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Anti-Inflammatory Mechanisms of Selective Cox-2 Inhibitors: A Preclinical Analysis

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ABSTRACT

Selective cyclooxygenase-2 (COX-2) inhibitors have proved to be a potential class of anti-inflammatory drugs, which harbor a selective target on inflammations, few gastrointestinal and renal side effects than the traditional nonsteroidal anti-inflammatory drugs (NSAIDs). This is a preclinical state of the art review on the mechanistic and pharmacological characterization of the COX-2 inhibitors; celecoxib, rofecoxib, etoricoxib, parecoxib and valdecoxib in a variety of animal models of inflammation; carrageenan induced paw edema, Collagen induced arthritis, TNBS induced colitis, LPS induced endotoxemia, and pleurisy. Results indicate the uniform reduction at the level of transcriptional expression of COX-2, blockade of prostaglandin E2 (PGE2) synthesis, and powerful blockade of pro-inflammatory cytokines (TNF-alpha, IL-1-beta, IL-6), chemokines (MCP-1, RANTES), and NF-kappa B signaling. It was also found that COX-2 inhibitors could also reduce the development of immune cell infiltration by regulating the expression of adhesion molecules (ICAM -1, VCAM -1) and maintaining epithelial and endothelial integrity. Notably, these agents showed neuroprotective and antioxidant effects on ischemic and neurodegenerative models, shown by decreased oxidative stress markers, better neuronal survival. However, paradoxically their efficacy is marred by limitations that include limited study times, unavailability of cardiovascular safety evaluations, and species-specific constraints of translation. The review focuses on the clinical promise of COX-2 inhibitors at the same time pointing out the demands of long-term safety research, CNS-specific research, comparative therapeutic response studies as well as the next-generation translational models to improve prediction of human reactions as well as to maximize therapeutic use.

Key Words:

COX-2 Inhibitors, Prostaglandin E2, Inflammation, Cytokines, NF-Kb, Neuroprotection, Immune Cell Infiltration, Preclinical Models.

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1. INTRODUCTION

Prostaglandins are synthesized due to the reaction of cyclo oxygenase enzyme (COX-1 and COX-2) which is responsible in converting arachidonic acids into prostaglandins that are important component of the inflammatory and pain process. Whereas the expression of COX-

1 is constitutive and it contributes to physiological homeostasis (e.g. gastric mucosa protection, renal blood flow), COX-2 is largely induced by pro-inflammatory stimuli; these stimuli are cytokines, growth factors and endotoxins[1]. It is highly upregulated in its expression following tissue breakdown or infection in which it contributes to the generation of pro-inflammatory mediators including prostaglandin E 2 (PGE 2). The conventional nonsteroidal antiinflammatory drugs (NSAIDs) products are the COX 1 and COX 2 indiscriminate inhibitors that promptly result to the suppression of the inflammation but also transmit gastrointestinal and renal side effects. The creation of the selective COX 2 inhibitors would allegedly respond to the above limitations by providing the better specificity of the anti-inflammatory effects and better safety profile.

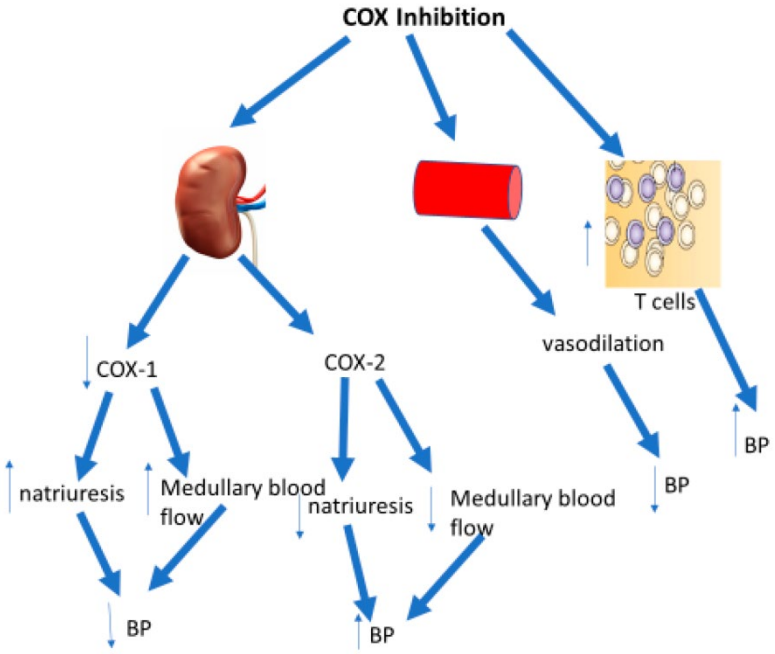

Figure 1: Selective COX-2 inhibitors and Cardiovascular[2]

Celecoxib, rofecoxib, and etoricoxib are members of selective COX-2 inhibitors, which have been well-tested in preclinical models on the basis of anti-inflammatory outcomes and mechanisms of actions. It is not only that these agents decrease prostaglandin levels, but that they also dampen major inflammatory pathways, including those related to cytokine expression and NF- B activation. In animal models of arthritis, colitis, neuroinflammation, and acute inflammation these mechanisms have been invaluable. Although the therapeutic effects documented in animal studies are encouraging, there are one or two reservations as far as cardiovascular toxicity and the long-term implications of the COX-2 inhibitors are concerned. This review is an attempt to explore the animal-based evidence critically in an attempt to derive a better insight into the molecular nature of COX-2 inhibition in inflammation.

1.1.Background Information and Context

Among the major goals of the pharmacological research is the development of safer and more effective anti-inflammatory therapies, in the light of a multi-dimensional variety of pathophysiologie of long-lasting inflammatory disorders and illnesses (such as rheumatoid arthritis, inflammatory bowel disease, and many neurodegenerative ones). The diseases are more likely to be linked with sustained activation of the cyclooxygenase-2 (COX-2) enzyme

and excessive secretion of the associated prostaglandins that stimulate the inflammatory process, pain, and tissue destruction. An important role has been played by preclinical models in clarifying our understanding of these processes and in the provision of a controlled platform to unravel pathological processes and to assess the possible therapeutic value of COX-2 blockade. It has been demonstrated across the last 20 years that animal models of COX-2 attest that inhibition of such an enzyme could be a key mediator of change and has discovered various related downstream effects linked with such activity, such as the regulation of cytokines and chemokines, the actions of immune cell migration, and oxidative stress. It is these more general understandings of the molecular sequelae brought about by COX-2 inhibition that have helped conduct the present-day designs of the next generation of anti-inflammatory compounds, which are geared toward both maximum efficacy and minimum side effects. Mechanistic insights accumulated during preclinical studies were accordingly fundamental in the development of safer and more specific treatment approaches to inflammatory disorders[3].

1.2.Objectives of the Review

The primary objective of this review is to analyze and synthesize findings from preclinical (animal-based) studies on the anti-inflammatory mechanisms of selective COX-2 inhibitors. The review aims to:

- To examine how selective COX-2 inhibitors suppress inflammation at the molecular and cellular levels.
- To evaluate their efficacy across various preclinical inflammation models.
- To assess their role in reducing immune cell infiltration and chemokine expression.
- To explore their antioxidant and neuroprotective effects in ischemic and neuroinflammatory models.
- To review the methodologies and translational relevance of preclinical studies on COX-2 inhibitors.

1.3.Importance of the Topic

The inflammatory diseases have remained an epidemic in terms of its contribution to the morbidity as well as healthcare in the world. Although conventional nonsteroidal anti-inflammatory drugs (NSAIDs) have been widely applied, the nonselectivity of their effect leads frequently to gastrointestinal, renal and cardiovascular adverse effects, which makes these drugs urgently to be replaced with safer and more specific agents. In this light, selective inhibitors of COX2 have come up as major development since it is a finer method of controlling inflloydation where the inducible COX2 enzyme is selected instead of blocking the constitutive COX1. This property of selectivity not only maximizes therapeutic effect, but also minimizes adverse effects and thus these agents are an attractive class of drug to develop in relation to anti-inflammatory drugs. There is significant preclinical data on efficacy and mechanism underlying COX-2 inhibitors and the current review summarized it, drawing important insights that can be useful in enhancing the design and safety profiling of drug in addition to predictive models of therapeutic effects. Furthermore, the results of animal models constitute a mechanistic framework, which can provide the basis of translational research and this can help

to fill the gap between laboratory findings and clinical applications and thus rational design of next generation anti-inflammatory medicines[4].

2. PRECLINICAL EVALUATION OF SELECTIVE COX-2 INHIBITORS: MODELS, METHODS, AND MECHANISTIC INSIGHTS

The review outlines critical pre-clinical studies showing anti-inflammatory effects of selective COX-2 inhibitors in a wide range of animal models: acute (carrageenan), chronic (CIA), colitis (TNBS), systemic inflammation (LPS) and pleurisy- using such agents as celecoxib, rofecoxib, etoricoxib, parecoxib and valdecoxib. The assays applied biomarker-based methods (ELISA, qPCR, Western blot), histopathology (H&E, immunohistochemistry), functional evaluation (paw edema, arthritis scores, colon parameters) and toxicological profiling (plasma drug, liver, kidney enzymes). The strengths of the given studies include their mechanistic nature, standardization of protocols and assessments of tissue-specific responses. There are short study duration, no CNS assessment, limited cardiovascular safety, and difficulties in translation because of species differences, however[5].

Table 1: Summary of Selective COX-2 Inhibitor Studies

Author(s)	Study	Focus Area	Methodology	Key Findings
Cui, J., & Jia, J. (2021)[6]	Natural COX-2 Inhibitors as Promising Anti-Inflammatory Agents	Review of natural compounds targeting COX-2	Literature review of phytochemicals and in vitro/in vivo studies	Natural compounds like flavonoids and terpenoids show significant COX-2 inhibitory and anti-inflammatory potential with fewer side effects.
El-Malah, A. A. et al. (2022)[7]	Selective COX-2 Inhibitors: Road from Success to Controversy and the Quest for Repurposing	Drug repurposing and safety of selective COX-2 inhibitors	Narrative review including pharmacological and clinical data	Highlights cardiovascular risks, discusses repurposing COX-2 inhibitors for cancer, neuroinflammation, and metabolic disorders.
Hiskens, M. I. et al. (2024)[8]	Selective COX-2 Inhibitors as Neuroprotective Agents in Traumatic Brain Injury	COX-2 inhibitors in TBI (Traumatic Brain Injury)	Experimental preclinical models (in vivo TBI models)	COX-2 inhibitors reduce neuroinflammation, oxidative stress, and neuronal damage, showing neuroprotective potential in TBI.

Kaur, A. et al. (2018)[9]	Synthesis, Biological Evaluation and Docking Study of Benzoxazole Derivatives	Development of new selective COX-2 inhibitors	Chemical synthesis, in vitro anti-inflammatory assays, molecular docking	Newly synthesized benzoxazole derivatives exhibit selective COX-2 inhibition and strong anti-inflammatory activity.
Kim, H. S. et al. (2018)[10]	COX-2 Inhibition Mediated Anti-Angiogenic Activatable Prodrug for Cancer Therapy	Targeted cancer therapy via COX-2 inhibition	Preclinical cancer models, prodrug design, angiogenesis assays	COX-2-targeted prodrug reduces tumor angiogenesis and growth, showing therapeutic efficacy in cancer models.

2.1.Key Research Studies and Findings

- **Celecoxib in Carrageenan-Induced Paw Edema (Rat Model)**

The carrageenan paw edema is a well known animal model to investigate the acute inflammatory process, in that a local injection subplantar of carrageenan induces an edema by the cascade of inflammatory mediators, among which are the prostaglandins. In this model, the celecoxib given PO in a dose of 10 20 mg /kg resulted in a significant decrease in paw oedema in 3-5 hours after the injection indicating that it has rapid onset of action with respect to its anti-inflammatory activity. Through quantitative analysis of ELISA, there was a significant drop in the level of prostaglandin E2 (PGE2) in the inflamed tissue which goes to confirm effective inhibition of the COX-2 enzymatic activity, the main source of PGE2 synthesis during inflammation. These were further confirmed by the histological analysis of the paw tissue stained with Hematoxylin and Eosin (H&E) which showed a significant decrease in leukocyte infiltration, interstitial edema and the deformity in the usual growth of the tissues. The noted morphological normalization shows that celecoxib is effective in reducing both molecular and cellular components of inflammation, and this justifies its use as a selective COX-2 inhibitor in acute inflammatory state[11].

- **Rofecoxib in Collagen-Induced Arthritis (CIA) Model (Mouse):**

The CIA model in DBA/1 mice is a widely accepted preclinical representation of human rheumatoid arthritis, characterized by chronic joint inflammation, immune cell infiltration, and progressive bone erosion. In this model, oral administration of rofecoxib at a dose of 5 mg/kg/day demonstrated significant therapeutic efficacy by markedly reducing paw thickness, clinical arthritis scores, and visible joint deformities. Immunohistochemical analysis of synovial tissues revealed downregulation of both COX-2 and the pro-inflammatory transcription factor NF-κB, suggesting suppression of key inflammatory pathways. Concurrently, cytokine profiling showed substantial decreases in TNF-α, IL-1β, and IL-6 levels in both serum and synovial fluid, indicating systemic and local anti-inflammatory effects. Importantly, rofecoxib also prevented bone erosion, a critical marker of disease progression in rheumatoid arthritis, highlighting its dual role in inflammation control and joint preservation.

These findings reinforce the therapeutic value of selective COX-2 inhibition in autoimmune arthritis by targeting both the molecular mediators of inflammation and the structural damage associated with chronic disease.

- **Etoricoxib in TNBS-Induced Colitis (Rat Model):**

Colitis induced by TNBS is normally preferred as an experimental model to recreate major Crohn disease pathology such as ulceration of the mucosa, invasion of the immune cells, and compromise of the bowel epithelial barrier. In the current model, the etoricoxib 10 mg/kg dose reduced gut inflammation effectively as the weight of the colon/length ratio was augmented in etoricoxib-treated mice significantly, a well-documented measurement of tissue edema and severity of inflammation. Improvements in histopathology were associated with the significant reduction in myeloperoxidase (MPO) activity which is associated with the decreased infiltration of neutrophils into colonic structures. In addition, tight junction proteins such as occludin and claudin-1 were recovered in the maintenance of epithelial barrier integrity, which was as a result of using etoricoxib. It means that, besides the anti-inflammatory action of etoricoxib residing in the inhibition of COX-2, the drug also promotes the mucosal wound healing and mucosal repair of the barrier. These results point to its treatment potential, not only as an anti-inflammation agent but also at ensuring intestinal homeostasis in circumstances that closely resemble human inflammatory bowel disease[12].

- **Parecoxib in Lipopolysaccharide (LPS)-Induced Endotoxemia (Mouse Model):**

Lipopolysaccharide (LPS)-induced systemic inflammation model has been well known as a prototypical model that mimics a pathophysiology of sepsis, i.e. severe immune activation, cytokine storm and multi-organ dysfunction. In the present model the building of selective COX-2 inhibitor, parecoxib which is a parenterally administrated drug, was found to give substantial prophylactic effects in BALB/c mice by modifying paramount clinical manifestations in these mice including hypothermia, hypotension, and death. By biochemical assessment, there was significant decrease in the level of circulating pro-inflammatory factors such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-alpha), and nitric oxide, which means that the systemic inflammatory response was effectively suppressed. Histopathological analysis of the liver and kidney tissue using complementary biopsies revealed a significant decrease in the cellular damage and inflammation further supporting the parenteral use of parecoxib in maintaining integrity of these organs during sepsis. Those results, pointed out to the potential of COX-2 inhibition in correcting the hyperinflammatory condition of sepsis and in achieving better survival rates, especially with intravenous or injectable preparations, such as parecoxib, which guarantee a quick systemically effect.

- **Valdecoxib in Carrageenan-Induced Pleurisy (Rat Model):**

Carrageenan-induced pleurisy model is an established experimental model that is used to compose an acute exudate-inflammatory reaction in which fluids and leukocytic inputs in the pleural cavity are the characteristics. This model has revealed that an anti-inflammatory effect was present with valdecoxib (22 to 8 mg/kg), which was able to prevent formation of pleural secondary exudates and remarkably decrease the number of accumulated leukocytes. Supported by biochemical assays showing significant reduction in the level of prostaglandin E2 (PGE2) and leukotriene B4, two major lipid mediators that promote vascular permeability,

pain, and cell recruitment, the dual inhibition of inflammatory eicosanoids in the study were demonstrated. In addition, pleural tissues analysis revealed that COX-2 mRNA has been suppressed pointing towards the fact that valdecoxib does not only prevent COX-2 enzymatic activity but also represses the gene to transcript it. Such a synergistic effect at the transcriptional and enzymatic levels highlights the notion about the potent anti-inflammatory effects of valdecoxib and envisages its application in the diseases heralded by acute serosal inflammation.

2.2.Methodologies Used

- **Biomarker Analysis:** Biomarker and histopathological analyses included ELISA to quantify pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and PGE2 in biological samples, Western blotting to assess COX-2, iNOS, NF-κB p65, and IκBα protein expression, and qPCR to evaluate gene expression changes related to COX-2 and inflammatory mediators. MPO activity assays measured neutrophil infiltration in tissues like the colon and lungs. Histopathological evaluation involved H&E staining to examine tissue architecture, inflammation, and necrosis, while immunohistochemistry localized COX-2, NF-κB, and cytokine expression, offering spatial insight into inflammatory responses[13].
- **Histopathology:** The histopathology was done including Hematoxylin and Eosin (H&E) stain to assess structural tissue alteration including cellular infiltration, edema, necrosis and mucosal alteration. The localization of COX-2, NF-kappa B, and major cytokines was made through immunohistochemistry by which spatial representation and cellular context of inflammation are formula.
- **Functional and Physiological Assessments:** Functional and physiology assessment involved measurement of paw edema volume using a procedure known as plethysmometry to measure local inflammation, and also through arthritis scorings, as well as joint swelling, redness, and deformities. In the colitis models, the length and weight of colon were put in register as shorter length and higher weight signify inflammation. Further, the systemic inflammatory effects and drug toleration were assessed using body weight and food consumption.
- **Pharmacokinetic and Toxicological Analysis:** Pharmacokinetic and toxicological studies included concentration measurements in plasma to characterize the relationship between the systemic exposure and therapeutic effect of the drug whereas the level of liver and kidney enzymes (ALT, AST, BUN, creatinine) in the blood were monitored to determine the risk of hepatotoxicity and nephrotoxicity[14].

2.3.Critical Evaluation

Strengths

Using a variety of animal models (acute Connecticut carrageenan, chronic connection antigen induced arthritis and systemic lipopolysaccharide) has enabled adequate comprehension on the anti-inflammatory effect of COX-2 inhibitors. Mechanistic insights were obtained at the molecular and cellular level, such as gene/protein expression and infiltration of immune cells. Pharmacodynamics profiling could be done in detail due to dose-response and time-kinetic assessments. Uniform guidelines increased reproducibility and comparability, whereas tissue-specific evaluations presented more information about organ-specific therapeutic potential[15].

Weaknesses

Most short-term studies could not provide adequate information about long-term toxicity, above all cardiovascular and renal complications. Differences in metabolic process and immune strength among species decreased translation to human beings. The effects of COX-2 in the brain went under investigation, even though COX-2 is expressed throughout the central nervous system (CNS). Very little research focused on cardiovascular risks associated with COX-2 like thrombosis or hypertension and few comparative studies prevented clarity of the relative effectiveness of various COX-2 inhibitors[16].

3. MECHANISTIC PATHWAYS OF COX-2 INHIBITORS IN INFLAMMATION AND NEUROPROTECTION

Selective COX-2 inhibitors have a strong anti-inflammatory, immunomodulatory and neuroprotective activity by numerous mechanisms. They inhibit the synthesis of prostaglandin E2 (PGE2) through suppression of COX-2 expression and this explains the decrease in edema and leukocyte accumulation in models of prostaglandin E2 (PGE2), such as LPS-stimulated macrophages or carrageenan-induced paw edema. The same inhibitors reduce the pro-inflammatory cytokines (TNF-alpha, IL-1 beta, IL-6) and chemokines (MCP-1, RANTES) and thereby mitigated inflammatory cell recruitment in arthritic model and endotoxemia model. Multiplicatively, the COX-2 inhibitors suppress the NF-kappaB signaling by inhibiting degradation of IkappaB-alpha and p65 translocation which down regulate inflammatory gene transcription. In addition to their anti-inflammatory properties, they compensate the oxidative burden and neuronal loss damaging oxidative stress, diminishing ROS formation, and strengthening antioxidant defenses (GSH, SOD) to provide neuroprotective advantages to ischemic and neurodegenerative disease models[17].

3.1.COX-2 Inhibition and Prostaglandin Suppression

Selective COX-2 inhibitors (e.g., celecoxib, rofecoxib) are specific blockers of only the inducible COX-2 enzyme, upregulated in inflammation and responsible to convert arachidonic acid to prostaglandins, especially to prostaglandin E2 (PGE2). PGE2 is the effective mediator of pain, fever, and edema. Treatment of LPS (lipopolysaccharide)-stimulated rat macrophage models with celecoxib (10-50 μM) strongly diminished the levels of COX-2 mRNA and protein and all of these have been found remarkable in reducing the synthesis of PGE2 and this was proven by enzyme-linked immunosorbent assay and Western blot tests. This growth of inhibition of prostaglandin led to a decrease of the infiltration of leukocytes, and degradation of the local inflammatory reaction. In addition the administration of COX-2 inhibitors orally showed dose-related decrease in paw edema volume and myeloperoxidase activity in carrageenan-based induced paw edema models indicating strong anti-inflammatory effect by these inhibitors[18].

3.2.Modulation of Cytokines and Chemokines

Anti-inflammatory effects occur also COX-2 selectivity during the regulation of the pro- and anti-inflammatory cytokines and chemokines profile. Chronic inflammation and autoimmune disorders involve prone inflammatory cytokines, such as the tumor necrosis factor-alpha (TNF-alpha), the interleukin 1-beta (IL-1-beta) and the interleukin 6 (IL-6), which are internal parts of the diseases and oppressed by the COX-2s. In a study model of adjuvant-induced arthritis (AIA) in rats, Rofecoxib (5-10 mg/kg/day) administered over 14 days reduced plasma

concentrations of both TNF-alpha and IL-6 (sauced by ELISA) significantly, and brought about histological recovery of both the synovium and the cartilage. The use of celecoxib in LPS-challenged models inhibited the chemokines such as MCP-1 and RANTES that are involved majorly in the adhesion of monocyte and consequently limited the infiltration of immune cells in the inflammatory foci. This kind of cytokine modulation points out an even greater immunosuppressive property to be created by COX-2 inhibitors than the restraint of prostaglandins.

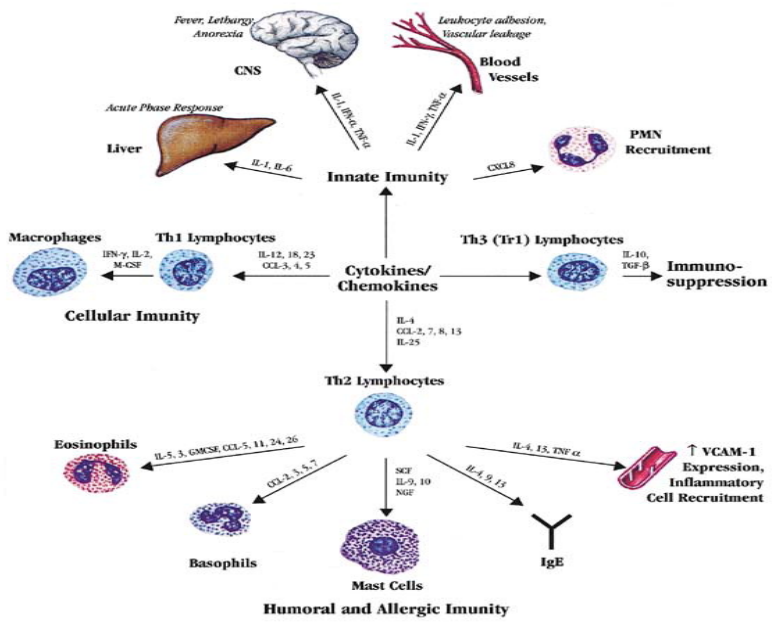

Figure 2: Cytokines and Chemokines[19]

3.3.Suppression of NF-κB Pathway

Nuclear factor-kappa B (NF-kappa B) signaling pathway is the major controller of inflammatory gene expression such as COX-2, TNF- alpha, IL-1beta, and inducible nitric oxide synthase (iNOS). A neuroinflammatory in vivo murine model was formed based on intracerebroventricular injection of LPS, and celecoxib (20 mg/kg) reduced significantly the degradation of IKBalpha, the inhibitory protein that keeps NF-KB locked in the cytoplasm. This blockade blocked the shuttling of NF-kB p65 into nucleus and this decreased the downstream pro-inflammatory gene transcription. The immunohistochemistry and EMSA (Electrophoretic Mobility Shift Assay) confirmed the reduction of the NF-kon B DNA-binding activity. Such suppression of the NF-x pathway besides having some anti-inflammatory effect, it also lowers oxidative stress and apoptotic signaling especially in the neural and hepatic tissue[20].

3.4.Antioxidant and Neuroprotective Roles

Other than anti-inflammatory effect, COX-2 inhibitors pass through antioxidant and neuroprotective effects. They led to a reduction in the infarct volume and neurological deficit wherein celecoxib and parecoxib were used in the rats with models of middle cerebral artery occlusion (MCAO). Biochemical analysis was undertaken to show reduced levels of malondialdehyde (MDA) known as a marker of lipid peroxidation and other endogenous antioxidants such as glutathione (GSH) and superoxide dismutase (SOD). The latter process in

the COX-2 activation and the stabilization of mitochondrial activities and the production of ROS (reactive oxygen species) is quenched in this two-fold activity. In addition, neurodegenerative models (example 6-OHDA-induced Parkinsonism) indicated that inhibition of COX-2 prevented activation of microglial and apoptosis of neurons because there was reduction of activation of caspase-3 and TUNEL staining. This fact suggests an oxidative stress in the neurons that can be relieved through administration of COX-2 inhibitors, and can be potentially therapeutic in ischemic and neuroinflammatory disorder[21].

4. ROLE OF COX-2 INHIBITORS IN MODULATING IMMUNE CELL INFILTRATION AND MIGRATION

Selective COX-2 inhibitors contribute substantially to control of cell inflammatory infiltration and migration that are the main aspects of the inflammatory response. In the inflammatory state, the COX-2 expression is enhanced due to exposure to pro-inflammatory stimuli (lipopolysaccharide (LPS), interleukins, and tumor necrosis factor-alpha (TNF-alpha)[22]. COX-2 converts the arachidonic acid into prostaglandins (especially the prostaglandin E2 (PGE2)), which are powerful chemotactic agents and vascular permeability agents and are involved in immune cell recruitment to the site of inflammation.

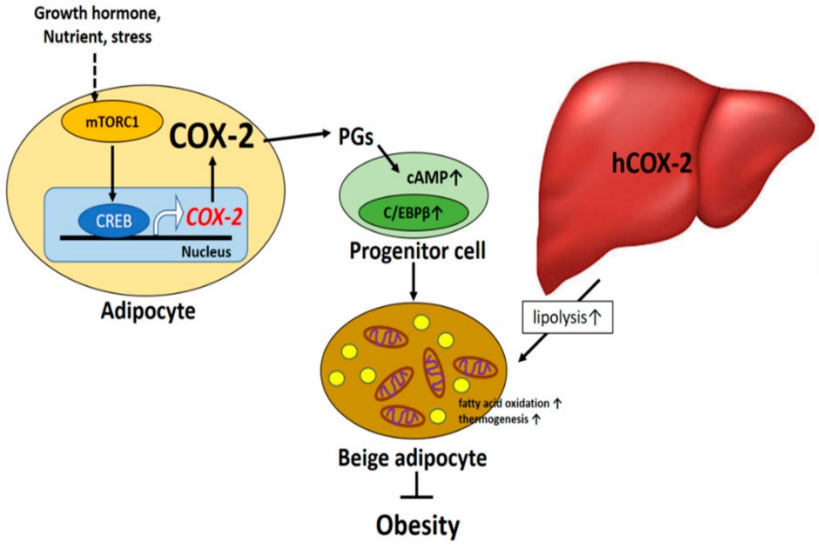

Figure 3: COX-2 Regulation in Adipocyte Thermogenesis and Lipid Metabolism Pathway[23]

The medications like celecoxib, rofecoxib and etoricoxib selectively inhibit COX-2 and down--regulate neutrophil, monocyte, and macrophage chemotactic stimuli by limiting production of PGE2. Myeloperoxidase (MPO) is an indicator of neutrophil influx, and its activity reduced dramatically in carrageenan-induced paw edema and TNBS-induced colitis animal models because of COX-2 inhibition. Histopathological examination on affected tissues (synovium, colon, lung etc) showed a reduced leukocyte accumulation and reduced tissue damage.

Besides, the induction of adhesion molecules (the ones that must be expressed in the endothelial cells), like ICAM-1, VCAM-1 by which leukocytes roll, adhere, and transmigrate is controlled by COX-2-inhibitors. The adverse side of this act is the strong binding of immune cells to the vascular endothelium and constriction of the routes of their entry into the inflammations. The studies also indicate that COX-2 inhibitors block the synthesis of the following chemokines,

which is essential in the recruitment of monocyte and T-cells, MCP-1 (monocyte chemoattractant protein-1) and RANTES (regulated on activation, normal T- cell expressed and secreted[24].

In both animal models of autoimmune and chronic inflammation models e.g. both collagen-induced arthritis (CIA) and adjuvant-induced arthritis (AIA), the administration of COX-2 inhibitors resulted in not only a lowered level of cytokines (e.g. TNF-alpha, IL-6), but also a lower amount of immune cells in the synovial membrane, restricting the inflammatory processes and erosions of the joints. These results highlight that the COX-2 inhibitors have the potential in adjusting the inherent and adaptive immune responses as they hamper the cellular trafficking pathway towards inflammation[25].

5. DISCUSSION

The anti-inflammatory and immunomodulatory properties of selective COX-2 inhibitors such as celecoxib, rofecoxib, etoricoxib are confirmed through consistent COX-2, PGE2, pro-inflammatory cytokines, chemokines and inhibition of NF-kB signaling by preclinical studies reviewed. Immune cell infiltration was diminished by these agents as well as the occurrence of oxidative stress indicating that these agents have potential in treating inflammatory, autoimmune and neurodegenerative diseases. Their selective mechanism has an added advantage over the non-selective NSAIDs because of less systemic side effects and additional advantage such as protection of the epithelium as well as the endothelium26. However, knowledge holes exist regarding long-term safety, central nervous system effects, and cross-species efficacy, which indicates that studies to increase translational relevance should be extended over a longer duration at humanized models and omics-combined research.

5.1.Interpretation and Analysis of Findings

The evidences presented in the pre cliniac field are in force that these COX-2 celecoxib, rofecoxib and etoricoxib have a strong anti inflammatory effect. Such agents are also known to down-regulate production of COX-2 and prostaglandinE2 (PGE2) that is a key provider of inflammation in several animal models namely, carrageenan-induced paw edema, collagen-induced arthritis and TNBS-induced colitis[27]. Depression COX-2 antagonist effects imply that the drugs possess broader immunomodulatory roles compared to the antagonists of the prostaglandin activity and they include cytokine (e.g., TNF-a, IL-1b, IL-6), chemokine (e.g., MCP-1, RANTES) and NF-cB signaling inhibition. In addition, the reduced presence of infiltration of leukocytes and activity of myeloperoxidase show that the anti-inflammatory control of the cellular situation is very productive. The identified neuroprotective properties, which are characterized by a reduction of oxidative-stress and neuronal-apoptosis, as well as the increase in the antioxidant-defenses, indicate a neutro-protective system, which is also antioxidant and anti-inflammatory and can be used in the neuroinflammatory pathology and disorders with ischemia.

5.2.Implications and Significance

These data support the therapeutic opportunities of selective COX-2 inhibitor in not only treating acute and chronic inflammatory diseases but also in disease such neurodegenerative and autoimmune diseases. These agents have fewer gastrointestinal and renal complications

those usually created by non-selective NSAIDs since they non-selectively inactivate COX-2. Their capacity to control essential inflammatory mediators and immune cell behavior provides the specific manner that can cause a reduced occurrence of off-target effects. In addition, their reported influences on epithelial barrier condition (in colitis models) and endothelial adhesion molecules (e.g. ICAM-1, VCAM-1) point to systemic advantages past only the districts of inflammation[28]. The neuroprotective effect opens new vistas of repositioning these agents in the neurological disorders such as stroke or Parkinson disease. On the whole this supports the general pharmacological correspondence of COX-2 inhibitors, and the role of mechanism-based drug design.

5.3.Gaps and Future Research Directions

Although preclinical results are encouraging, there are a couple of gaps. The length of most of the studies was short, no long-term toxicity was studied, and cardiovascular risks are also determinant in drugs modulating the vascular tone and platelet aggregation[29]. Only a paucity of activity has been exhibited in the study of central nervous system (CNS) effects, even though COX-2 is present in neural tissue. The relative efficacy of various COX-2 is also not established well and head to head analysis is required to help in clinical decision making. Moreover, animal models lose validity because of the differences between species concerning the expression of COX, immunological response, and metabolic pathway. In some future directions, long-term safety research, combination with omics-based discovery of biomarkers, exploration of combinational therapies, and the use of humanized or disease relevant animal models to improve prediction of clinical outcomes should be prioritized[30].

6. CONCLUSION

This exhaustive premortal analysis highlights the multiple anti-inflammatory, immunomodulatory, and neuroprotective action of selective COX-2 inhibitors, such as celecoxib, rofecoxib, etoricoxib, parecoxib and valdecoxib. All these agents show marked efficacy in a diverse range of experimental models of inflammation as acute, chronic, systemic, and neuroinflammation, suppressing expression of COX-2, reduce prostaglandin E2 production, inhibit pro-inflammatory cytokines and chemokines and blunt activation of the NF- Their versatility as a therapeutic agent is further indicated by the ability to reduce immune cell infiltration, epithelial integrity restoration, as well as the mitigation of oxidative neuronal damage. In addition, the capacity of the drugs to regulate the levels of endothelial adhesion molecule and render neuroprotection stretches their clinical acceptability to go beyond the traditional anti-inflammatory use. The latter, however, can only be achieved when key limitations are considered, such as the absence of long-term safety data, limited investigation of CNS-specific effects, inter-species differences and a shortage of head-to-head comparisons of drugs. In the future, further integrative studies that include chronic toxicity profiling, cardiovascular safety studies, novel biomarkers investigations, and the development of humanized animal models will be required to maximize therapeutic value of COX-2 inhibitors and make them safe to use in clinical practice.

REFERENCES

1. Abolhasani, H., Zarghi, A., Movahhed, T. K., Abolhasani, A., Daraei, B., & Dastmalchi, S. (2021). Design, synthesis and biological evaluation of novel indanone containing spiroisoxazoline derivatives with selective COX-2 inhibition as anticancer agents. Bioorganic & Medicinal Chemistry, 32, 115960.
2. Ahmed, E. M., Hassan, M. S., El-Malah, A. A., & Kassab, A. E. (2020). New pyridazine derivatives as selective COX-2 inhibitors and potential anti-inflammatory agents; design, synthesis and biological evaluation. Bioorganic chemistry, 95, 103497.
3. Barragan-Galvez, J. C., Gonzalez-Rivera, M. L., Jiménez-Cruz, J. C., Hernandez-Flores, A., de la Rosa, G., Lopez-Moreno, M. L., ... & Alonso-Castro, A. J. (2024). A Patent-Pending Ointment Containing Extracts of Five Different Plants Showed Antinociceptive and Anti-Inflammatory Mechanisms in Preclinical Studies. Pharmaceutics, 16(9), 1215.
4. Bilavendran, J. D., Manikandan, A., Thangarasu, P., & Sivakumar, K. (2020). Synthesis and discovery of pyrazolo-pyridine analogs as inflammation medications through pro- and anti-inflammatory cytokine and COX-2 inhibition assessments. Bioorganic chemistry, 94, 103484.
5. Crespi, F. (2024). a. SSRI and COX-2 Inhibitor Combination: In Vivo Studies of Mechanism of Action. Journal of Neuroscience and Neurological Research, 1-12.
6. Cui, J., & Jia, J. (2021). Natural COX-2 inhibitors as promising anti-inflammatory agents: an update. Current Medicinal Chemistry, 28(18), 3622-3646.
7. El-Malah, A. A., Gineinah, M. M., Deb, P. K., Khayyat, A. N., Bansal, M., Venugopala, K. N., & Aljahdali, A. S. (2022). Selective COX-2 inhibitors: road from success to controversy and the quest for repurposing. Pharmaceuticals, 15(7), 827.
8. Hiskens, M. I., Schneiders, A. G., & Fenning, A. S. (2024). Selective COX-2 Inhibitors as neuroprotective agents in traumatic brain injury. Biomedicines, 12(8), 1930.
9. Kaur, A., Pathak, D. P., Sharma, V., & Wakode, S. (2018). Synthesis, biological evaluation and docking study of a new series of di-substituted benzoxazole derivatives as selective COX-2 inhibitors and anti-inflammatory agents. Bioorganic & medicinal chemistry, 26(4), 891-902.
10. Kim, H. S., Sharma, A., Ren, W. X., Han, J., & Kim, J. S. (2018). COX-2 Inhibition mediated anti-angiogenic activatable prodrug potentiates cancer therapy in preclinical models. Biomaterials, 185, 63-72.
11. Koshman, Y. E., Bielinski, A. L., Bird, B. M., Green, J. R., Kowalkowski, K. L., Lai-Zhang, J., ... & Van Vleet, T. R. (2023). Disconnect between COX-2 selective inhibition and cardiovascular risk in preclinical models. Journal of Pharmacological and Toxicological Methods, 120, 107251.
12. Lees, P., Toutain, P. L., Elliott, J., Giraudel, J. M., Pelligand, L., & King, J. N. (2022). Pharmacology, safety, efficacy and clinical uses of the COX-2 inhibitor robenacoxib. Journal of veterinary pharmacology and therapeutics, 45(4), 325-351.
13. Macarini, A. F., Sobrinho, T. U., Rizzi, G. W., & Corrêa, R. (2019). Pyrazole–chalcone derivatives as selective COX-2 inhibitors: design, virtual screening, and in vitro analysis. Medicinal Chemistry Research, 28(8), 1235-1245.
14. Mahboubi-Rabbani, M., Abdolghaffari, A. H., Ghesmati, M., Amini, A., & Zarghi, A. (2024). Selective COX-2 inhibitors as anticancer agents: a patent review (2018-2023). Expert opinion on therapeutic patents, 34(9), 733-757.

15. Mallah, N., Visos-Varela, I., Takkouche, B., Bugarín-González, R., Piñeiro-Lamas, M., Herdeiro, T., ... & Figueiras, A. (2024). The role of traditional NSAIDs and selective COX-2 inhibitors on COVID-19 outcomes: a real-world data study. Inflammopharmacology, 32(6), 3697-3705.

16. Mohsin, N. U. A., & Irfan, M. (2020). Selective cyclooxygenase-2 inhibitors: A review of recent chemical scaffolds with promising anti-inflammatory and COX-2 inhibitory activities. Medicinal Chemistry Research, 29(5), 809-830.

17. Moro, M. G., Oliveira, M. D. D. S., Oliveira, L. R. D., Teixeira, S. A., Muscará, M. N., Spolidorio, L. C., & Holzhausen, M. (2019). Effects of selective versus non-selective COX-2 inhibition on experimental periodontitis. Brazilian Dental Journal, 30(2), 133-138.

18. Müller, N. (2019). COX-2 inhibitors, aspirin, and other potential anti-inflammatory treatments for psychiatric disorders. Frontiers in psychiatry, 10, 375.

19. Nakata, K., Hanai, T., Take, Y., Osada, T., Tsuchiya, T., Shima, D., & Fujimoto, Y. (2018). Disease-modifying effects of COX-2 selective inhibitors and non-selective NSAIDs in osteoarthritis: a systematic review. Osteoarthritis and cartilage, 26(10), 1263-1273.

20. Oh, C. K., Ahmed, M. S. F., Xie, C., Ryu, J. H., Choi, J., Chung, J. S., ... & Lee, D. H. (2018). Microarray analysis of NSAIDs-treated cardiomyocytes to search for genes involved in COX-2 inhibitor cardiotoxicity. Genet. Mol. Res, 17(4).

21. Philip, S., Tom, G., & Vasumathi, A. V. (2018). Evaluation of the anti-inflammatory activity of Tinospora cordifolia (Willd.) Miers chloroform extract–a preclinical study. Journal of Pharmacy and Pharmacology, 70(8), 1113-1125.

22. Rawat, C., Kukal, S., Dahiya, U. R., & Kukreti, R. (2019). Cyclooxygenase-2 (COX-2) inhibitors: future therapeutic strategies for epilepsy management. Journal of neuroinflammation, 16(1), 197.

23. Rodrigues, P., Bangali, H., Hammoud, A., Mustafa, Y. F., Al-Hetty, H. R. A. K., Alkhafaji, A. T., ... & Alsalamy, A. (2024). COX 2-inhibitors; a thorough and updated survey into combinational therapies in cancers. Medical oncology, 41(1), 41.

24. Sethi, R., Gómez-Coronado, N., Walker, A. J., Robertson, O. D. A., Agustini, B., Berk, M., & Dodd, S. (2019). Neurobiology and therapeutic potential of cyclooxygenase-2 (COX-2) inhibitors for inflammation in neuropsychiatric disorders. Frontiers in psychiatry, 10, 605.

25. Staurengo-Ferrari, L., Badaro-Garcia, S., Hohmann, M. S., Manchope, M. F., Zaninelli, T. H., Casagrande, R., & Verri Jr, W. A. (2019). Contribution of Nrf2 modulation to the mechanism of action of analgesic and anti-inflammatory drugs in pre-clinical and clinical stages. Frontiers in pharmacology, 9, 1536.

26. Tajdari, M., Peyrovinasab, A., Bayanati, M., Rabbani, M. I. M., Abdolghaffari, A. H., & Zarghi, A. (2024). Dual COX-2/TNF-α Inhibitors as promising anti-inflammatory and cancer chemopreventive agents: A review. Iranian Journal of Pharmaceutical Research: IJPR, 23(1), e151312.

27. Tellegen, A. R., Rudnik-Jansen, I., Beukers, M., Miranda-Bedate, A., Bach, F. C., De Jong, W., ... & Tryfonidou, M. A. (2018). Intradiscal delivery of celecoxib-loaded microspheres restores intervertebral disc integrity in a preclinical canine model. Journal of Controlled Release, 286, 439-450.

28. Timur, U. T., Caron, M. M., Jeuken, R. M., Bastiaansen-Jenniskens, Y. M., Welting, T. J., van Rhijn, L. W., ... & Emans, P. J. (2020). Chondroprotective actions of selective COX-2 inhibitors in vivo: a systematic review. International journal of molecular sciences, 21(18), 6962.

29. Vishwakarma, R. K., Negi, D. S., & Negi, A. (2023). Abortitristoside A and desrhamnosylverbanscoside: the potential COX-2 inhibitor from the leaves of Nyctanthes arbor-tristis as anti-inflammatory agents based on the in vitro assay, molecular docking and ADMET prediction. Chemical Papers, 77(6), 3035-3049.

30. Westwell-Roper, C., & Stewart, S. E. (2020). Commentary: neurobiology and therapeutic potential of cyclooxygenase-2 (COX-2) inhibitors for inflammation in neuropsychiatric disorders. Frontiers in Psychiatry, 11, 264.