=0). The carotid artery was used to draw 300 ml of blood at t=760, 5, 60, 120, 180, 240, 300, and 360 minutes. The samples were placed in a 12000 g centrifuge for two minutes at 48 degrees Celsius. The plasma was extracted and then snap-frozen in liquid nitrogen with heparin (15 U ml71, National Veterinary Supplies, Stoke on Trent) added. Following each blood draw, an intravenous injection of 300 milliliters of warm saline was given.

Cell culture: When exposed to IL-1b, the human epithelial carcinoma cell line Cell culture A549 (ECACC Ref. No. 86012804) expresses COX-2 (Mitchell et al., 1994). This cell line's ability to produce PGE2 makes it a useful indicator of COX-2 activity. A549 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Sigma, Poole, U.K.) supplemented with 10% fetal bovine serum (FBS; Sigma, Poole, U.K.) and kept in a humidified environment of 5% CO2-95% air at 378C. Prior to use in the experimental methods, cells were grown to confluence in 96-well plates using seeding. The cells were cultured in fresh DMEM supplemented with 10% FBS and 10 mg/ml of IL1b for 24 hours in order to promote COX-2 expression. The media was changed out with new DMEM prior to the experiment: Ca2+- free modified Krebs-Ringer solution (see below) (4 : 1, v/v) at 37° C.

Washed platelets: The thromboxane (TX) B2 generation by platelets served as a COX-1 activity index. Blood was drawn by venepuncture into plastic tubes covered with 0.1% porcine gelatine in water, at 37°C, for 1 ± 3 hours. The subjects were healthy and had not used NSAIDs for at least two weeks. The tubes also contained 3.15% trisodium

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citrate (1: 9, v/v) trisodium citrate. To create platelet rich plasma, the blood was centrifuged at 2006g for 7 minutes (PRP). After that, 300 ng ml71 of prostacyclin was added to the PRP, and then % bovine serum albumin. To sediment the platelets, the pellet was gently resuspended and then 300 ng of prostacyclin (ml71) was added by centrifugation at 10006g for 15 minutes. After that, the supernatant was discarded and replaced with the same volume of modified KrebsRinger solution that was free of Ca2+ at 37° C (10 mM NaHCO3, 20 mM HEPES, 120 mM NaCl, 2 mM Na2SO4, and 4 mM KCl). 0.1 glucose, 0.1%. After being pelleted once more, the platelets were resuspended at 37°C in a modified Krebs Ringer buffer free of Ca2+, matching half of the original plasma volume. After 30 minutes, the platelet solution was diluted 1: 5 in DMEM with 10% FBS added, and it was then plated into 96-well plates with gelatin coating (100 ml well 71). Analyzing the effects of NSAIDs on COX-1 and COX-2 Ten milliliters of plasma were introduced to a medium containing either preinduced A549 cells or washed platelets in order to measure the activity of NSAIDs in the plasma obtained from the rats.

II. CONCLUSION

Despite the large number of documented in vitro tests and the differences between these systems, the most relevant models exhibit a similar trend in non-steroidal anti-inflammatory drug selectivity. The results of studies using human recombinant enzymes in whole cells or in the human whole-blood test determine whether non-steroidal anti-inflammatory drugs are nonselective, COX-2 preferential, or COX-2 selective. Although in vitro methods are useful for measuring activity, it is important to carefully analyze how these data may be used therapeutically. Based only on in vitro research, it is impossible to predict the level of COX-1 and COX-2 inhibition in vivo at a certain dosage. It is necessary to take into account the pharmacokinetic properties of each chemical, including its distribution, plasma levels, and capacity to bind to plasma proteins. Many Drugs have continuously shown preferential or selective inhibition of COX-2 in relevant in vitro test systems. Human pharmacological studies also showed a COX-1 sparing effect for several of these medications using markers such platelet aggregation, blood TXB2 levels, or urine excretion of PGE2. More study is needed to completely comprehend how these compounds impact the production of prostaglandins in other organs, such as the stomach mucosa and synovium. However, the ultimate evaluation of the therapeutic importance of COX-2 selectivity requires head-to-head comparisons in large clinical investigations.

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