Review Article

Therapeutic Activity of Collagen with Analgesics: A Review

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ABSTRACT

This article is an examination of the therapeutic activity of collagen with analgesics. The scientific development and subsequent activity of collagen continues to influence the researchers all over the globe today. This article examines the research done and published by researchers and scientists. Consideration of current trends and data in scientific queries and demonstrates further aspects of collagen and analgesics. Additionally, this article explores options for therapeutic activity of cox inhibitors like etoricoxib, celecoxib, diclofenac and ibuprofen.

Keywords: Collagen, Analgesics, Cox-Inhibitors, Celecoxib, Etoricoxib, Diclofenac, Ibuprofen, Review, Therapeutic activity.

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INTRODUCTION

Collagen is a major component of human connective tissue that forms several body parts especially bone, skin and muscles. Collagen is of great importance because of its inflammation reducing properties and helps in ease of pain. Collagen supplements have gained popularity in the recent years because as we grow older, collagen deteriorates which results in various diseases and problems. These tends to accumulate in the tissues and enhances collagen production in the body which results in reduced pain and inflammation.

Analgesics are pain killers which functions without affecting the consciousness of a person and provides relief from pain without putting the person to sleep. Most common group of pain killers are Non-steroidal anti-inflammatory drugs (NSAIDs) and collagen. Experts have grouped them together because they work in a similar way and have a similar potency. There are various types of analgesics like CGRP inhibitors, COX-2 inhibitors etc. Cyclooxygenase (COX) are enzymes that produces prostaglandins. There are 2 types of COX- Enzymes, COX-1 and COX-2 that promotes inflammation, pain and fever by producing prostaglandins. While COX-1 produces prostaglandins that activates platelets and provide protection to stomach and intestinal lining.COX-2 inhibitors are a sub-class of non-steroidal anti-inflammatory drugs (NSAIDs) which block the COX-enzymes that leads to reduced production of prostaglandins thereby reducing inflammation, fever and pain. Therefore COX-2 inhibitors are used for the treatment of sports related injuries, osteoarthritis, rheumatoid arthritis, menstrual cramps etc. which are characterized by inflammation, mild to moderate pain and fever.

In a study conducted by Weng Chu Sai, (2009) studied the effects of COX-2 inhibitor celecoxib on the repair process. Rat Achilles tendons were collected and the three properties were checked at various celecoxib doses. The observations showed that celecoxib prevented cell migration in proliferation, whereas the collagen content remained unchanged.

In another study conducted by Yun-Sheng Ou (2011) stated that COX-2 selective inhibitor celecoxib is known for its anti-inflammatory effects in rheumatoid arthritis and OA patients. It was hypothesized that NSAIDs can help to influence collagen metabolism and improve the osteoarthritic cartilage. This study was designed to study the long-term effects of three NSAIDs namely celecoxib, Ibuprofen, indomethacin on the collagen I, II & III using rat models with induced OA. The results explained that celecoxib had no remarkable effect, ibuprofen increased the synthesis of I, II, III types of collagens. Indomethacin suppressed the synthesis of collagen type II and enhanced type I and III. Hence it can be concluded that celecoxib has no influence on collagen synthesis, ibuprofen can be critical and indomethacin could be damaging.

Thus, in this study our aim is to reflect the combined activity of collagen with COX-inhibitors and NSAIDs like Celecoxib, Etoricoxib, diclofenac and ibuprofen as a pain and inflammation reliever in case of acute pain in case of adults, rheumatoid arthritis, spondylitis, fever etc.

1. METHODS

The study was conducted using four databases Google Scholars SAGE, DOAJ and PubMed. Selection of papers were done based on keywords and theme relevant to this review. Further the published papers from these databases

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were arranged in systematic order with respect to year of publication.

RESULTS AND DISCUSSION

Table 1: Therapeutic Activity of Collagen with analgesics-cox- inhibitors like Celecoxib and Etoricoxib

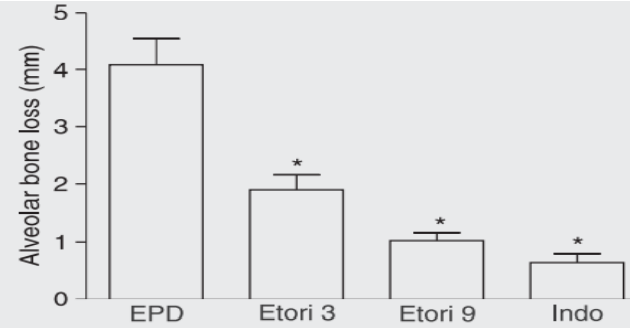
Sr.No.	Paper Title	Year
1	Comparison of etoricoxib and indomethacin for the treatment of experimental periodontitis in rats	2006
2	The Effects of Common Anti-Inflammatory Drugs on the Healing Rat Patellar Tendon	2007
3	Effects of Celecoxib on Migration, Proliferation and Collagen Expression of Tendon Cells	2009
4	Celecoxib suppresses fibroblast proliferation and collagen expression by inhibiting ERK1/2 and SMAD2/3 phosphorylation	2011
5	Effect of celecoxib on proliferation, collagen expression, ERK1/2 and SMAD2/3 phosphorylation in NIH/3T3 fibroblasts	2011
6	Study of Osteoarthritis Treatment with Anti-Inflammatory Drugs: Cyclooxygenase-2 Inhibitor and Steroids	2015
7	Do Different Cyclooxygenase Inhibitors Impair Rotator Cuff Healing in a Rabbit Model?	2015
8	Effects of the Highly COX-2-Selective Analgesic NSAID Etoricoxib on Human Periodontal Ligament Fibroblasts during Compressive Orthodontic Mechanical Strain	2019
9	Etoricoxib treatment prevented body weight gain and ameliorated oxidative stress in the liver of high-fat diet–fed rats	2020

Comparison of treatment of Periodontitis using etoricoxib and indomethacin

This study compared the activities of etoricoxib COX-2 selective and indomethacin COX-2 non-selective NSAID on the Periodontitis in the rat models. Six female mice were used for this experiment, after which further tests were performed. Inflammatory cell infiltration, ABL, cementum and collagen fiber destruction were reduced both by etoricoxib and indomethacin, as shown by the histopathology of the periodontium. Etoricoxib induced lesser damage than indomethacin in the intestinal and gastric mucosa. Nylon thread ligature was sterilized and placed around the cervix of the second left upper molar of anesthetized rats. Their survival rates and weights we recorded daily. Alveolar bone loss was measured using a stereoscope loupe (4X magnification).

Tissue Analysis: Histopathological analysis was used to quantify the inflammatory cell infiltration. The stomach and intestines of the rats were analyzed using the microscope. For statistical analysis, results were represented as mean ± standard error of the mean. If the p-value < 0.05, then the values are statistically significant. The treatment of EPD was initiated after 5 days to make it a more realistic model. Microscopic analysis showed retention of bone loss with both the drugs. Alveolar process, reduction of the inflammatory cell infiltration, and partial preservation of collagen fibers of the periodontal ligament were considerably preserved. Etoricoxib reduced the intestinal and gastrointestinal mucosal damage almost same as non-EPD mice (Figure 1.) Hence, therapy with etoricoxib helped retain survivability, reducing inflammation, collagen and bone loss, and the health of the intestinal and gastrointestinal regions.[1]

Figure 1: Comparison of the effect of etoricoxib (3mg/Kg and 9mg/Kg) and indomethacin on alveolar bone loss. (M.C.F. Azoubel1, 2006)



Study of Effects of NSAIDs on healing Rat Patellar Tendon

The effect of various NSAIDs, often prescribed during tendon injuries is unclear. The study was conducted on 215 rats whose patellar tendons were transected and sutured, then distributed into seven groups and given ibuprofen, acetaminophen, naproxen, piroxicam, celecoxib, valdecoxib, or control, respectively, for 14days. Results suggested, all the anti-inflammatory drugs except ibuprofen showed ill effects on the healing strength at the bone tendon junction, with decrease in collagen content at the injury site. The patellar tendon was exposed using a 10mm long incision made at the anterior portion of the knee using a No. 11 blade scalpel and the transection was sutured. After division into groups, they received; acetaminophen (60 mg/kg), ibuprofen (30 mg/kg), piroxicam (2.5 mg/kg), naproxen (24 mg/kg), celecoxib (10 mg/kg), and valdecoxib (3 mg/kg) and control (no drug) respectively. The activity of rats was monitored using photoelectric sensors. After 14 days, all rats were euthanized with carbon dioxide overdose, after which their tissues were collected for the biomechanical test. Hydroxyproline concentration was used to measure the collagen content. Dimethylmethylene blue assay was used

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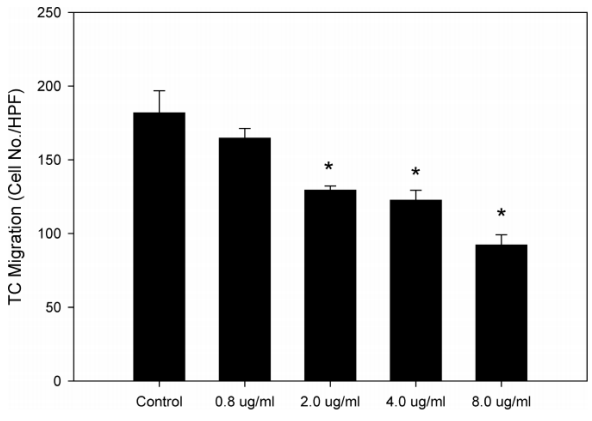
to quantify proteoglycan content. Standard curves were used to find sulfated glycosaminoglycan concentration. Histological examination was conducted on the quadricep muscles and tendon using the H&E test. For statistical analysis, all the results were represented as mean ± standard error of the mean. It was determined that if the p-value < 0.05, then the values are statistically significant. From earlier studies, it was revealed that the anti-inflammatory medicines showed contradictory results for tissue injury treatments. This study showed that early treatment of therapeutic doses of cox-2 inhibitors had destructive effects at the injury sites. The control group also showed higher hydroxyproline content (major component of collagen) compared to celecoxib, piroxicam, naproxen, and valdecoxib. Overall, these anti-inflammatory drugs did not help in the healing process and had the opposite effect. As the NSAIDs administrations alter the cell proliferation and repair of tendon, it leads to reduced collagen content because of them. [2]

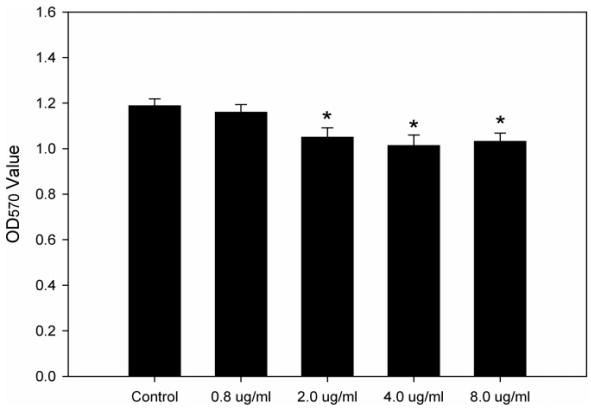
Study of Migration, Proliferation and collagen expression in tendon cells using celecoxib

During the regenerative phase of tendon injury, the tendon cells are transported to the repair site to proliferate actively and deposit plenty of extracellular matrix, necessary for the healing process. This study aims to study the effects of COX-2 inhibitor celecoxib on the repair process. Rat Achilles tendons were collected and the three properties were checked at various celecoxib doses. The observations showed that celecoxib prevented cell migration in proliferation, whereas the collagen content remained unchanged.20 Sprague-Dawley rats were used to obtain the Achilles tendons, which were cultured for further tests. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue (MTT) assay was conducted to detect cell proliferation and cell viability with various celecoxib doses. Transwell Filter Migration Assay was conducted on tendons with and without celecoxib doses to check the migration of tendon cells. RT-PCR analysis was performed to assess the gene expression of α1 (I) procollagen and α1 (III) procollagen. The gene expression was recorded to be constant throughout. Immunocytochemistry was used to study collagen I and II protein levels in the tendon cells at various celecoxib concentrations (Figure 2) The results showed constant levels throughout. For statistical analysis, all the results were represented as mean ± standard error of the mean. It was determined that if the p-value < 0.05, then the values are statistically significant. Inflammation, regeneration, and remodeling/maturation are the three phases involved in the tendon injury healing process. The main steps involve the migration and proliferation of tendon cells and collagen I and III synthesis. This study was aimed to study the effects of COX-2 selective inhibitor celecoxib's effects on the same. Celecoxib was shown to inhibit the tendon cell proliferation and migration, whereas it did not affect the collagen type I and II expression.[3]

Figure 2: Shows the change in migration of tendon cells (a) and cell proliferation (b) at different celecoxib doses. (Effects of celecoxib on migration, 2007)



(a)



(b)

Study of collagen suppression using celecoxib which inhibits ERK1/2 and SMAD2/3 phosphorylation

The aim of this study was to determine if celecoxib suppresses collagen by inhibiting ERK1/2 and SMAD2/3. For this, NIH/3T3 fibroblasts were stimulated with fibroblast growth factor-2 (FGF-2) or transforming growth factor-β1 (TGF-β1) and then treated with celecoxib. The results showed celecoxib to suppress cell proliferation, cell viability by inhibiting ERK1/2 phosphorylation but not its expression. Along with this, celecoxib also inhibited collagen expression by inhibiting ERK1/2 and SMAD2/3 phosphorylation. Celecoxib was also shown to induce apoptosis of NIH/3T3 fibroblasts. Sigma-Aldrich supplied NIH/3T3 cells were maintained and grown properly. Methylthiazoletetrazolium assay was conducted with and without simulation using FGF-2 and treated with increasing levels of celecoxib. It was to assess the cell proliferation and cell viability. TRIzol reagent was used to isolate RNA from NIH/3T3 cells and the quantitative RT-PCR was conducted. This test was to quantify gene expression. The cultured cells were lysed and Western blot analysis was performed to assess the gene expression. For statistical analysis, all the results were represented as mean ± standard error of the mean. It was determined that if the p-value < 0.05, then the values are statistically significant. In this study,

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celecoxib inhibited NIH/3T3 fibroblast proliferation, viability, collagen expression up-regulated p-SMAD2/3 and up-regulated p-ERK1/2. This implies that celecoxib could inhibit adhesion formation through other mechanisms apart from inhibiting COX-2, where COX-2 is known to play important role in the healing process of injuries. Adhesion formation takes place through activation of SMAD2/3 and ERK1/2 by TGF-β1 and FGF-2 which promotes collagen expression and fibroblast proliferation.[4]

Study of collagen content, proliferation, and ERK1/2 and SMAD2/3 phosphorylation in presence of Celecoxib

The aim of this study was to check the effects of celecoxib in NIH/3T3 fibroblasts on cell proliferation, collagen expression, and ERK1/2 and SMAD2/3 phosphorylation. Transforming growth factor-β1 (TGF-β1) or fibroblast growth factor-2 (FGF-2) was used to stimulate the cultured NIH/3T3 fibroblast cells. The results indicated the celecoxib inhibited cell proliferation and collagen expression which is due to its activity in inhibiting the ERK1/2, and SMAD2/3 phosphorylation. The purchased NIH/3T3 fibroblast cells were cultured in the appropriate medium and then used for further assays. Methyl thiazole tetrazolium assay was conducted to study cell proliferation with and without celecoxib and with different concentrations respectively. Western Blot analysis was used to study the expression of ERK1/2 and SMAD2/3in the cells. Quantitative RT-PCR was performed to obtain COL3 and COL1 expression levels in the cells. Celecoxib was shown to inhibit NIH/3T3 fibroblast proliferation, collagen expression (COL1&3), the up-regulated p-ERK1/2 & p-SMAD2/3 stimulated by FGF-2 & TGF-β1. The main reason for inhibition of proliferation and collagen expression could be because the celecoxib inhibits the p-ERK1/2 and SMAD2/3 phosphorylation which was originally to help in proliferation and collagen formation. This could imply that fibrosis processes correlated with fibroblasts, such as wound healing, scar, and adhesion formation could be influenced by the celecoxib administration. [5]

Effects of COX-2 inhibitors and Steroids on Osteoarthritis

Cyclooxygenase-2 (COX-2) inhibitors are used extensively to treat pain and inflammation and are known to have beneficial effects on cartilage damage by inhibiting the PGE2 production, by reducing the proteoglycan content in cartilage explants. This study compares the activity of prednisone, a steroid, Celecoxib, a COX-2 selective inhibitor, and piroxicam, a nonselective COX inhibitor, in reducing the MMP and PGE2 production and stimulate anabolic CII and aggrecan. An in-vitro experiment was conducted on chondrocytes by exposing them to TNF-α to mimic the activated chondrocyte. The study found steroid (prednisone) and celecoxib decreased MMP-1 and PGE2 production, whereas increased expression of aggrecan, piroxicam reduced only PGE2. Celecoxib showed increased gene expression of collagen type II. Another experiment was conducted in vivo to determine the activity of celecoxib on the posttraumatic OA (PTOA) mouse model. Results confirmed that celecoxib reduced the MMP1 and increased the aggrecan.

ELISA was conducted for PGE2 concentration. TNF-α showed increased production of PGE2, shown in figure3. The western blot test was conducted for MMP1 to see how it was affected by pharmacologic treatment on the stimulated chondrocytes. MMP1 concentrations were increased because of TNF-α, also shown in figure 2. Optical and histopathological analysis was used to quantify the cartilage damage in early osteoarthritis (OA).

RT-PCR test was conducted to quantify gene expression for MMP1, PGE2, and aggrecan.

MabCII-NIF: A non-invasive monitoring technique for the in vivo study is effective in identifying OA in the early stages (Figure 2 and 3).

P-value: For statistical analysis, all the results were represented as mean ± standard error of the mean. It was determined that if the p-value < 0.05, then the values are statistically significant. All drugs showed increased gene expression of CII and aggrecan, whereas only celecoxib and prednisone reduced MMP-1 and MMP13. All three drugs showed the reduced production of PGE2. In the in-vitro study of the PTOA mouse model, the most effective activity was demonstrated by celecoxib by decreasing the inflammatory cytokines and PGE2 concentration (by 90%). It also showed increased production of aggrecan, a minor increase in the CII (collagen type II). During OA, there is a disturbance in the homeostasis of chondrocytes, leading to the degradation of ECM (extracellular matrix) and articular cartilage. Proinflammatory cytokines like TNF-α increase the rate of this destruction by releasing MMPs, which degrade collagen. It was noticed that COX-2 expression is increased in eh OA affected chondrocytes which is celecoxib could show effective improvement in proteoglycan synthesis.[6]

Figure 3: Gene expression graph with (a) collagen II, (b) aggrecan, (c) MMP-1 and (d) MMP13. (Hongsik Cho, 2015)

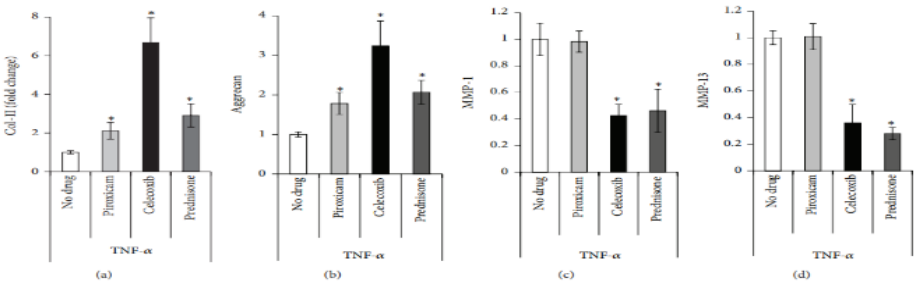

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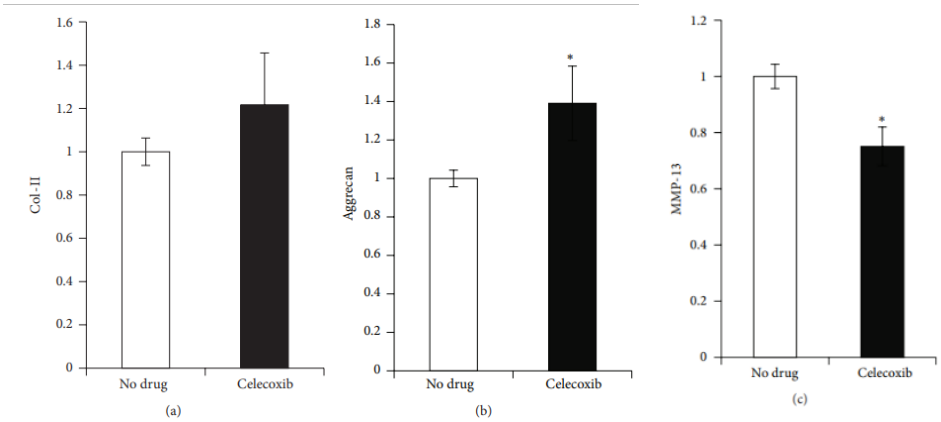
Figure 4: Effect of the celecoxib on MMP12, Coll-II, aggrecan in the in vivo study. (Hongsik Cho, 2015)



Effects of NSAIDs on Rotator cuff healing in rabbits

Tendon healing involves inflammatory reactions, and any factors that delay this can delay the healing process which can be done by COX inhibitors. The experiment was conducted on 96 New Zealand rabbits, for a massive tear, the right supraspinatus tendon of each rabbit was detached. Ibuprofen, celecoxib, flurbiprofen axetil, and the control were the four groups formed to divide the rabbits. Histological and biomechanical testing was used to analyze the tissues in 3, 6, 12 weeks. Celecoxib group showed a lower failure load compared to others and no cartilage or new bone formation, with collagen oriented out of order. The other two groups showed a mixture of fibroblastic cells and hypercellular tendons and the four zones of the bone tender interface similar to the control group. All groups showed increase in collagen I with time except celecoxib. Rabbits were anesthetized, the tendon was dissected from the greater tuberosity (GT), and tissue was removed. Orthocord suture was used for a modified Mason-Allen stitch. After division into four groups, they received; ibuprofen (10 mg·kg−1·d−1), celecoxib (8 mg·kg−1·d−1), flurbiprofen axetil (2 mg·kg−1·d−1), and control (no drug) respectively. In comparison to the normal side, the repair percentage on the load side was calculated. Failure load is the percentage ratio of load to failure on surgery to the normal side. Picric acid Sirius red staining test was used to analyze the collagen content in the bone tendon insertion. With polarized light illumination, collagen I looked yellowish red, and collagen III looked green. NSAIDs treat pain caused by the inflammatory reactions after the rotator cuff treatment, which involves the COX enzymes. It is known that the inflammation reaction is required for the tendon healing process and disrupting its mechanism causes the healing to delay. Celecoxib showed lower failure load (p=0.002) ibuprofen or flurbiprofen, showing no significant difference compared to the control group. This study showed that celecoxib affected the healing process and collagen formation much higher than the nonselective COX inhibitors. Hence it can be said that COX inhibitors affect the healing process if administered in the early stages after rotator cuff repair.[7]

Interpret role of etoricoxib on HPL (human Periodontal Ligament) fibroblasts

The study focused to determine the role of etoricoxib on Human periodontal ligament (hPDL) fibroblasts, in vitro experiments were conducted with etoricoxib treatment during orthodontic tooth movement. Experiment included simulation of compressive orthodontic mechanical strain on hPDL fibroblast cell lines. The cell-lines were incubated at various concentrations of etoricoxib for 72hrs under normal physiological conditions after which mechanical stress ($2g/cm^2$) was applied to half samples for 48 hours. Various tests and assays concluded that there were no detrimental effects of etoricoxib on the hPDL fibroblasts including the periodontal inflammation, extracellular matrix remodeling, RANK-L/OPG expression, and osteoclastogenesis.

Gene expression quantification using RT-PCR: MIQE guidelines were followed to perform RT-qPCR, RNA isolation and quality assessment.

Quantification of RANK-L Protein Expression using Western Blot: Proteins were extracted from the cell lines and quantified using western blot using anti rabbit IgG. Etoricoxib had no effects on RANK-L production at any concentrations. **ELISA** was performed to quantify and detect PG-E2(prostaglandin E2), sRANK-L (soluble receptor activator of nuclear factor kappa b ligand), OPG (osteoprotegerin), and ALPL (alkaline phosphatase).

TRAP Histochemistry was to assess hPDL fibroblasts mediated Osteoclastogenesis in Coculture. Etoricoxib had no effects on Osteoclastogenesis at any concentrations **Flow cytometry** was employed to check cell apoptosis and necrosis which didn't not show any significant changes to etoricoxib. Cell cytotoxicity was estimated using LDH assay and it didn't not show any significant changes to etoricoxib. Cell viability was checked using MTT assay and etoricoxib did not affect it at any concentration levels. For statistical analysis, all the results were represented as mean ± standard deviation of the mean. It was determined that if p value < 5, then the values are statistically significant. Etoricoxib was found to have no improvement in

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periodontal inflammation, extracellular matrix remodeling, RANK-L/OPG expression, and osteoclastogenesis during simulated orthodontic compressive strain on the hPDL fibroblast cell lines. It was quite opposite of the expected results as etoricoxib a selective cox 2 inhibitor is known for its activity against inflammation in various other parts of the body.[8]

Effects of etoricoxib to stabilize various factors in high-fat diet induced obese rats

The goal of this study was to determine the therapeutic effects of etoricoxib on high-fat diet mice since obesity is known to increase the production of COX2 enzymes and further cause various health complications and enhanced inflammation. Twenty-eight, (10-12 weeks old) male Wistar rats were distributed into four groups with conditions; control, control + etoricoxib, HF and HF + etoricoxib for 8weeks during the study. Tests were conducted and results revealed that etoricoxib treatment lowered the weight gain, (peritoneal, epididymal, mesenteric) fat wet weights, blood glucose, cholesterol, LDL and triglyceride levels, AST, ALP, ALT and MPO levels, oxidative stress indicators like MDA, NO, APOP levels, evasion of inflammatory cells in liver tissue and collagen deposition in hepatic tissues which were found in increased levels in the HF rats without etoricoxib. Whereas etoricoxib increased the SOD activity and GSH levels in HF mice which were found reduced in its absence. Overall, the research suggested beneficial role of etoricoxib on obesity induced problems in animal model. Oral glucose tolerance test (OGTT) was performed twice before and after experiment. Fasted for 12 hours before the test. Use of diagnostic kits (Hungary) were used to determine activities ALP, AST, ALT and concentrations of total cholesterol HDL, LDL, and tri-glycerides. Liver tissue sample was prepared and plasma and Liver's lipid peroxidation were measured as MDA content, also NO and APOP concentrations were determined. Catalase activity was deduced using similar method of absorption at 240nm per min with absorbance change of 0.01units/min. SOD levels were interpreted using another method and absorbance recorded at 480nm. MPO (myeloperoxidase) levels were found using a slightly modified 96-well plate-based assay. Etoricoxib reduced MPO activity. Hematoxylin/eosin staining technique was used to study the tissue architecture. Excess collagen deposits found in HF mice were reduced in etoricoxib treated mice. For statistical analysis, all the results were represented as mean ± standard error of the mean. It was determined that if p value < 5, then the values are statistically significant. Etoricoxib a cox 2 inhibitor was found to perform therapeutic activity in high fat obese conditions to reduce body weight, fat, cholesterol, ROS production, oxidative stress, inflammatory cells and excess collagen close to the normal levels. It was also found that this specific inhibitor increased the antioxidant activity and GSH levels which was found to be reduced in obese conditions. Inhibitors of COX enzymes could be potential solution to these disabilities, and etoricoxib proves to do the job through the experiment and the analysis.[9]

Table 2: Therapeutic Activity of Collagen with analgesics like Diclofenac and Ibuprofen

Sr. no.	Paper Title	Year
1	The influence of some non-steroidal anti-inflammatory drugs on the retraction of collagen lattices	2002
2	Effects of ibuprofen on molecular markers of cartilage and synovium turnover in patients with knee osteoarthritis	2004
3	Effects of diclofenac sodium on bursting pressures of anastomoses and hydroxyproline contents of perianastomotic tissues in a laboratory study	2006
4	Cyclooxygenase inhibition by diclofenac formulated in bio adhesive carriers	2008
5	Ibuprofen and diclofenac sodium in the treatment of osteoarthritis: a comparative trial of two once-daily sustained-release NSAID formulations	2008
6	The effects of NSAIDs on types I, II, and III collagen metabolism in a rat osteoarthritis model	2011
7	Effect of Postoperative Diclofenac on Anastomotic Healing, Skin Wounds and Subcutaneous Collagen Accumulation: A Randomized, Blinded, Placebo-Controlled, Experimental Study	2012
8	Therapeutic mechanisms of ibuprofen, prednisone and betamethasone in osteoarthritis	2016
9	Tendon collagen synthesis declines with immobilization in elderly humans: no effect of anti-inflammatory medication	2017
10	Effects of anti-inflammatory (NSAID) treatment on human tendinopathic tissue	2017

Withdrawal of collagen lattices altered by non-steroidal anti-inflammatory drugs

This study demonstrates the repercussions of some non-steroidal anti-inflammatory drugs on the generation and retraction of collagen lattices seeded with fibroblasts. Diclofenac was observed to be the most effective retardant among the NSAIDs. Ibuprofen was observed to notably intensify the lattice retraction when used in 10 µg/ml and 50 µg/ml.

Direct effect of NSAIDs on collagen lattice: At 10 µg/ml concentration of diclofenac no effect was observed. The concentration was observed to be almost totally inhibited (lattice width 97.4% and 90.7% of the day 0) at

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concentrations 50 µg/ml and 100 µg/ml. Ibuprofen escalated the lattice retraction at both 10 and 50 µg/ml concentrations whereas no effect was observed at 100 µg/ml.

Withdrawal of lattice in existence of cells preincubated with NSAIDs: Diclofenac preincubated cells were observed to inhibit only early lattice retraction (day 2) at 100 µg/ml whereas no effect of lower concentrations. At day 8 no further changes in lattice width were observed between control and preincubated cells. Ibuprofen incubated cells demonstrated lattice retraction initially at 50 and 100 µg/ml concentrations (day 2). A minor stimulation was also observed at day 8 at 100 µg/ml concentration only.

Withdrawal of lattice after ASAIDs elimination during culturing: Diclofenac dynamically inhibits the lattice retraction. Retraction can be observed continuing extensively after replacement of medium followed by one week recovery of cells. It was observed that ibuprofen is an incapacitated inhibitor of lattice retraction. Toxic effect possibility was taken into inspection as after washing off the drugs from the lattice the retraction was again seen. It can be hypothesized that these drugs work by hampering the formation of some proteins vital for retraction of lattices. The results of this study demonstrates that cell matrix interactions of connective tissues can be modulated by non-steroidal anti-inflammatory drugs.[10]

Ibuprofen consequence on molecular markers of cartilage and synovium turnover in patients with knee osteoarthritis.

The objective of this experimental study was the evaluation of ibuprofen effects on the urinary transudation of C-terminal crosslinking telopeptide of collagen type II and the urinary glucosyl galactosyl pyridinoline, two advanced cartilage molecular markers and synovial tissue metabolism respectively in patients affected with knee osteoarthritis. 201 people with knee osteoarthritis were executed with non-steroidal anti-inflammatory drugs. Urinary transudation of C-terminal crosslinking telopeptide of collagen type II (CTX II) and the urinary glucosyl galactosyl pyridinoline (Glc-Gal-PYD) were measured after 4-6 weeks of treatment and a major increase was observed from the reference in the placebo group whereas no remarkable increase was noted in the ibuprofen group. Exceptional variation was observed from the reference between the placebo and ibuprofen group for CTX II. In conclusion, major elevation in markers indicating cartilage and synovial metabolism in patients with knee osteoarthritis was observed which could be prevented by inflated doses of ibuprofen. No major variation at the baseline ($p>0.05$) was observed between placebo and ibuprofen groups in the urinary levels of CTX II and Glc-Gal-PYD. Significant elevation in CTX II and Glc-Gal-PYD during a reexamination were observed in the placebo group with 17% mean increase ($p=0.023$ *vs* reference), and 10% ($p=0.020$ *vs* reference), respectively. The ibuprofen group showed slight increase of around 4% ($p=0.045$) in Glc-Gal-PYD and no notable elevation in CTX II was observed (0>0.05 *vs* reference). Remarkably higher increase in placebo group was observed in CTX II from reference in comparison to the ibuprofen group (+13%, $p=0.017$) after 4-6 weeks of treatment whereas the variation between groups was insufficient to reach statistical significance for Glc-Gal-PYD. Patients were further divided into groups according to the presence and absence of swelling of knee at the baseline (n=127 with swelling, n=74 swelling absent), urinary CTX II and Glc-Gal-PYD were observed to be significantly increased in the group with knee swelling. No major change in the group with absence of swelling was observed. No increase was observed in patients with knee swelling in the ibuprofen group and the variation from the placebo group was remarkable for CTX II ($p=0.014$) whereas only partial correlation was noted between Glc-Gal-PYD and CTX II levels at reference ($r^2= 0.17$, $p<0.001$). Observation was made that the patients with presence of knee swelling were identified by elevated urinary excretion of markers representing cartilage and synovium deterioration respectively. Over the course of 4-6 weeks, the amount of these indicators rose in the placebo group and the increase was somewhat prevented by the high dosage of ibuprofen indicating possible effect on cartilage and synovium metabolism in patients with active knee osteoarthritis. The fundamental hypothesis is that reflection of metabolic processes in joint tissues could be done by such markers and also could be used for diagnosis, and monitoring of osteoarthritis. The study concluded that that greater urinary excretion of CTX II and Glc-Gal-PYD is linked to a faster course of joint destruction in vivo in patients with early arthritis and knee osteoarthritis, implying that these markers are linked to joint damage. There were a few drawbacks to this study. [11]

Diclofenac sodium effects on bursting pressures of anastomoses and hydroxyproline contents of perianastomotic tissues.

Diclofenac sodium is a non-steroidal anti-inflammatory medication (NSAID) often used as an analgesic with anti-inflammatory characteristics. The effects of diclofenac on the rejuvenation of colonic anastomosis in rats were investigated in this study, which included 36 rats. The distal colons of rats were resectioned and anastomosed. Diclofenac was given intramuscularly at a dose of 4 mg/kg/day, while the control group received 0.1 mL/day of 0.9 percent NaCl. On the third and seventh postoperative days, the bursting pressure of the anastomoses and the hydroxyproline level of the perianastomotic tissues were measured. The Mann-Whitney U-test was employed for statistical analysis. On the third and seventh days, the diclofenac group of rats had lower bursting pressures than the control group. The average hydroxyproline content of diclofenac-treated rats' perianastomotic tissues was lower than controls on the 3rd and 7th days, which was similar to bursting pressures. Finally, diclofenac sodium has a deleterious influence on the bursting pressures of the colonic anastomosis and the hydroxyproline content of perianastomotic tissues in rats. Anastomotic leak rates have little effect. Infectious problems were followed by anastomotic leaks in three rats from each group. After that,

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the number of rats in each group was reported to be eight on day three and seven on day seven. There were no substantial differences in anastomotic leakage across the groups (p>0.05). [12]

The diclofenac group had lower rupturing pressures than the control group on days 3 and 7, which was statistically significant (day 3 p=0.01 and day 7 p=0.03). On days 3 and 7, the diclofenac group's hydroxyproline contents in perianastomotic tissues were lower than the control group, and these differences were also significant as rupturing pressures (day 3 p=0.01; day 7 p=0.01). Finally, this investigation showed that diclofenac had a deleterious effect on anastomosis bursting pressures and the hydroxyproline content of perianastomotic tissues in rats' colons. Diclofenac has also been discovered to have no effect on the rate of leakage of colonic anastomosis in rats.

Diclofenac formulated in bio adhesive carriers inhibits cyclooxygenase

Diclofenac is commonly used to treat rheumatic illnesses and other inflammatory diseases. The biophysical and biochemical properties of diclofenac formulated in previously created bio adhesive liposomes with hyaluronan (HA-BAL) or collagen (COL-BAL) on their surface are investigated in this study. Both types of liposomes efficiently encapsulated diclofenac, with encapsulated dosages reaching 13 mg/ml and acting as sustained release diclofenac depots with half-lives of drug release ranging from 1 to 3 days (under fast circumstances). In CT-26 cells with CD44 Hyaluronan receptors and integrins, liposomal diclofenac showed remedial efficacy. HA-BAL and COL-BAL had 40 and 6-fold higher cellular affinity than ordinary liposomes, respectively. Diclofenac encapsulated in liposomes and diclofenac free were shown to have similar actions. This study suggests that new diclofenac formulations have important physiochemical and biochemical properties.

Molecular characteristics of diclofenac-liposome formulations: At low drug concentrations (0.1–5 mg/ml) and 20 mg lipid/ml of liposome concentration, diclofenac enclosing efficiency was found to be 40-50 percent. At low drug concentrations, encapsulation efficiency is quite high and practically complete; however, as drug concentration increases, encapsulation efficiency decreases. Based on physicochemical considerations, directed local administration of diclofenac–liposome formulations, where dilution is substantially lower than in systemic administration, is projected to increase the time duration for drug delivery from each dosage well beyond 1–3 days.

Diclofenac-liposome compositions have cellular characteristics: The expression of CD44 on the CT-26 cell line was chosen for this study, and it is believed to have a relationship with the preferred binding of HA-BAL. All of the liposome types tested (RL, HA-BAL, and COL-BAL) were found to bind to CT-26 cell monolayers in a saturating manner. Regular liposomes, on the other hand, have a non-specific and weak contact with the CT-26. The binding of COL-BAL to these cells is intermediate between that of RL and HA-BAL, with an affinity that is 6-fold higher than that of RL and a capacity decline that is less pronounced than that of HA-BAL. These findings point to the presence of components on the CT-26 cells' surface that can bind collagen (thus COL-BAL), such as integrin and ECM, although with a lower specificity than HA-BAL. Nonetheless, both forms of bio adhesive liposomes demonstrate adequate binding to act as Diclofenac depots with site retention. Finally, at remedially-relevant doses, SD's HA-BAL and COL-BAL have a high enclosing capability. The bio adhesive CT-26 binds to it with a high affinity.[13]

Osteoarthritis treatment using NSAID (Ibuprofen and diclofenac sodium)

A study was conducted for the comparison of the effectiveness and tolerability of non-steroidal anti-inflammatory drugs such as ibuprofen and diclofenac sodium in patients suffering from osteoarthritis primarily concerning the knee and/or hip. Patients were given either two tablets of ibuprofen each evening for 21 days or a single 100mg diclofenac tablet. The result concluded that both therapies were effective but not equally. By day 21 much improvement was seen in ibuprofen-treated patients i.e., about 37% whereas only 10% diclofenac treated patients were improved (p = 0.04). The overall treatment was also rated good or excellent by 80 % patients in favor of ibuprofen.

Patients: 30 patients were randomly chosen to be administered with two sustained release 1600 mg ibuprofen tablets and other 31 patients were administered with one 100 mg diclofenac tablet. From the total of 61 patients, 51% patients reported pain from knee only or from both knee and other sites, 25% reported pain from hip or both hip and knee. The remaining 18% of the patients did not report any site of pain. 63% of the patients from the ibuprofen group and 55% from the diclofenac group had previously been prescribed non-steroidal anti-inflammatory drugs as treatment.

Potency: The degree of improvement was noted to be increased from 13% at day & to 37% at day 21 in patients administered with ibuprofen and in diclofenac group it was from 3% at day 7 to 10% at day 21. The measure of potency at day 7 and day 21 was 0.04. Several other parameters were evaluated wherein significant statistical differences were observed in favor of ibuprofen such as severity of day pain at Day 21 (p=0.006), severity of night pain at Day 7 (p=0.048), better quality of sleep at Day 7 (p=0.04) and at Day 21 (p=0.03), followed by their overall ability to carry out normal activities at Day 21 (p=0.01).

Unfavorable effects: In the ibuprofen treated group three unfavorable effects were reported by 10% of the patients which were mild abdominal pain (unrelated to the treatment), mild insomnia and lastly moderately severe constipation. In comparison 12 adverse effects of gastrointestinal nature were reported by the diclofenac treated groups by 32% of the patients (3 mild, 8 moderate

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severity and 1 severe). Two patients of this group were withdrawn from the study due to diarrhea and dizziness.[14]

Collagen metabolism of type I, II and III affected by NSAIDs: A study using rat osteoarthritis model.

A rat osteoarthritis model was used to observe the effects of extended use of NSAIDs such as ibuprofen on types I, II and III of collagen metabolism. A total of 130 rats were randomly divided into 4 groups, two of which were the ibuprofen group and the normal saline group. Osteoarthritis was instigated in the rats surgically. The expression of collagen type I, II and III were observed with the help of immunohistochemistry after 3 months followed by 6 and 9 months of the induction of osteoarthritis in rats. The results suggested that ibuprofen increased the synthesis followed by the expression of types I, II and III collagens.

Observation of rats after surgical induction of osteoarthritis: The change could be observed after two months of the surgical induction. Light was displayed after HE staining and cartilage cells expressed mild hyperplasia. The toluidine blue staining was observed slightly uneven. The experiment couldn't be completed in the 9th month due to substantial number of rat deaths.

Micro anatomical observation of articular cartilage: In the 9th month of surgical induction of OA the cartilage layer was observed to be thinner and degenerated in the ibuprofen group. The chondrocytes were observed to be arranged irregularly.

Proteoglycan expression quantification in cartilage: For the observation of expression of proteoglycan, it was stained with toluidine blue. And the result of ibuprofen group showed no exceptional effect on the expression.

Demonstration of type I collagen: It was observed that ibuprofen notably increased the expression of type I collagen and in the 9th month it inhibited the expression ($P < 0.01$)

Demonstration of type II collagen: It was observed that ibuprofen elevated the expression of type II collagen.

Demonstration of type III collagen: No exceptional change was observed in the expression during the 3rd month by ibuprofen but in 9th month major elevation of expression was observed ($P<0.01$). Surgical induction of OA represents an ideal model for this study. The initial type of damage of type II collagen is the damage of articulate cartilage. In this study osteoarthritis chondrocytes co-expressed themselves in type I and type III collagens which were initially observed in the superficial layer and the transitional layer of cartilage and then in all layers. As the intensity of OA increased its expression also increases. In conclusion this study demonstrated that ibuprofen is not the ideal choice for the treatment of osteoarthritis.[15]

A randomized and placebo-controlled experimental study to investigate the effect of diclofenac on anastomotic healing, skin injuries and subcutaneous increase of collagen.

This study inspects the effects of diclofenac on the fracture strength of colonic anastomosis, a skin wound and subcutaneous accumulation of collagen. The experimental study was randomized, placebo-controlled and blinded study in which 60 male rats were chosen and were administered with a dose of 4mg/kg/day diclofenac or placebo. Colonic anastomosis was conducted for individual rat and an expanded polytetrafluoroethylene (ePTFE) tube was accommodated hypodermically. Hydroxyproline content in ePTFE tubes including wound breaking strength of anastomotic and incisional strength were measured after 7 days. No major differences were observed in any strength measurements but a 38% decline in hydroxyproline accumulation as a consequence of treatment with diclofenac was observed ($p=0.03$). Hypodermal collagen accumulation in the placebo group corresponded positively with skin wound but negatively with anastomotic biomechanical strength. 8 rats (4 from each group) died in the initial hours. Autopsy concluded the reason to be respiratory failure as a consequence of anesthesia thus the experimentation was performed on remaining rats. No major differences in breaking strength intergroup was observed for anastomotic and skin injuries. The diclofenac group was observed to have declined collagen accumulation by 38% measured by hydroxyproline content in the ePTFE tubes in comparison with the placebo group. The decline in anastomotic strength in the placebo group was majorly correlated with increasing hydroxyproline accumulation in hypodermis and a tendency of positive correlation was noted between hypodermis accumulation of hydroxyproline and skin injury strength whereas no alike changes were observed in the diclofenac group. A dose of diclofenac which had earlier been found to be an efficient decliner of cyclo-oxygenase levels in anastomotic tissue did not affect the failure strength of colonic anastomoses or skin injuries. However, the inhibition of collagen accumulation in the ePTFE tubes was observed. The anastomotic strength is not determined by the total accumulation of collagen but by the level of arrangement, full-growth and cross-linking of collagen and fibrils. Therefore, the major focus of this study is on the accumulation using the ePTFE tube. Since the failure strength evaluations are the major criteria of anastomotic quality, it is concluded that the effective prohibiting effect on the hypodermis collagen formation was not significant in this study. This study observed and provided the inverse relation between the hypodermis accumulation of hydroxyproline and anastomotic strength among the rats of placebo group. It was observed that the effect on diclofenac on generation of collagen was organ dependent. In conclusion, diclofenac showed no impact on the breaking strength of colonic anastomoses or skin injuries. However, an inhibitory effect of relevant dose of diclofenac on accumulation of collagen in ePTFE tubes was observed.[16]

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Remedial mechanisms of non-steroidal anti-inflammatory drugs, in particular ibuprofen in osteoarthritis.

The goal of this research is to look at the remedial mechanisms of NSAIDs and steroids in the treatment of osteoarthritis. Human chondrocytes CHON-002 cell line was used in this study. ELISA was used to assess the cytokines and interleukins produced by cells treated with tumor necrosis factor α (TNF- α). Following treatment with interleukins, Western blot was used to determine the levels of collagen I, aggrecan, matrix metalloproteinase (MMP1), MMP-13, signal transducer and activator of transcription (STAT) 3, nuclear factor component p65, and inhibitory subunit of nuclear factor. Interleukin 6 or 8 was found to raise the protein levels of collagen I, MMP 1 and MMP 13, as well as enhance STAT3 phosphorylation and increase the expression of nuclear factor (NK) subunit p65, but had no effect on aggrecan protein levels. The levels of collagen 1, MMP 1 and MMP 13 were observed to be declining after combined treatment with siSTAT3 and MG132. The levels of interleukin 6 and 8 were also noted to be crucially dropping by ibuprofen. Ibuprofen reduced collagen 1, MMP 1 and MMP 13 levels and inactivated the NF and STAT3 pathways when combined with interleukin 6 or 8. It is concluded that ibuprofen may avert osteoarthritis by suppressing the expression of interleukins 6 and 8 and subsequently inactivating NF and STAT3 pathways therefore eventually resulting in the reduced levels of collagen 1, MMP 1 and MMP 13.

Levels of IF-1 β, IL-6, IL-8, and IL-10 expression with and without TNF- α treatment: The study used TNF- α levels of 5 ng/ml to activate cultured chondrocytes and restore an inflammatory response in vitro. Cultures not treated with TNF- α served as control group. The levels of interleukins were measured using ELISA. TNF- α significantly increased the expression levels of interleukins ($p<0.05$) when compared to the control group.

Collagen I, aggrecan, and MMP1 and MMP13 expression with or without cytokines: The western blotting results exhibited significant increase in the expression levels of collagen I, MMP 1 and MMP 13 by the treatment with IL 6 or 8. No remarkable changes were observed in the protein levels of aggrecan when treated with IF-1β, IL-6, IL-8 or IL-10 in comparison with the control group. Treatment with IL-1β or IL 10 increased collagen I, MMP-1, and MMP-13 expression, but there was no statistically significant difference between the experimental and control groups.

IL 6 and IL 8 promote STAT3 phosphorylation and NF-κB subunit p65 activation: In comparison to the control group, the level of expression of $pSTAT3^{Ser727}$ and $pSTAT3^{Tyr705}$ was found to be higher after treatment with IL 6 or 8, showing that IL 6 and 8 promote STAT3 phosphorylation. Furthermore, after treatment with IL-6 or IL-8, the expression of IκB was lower than the control group, although the expression of p65 (both in the cell nucleus and throughout the cell) was higher, indicating that NF-κB p65 was activated by IL-6 or IL-8 stimulation.

The effects of inhibiting STAT3 and NF κB on collagen I, aggrecan, and MMP 1 and MMP 13 expression: In comparison to the control group, the expression of $pSTAT3^{Ser727}$ and $pSTAT3^{Tyr705}$ was significantly reduced after transfection with siSTAT3. After transfection with siSTAT3, the effects of IL 6 and 8 on the expression of collagen I, MMP 1 and MMP 13 were reduced but not totally eliminated, showing the presence of additional mechanisms for the control of collagen I, MMP-1 and MMP-13 expression. In comparison to the IL-6/IL-8+ siSTAT3 group, the expression of collagen I, MMP-1, and MMP-13 was reduced after treatment with MG132 followed by transfection with siSTAT3. The findings showed that the effects of IL-6 and IL-8 on collagen I, MMP-1, and MMP-13 production may be mediated by STAT3 phosphorylation and activation of NF-κB p65.

Effects of NSAIDs and cytokines on cell viability and IL 6 and IL 8 expression: After treatment with cytokines or NSAIDs, there was no difference in cell viability when compared to the control group (ibuprofen). In the expression of IL 6 and IL 8, ELISA followed by treatment with ibuprofen alone revealed no significant changes. When TNF-α was coupled with ibuprofen, however, the elevated levels of IL-6 and IL-8 generated by TNF-α were significantly reduced ($p<0.05$).

STAT3, NF kB p65, collagen I, MMP 1 and MMP 13 expression in response to IL 6 or IL 8 in combination with medications: Ibuprofen administration decreased the expression of $pSTAT3^{Ser727}$, $pSTAT3^{Tyr705}$, p65 (both in the cell nucleus and whole-cell), collagen I, MMP-1, and MMP-13, but it increased the levels of IκB. These results suggested that ibuprofen reduced the inflammatory response and prevented abnormal catabolism. TNF-α activates cultured chondrocytes, resulting in increased expression of interleukins linked to osteoarthritis, according to this research. Finally, the findings of this study showed that ibuprofen's capacity to protect against osteoarthritis was due to suppression of IL 6 and IL expression, followed by suppression of the NF-κB and STAT3 signaling pathways, and finally, reduction of collagen I, MMP-1, and MMP-13 production.[17]

In older adults, tendon collagen production decreases with immobilization: anti-inflammatory medicine has no effect.

In this investigation, the effects of NSAIDs on human patellar tendon protein production and mechanical characteristics during immobilization and readapting resistance training, as well as the impacts of NSAIDs on these criteria, were investigated. 19 men between the ages of 60 and 80 were randomly assigned to receive either NSAIDs (1200mg/day ibuprofen) or a placebo. For two weeks, one lower leg was immobilized in a cast and then retrained for six weeks. Tendon collagen protein production, mechanical characteristics, size, expression of genes involved in collagen turnover and remodeling, and signal intensity were all studied. There was a decrease in tendon collagen synthesis ($P<0.001$), but mechanical characteristics and tendon size were unchanged, and no effect of NSAIDs was found. In both groups, there was an increase in matrix

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metalloproteinase-2 mRNA (P<0.1), but only in the placebo group was there was a drop in scleraxis mRNA (P<0.05). Collagen protein production was reduced in mature people's tendons after 2 weeks of immobilization; however, tendon stiffness and modulus were only moderately reduced, and NSAIDs had no effect on this.

Characteristics of the subject: Post hoc testing revealed that the placebo group's body weight increased after immobilisation and two weeks of retraining, then returned to normal after six weeks of retraining, whereas the ibuprofen group's body weight increased after six weeks of retraining and two weeks of retraining.

Mechanical properties of tendon: There was no difference in tendon stiffness between the groups. Despite the fact that there was no difference in mechanical properties between groups at the baseline, a group effect was observed for tendon deformation and a tendency to a group effect (P<0.1) was observed for tendon strain, both of which were higher in the ibuprofen group compared to the placebo group.

Intensity of the magnetic resonance imaging signal in the tendons: After immobilization, the distant tendon signal potency decreased, as revealed by post hoc tests, and restored to reference values after 2 weeks of retraining. Throughout the trial, there was no change in signal intensity in the central part of the tendon, but there was a decrease in the proximal section of the tendon, which remained reduced throughout the retraining period.

Synthesis of tendon collagen: Tendon collagen FSR was found to be 0.037 ± 0.003 percent /h in the placebo group and 0.032 ± 0.003 percent /h in the ibuprofen group at the reference line. After immobilization, the FSR in the placebo group was 0.007 ± 0.001 percent per hour, while in the ibuprofen group it was 0.006 ± 0.002 percent per hour. There was no evidence of a group impact. Furthermore, after immobilization, there was no change in the FSR from the reference line. At baseline, the 95 percent confidence intervals for group differences in tendon collagen FSR were -0.003 to 0.012, and after immobilization, they were -0.007 to 0.008.

Gene expression in tendon: After immobilization, the placebo group showed a decrease in scleraxis expression, whereas the ibuprofen group showed no change, leading in a group variance after immobilization.

Proportions of tendon: At baseline, no differences in tendon length or CSA were noted across groups, and the data for each independent individual were set to 100 percent. The distal regions showed little modification over time or between groups. There was no variation in the proximal part. Finally, there were no differences in tendon length or mean CSA between groups or over time (P > 0.05). The key findings were that after 2 weeks of immobilization, a decrease in tendon collagen synthesis was observed in mature humans, while there was a negligible reduction in tendon stiffness and modulus, on which NSAIDs had no effect, indicating the importance of mechanical loading for the maintenance of tendon collagen turnover. Furthermore, translational activity of tendon collagen protein formation may be decreased due to immobilization. Furthermore, the current findings suggest that NSAIDs may be used as an analgesic treatment during times of unloading in older individuals without causing unfavorable mechanical repercussions for healthy tendon adaptation. [18]

Non-steroidal anti-inflammatory drugs effect on tendinopathic tissue of humans.

Tendinopathy treatment can be done with the use of NSAIDs but the detailed effects of these drugs are not yet clear on the tendon tissue. 26 adults (16 men and 10 women) are chosen for this study with tendinopathy. The methods adopted in this study includes ultrasonography examination followed by tendon biopsies, RNA extraction and RT-PCR. In conclusion no change in the amount of RNA was observed in response to treatment with ibuprofen.

Circulating ibuprofen: After one week of treatment no ibuprofen samples were detected in the placebo group whereas all the samples from the ibuprofen group contained measurable levels of ibuprofen i.e., 30 ± 26µg/ml (means ± SD).

Gene expression and RNA content: No change in the total amount of RNA was observed in response to the treatment with ibuprofen and similarly no change in the gene expression was observed.

Tendon pain, function and thickness: No variation was observed between groups in the post treatment values or in the pre-to-post changes in function of tendon or the thickness of tendon. Similarly, no changes were observed in the pre-to-post changes in the tendon pain scores between groups.

Doppler activity: After the treatment period the Doppler signal was observed to be significantly lower in the placebo group in comparison with the ibuprofen group (p<0.05) and the pre-to-pot changes in the total Doppler signal also differed between groups (p<0.01). However, no significant difference between the treatment groups could be detected in the Doppler within the tendon since on patient in the placebo group demonstrated an increase in signal after the treatment in contrast to other patients in this group. The study suggests that the cell activity of tendon is not noticeably altered and no remedial response was induced by the treatment. Interestingly reduced blood flow in the tendon was noted (measured as Doppler signal) in comparison with the ibuprofen group whereas the pain perception was observed to be elevated in the placebo group.

Cellular response to ibuprofen: No evidence of the cell activity changes or number was found in response to ibuprofen. The similar total RNA content in both groups suggested that the ribosomal content and thus the translational potential/activity of the cells was stable. Therefore, either the cells investigated were not responsive to ibuprofen treatment or it didn't reach the tendon cells.

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Tendon pain and function: No significant changes in the tendon function were observed. Also, same levels of pain were experienced by patients during running of both groups. However, it is suggested that some extent of pain relief may be induced by treatment with ibuprofen.

Blood flow in tendon: Doppler signal was found to be reduced in the placebo group after treatment and a positive relation was observed between the physical activity and the amount of Doppler signal within the ibuprofen group. [19]

Table 3: Therapeutic Activity of Collagen with analgesics like Diclofenac and Ibuprofen

Sr No.	Paper Title	Year
1	Persistent Analgesic Effect of Sustained Release Diclofenac Sodium Preparation on Bovine Type II Collagen-Induced Arthritis	2002
2	A novel Diclofenac-carrier for local treatment of osteoarthritis applying live-animal MRI	2009
3	Liposomal dexamethasone–diclofenac combinations for local osteoarthritis treatment	2009
4	An Overview of Clinical Pharmacology of Ibuprofen	2010
5	Efficacy and tolerance of enzymatic hydrolyzed collagen (EHC) vs. glucosamine sulphate (GS) in the treatment of knee osteoarthritis (KOA)	2010
6	Comparative Effect of Nimesulide and Ibuprofen on the Urinary Levels of Collagen Type II C-Telopeptide Degradation Products and on the Serum Levels of Hyaluronan and Matrix Metalloproteinases-3 and -13 in Patients with Flare-Up of Osteoarthritis	2012
7	Intra-articular injection of collagenase in the knee of rats as an alternative model to study nociception associated with osteoarthritis	2014
8	Collagen-based biomaterials for ibuprofen delivery	2016
9	Comparison of analgesic effect of preoperative topical Diclofenac versus Ketorolac on postoperative pain after Corneal Collagen Cross Linkage	2017

Analgesic effect of sr318b diclofenac preparation on Bovine type ii collagen-induced arthritis

Diclofenac sodium is a robust non-steroidal anti-inflammatory prescription medication used to treat pain. It exhibits strong analgesic effects on the treatment of rheumatoid arthritis and must be taken twice each day. This study focused on preparing a single-dose analgesic to reduce the adverse side effects like gastrointestinal effects. The analgesic efficacy was investigated in monkeys bearing bovine type II collagen-induced arthritis. Methodology: At room temperature, SR318B rapid-release granules were synthesized and stored. Bovine type II collagen-induced arthritis was induced in twenty female monkeys aged 5 years or older. Once the arthritis was confirmed, SR318B was administrated orally once daily for two weeks. A control group received empty capsules. The Ellipsoid area of the proximal interphalangeal joints was measured. The mean ellipsoid area of the proximal interphalangeal joints reported to be 101.6 percent greater than it was before injection of SR318B.Moreover, the size of inflammation was seen to be reduced. The results suggest that SR318B has therapeutic effects on collagen-induced arthritis. Hence the preparation may be used as a safer analgesic to treat rheumatoid arthritis in humans too. [20]

Diclofenac Carrier for Treatment of Osteoarthritis

Osteoarthritis is a disease that impacts our body's joints & is presently treated with the oral administration of NSAIDs. This, however, may result in increased gastrointestinal toxicity. This study studies whether a new slow-release NSAID carrier formulation will work more efficiently and reduce the toxic and adverse side effects. The test NSAID was diclofenac and a carrier was formulated by collagen lipid conjugates. The in vivo activity of this was studied in osteoarthritis-induced rats, using MRI scans. Methodology: The collagomers were first prepared by dissolving collagen in DPPE in the ratio of 1:5(w/w), in a lipid concentration. The lipid solution and collagen solution were combined to form a homogenous suspension and made into pellets. This was followed by dialysis and the collagomer suspension was lyophilized and stored. Rats were induced with osteoarthritis. Each animal was given a single intraarticular injection of diclofenac collagomers to the left knee. MRI was used to determine the inflammation. The test subjects showed an increase in inflammation which reduced in time indicating that the collagomers worked. Moreover, the inflammation contradiction was more than that of normal NSAID administration, with over 25% decrease in the inflammation. In conclusion, the collagomers did perform well as a carrier for diclofenac and thus will help avoid adverse gastrointestinal effects of NSAID. These carriers target local cells. Hence, with more scientific study, this can be used as a potential treatment in the future. [21]

Liposomal Diclofenac for Osteoarthritis Treatment

Treatments for osteoarthritis such as oral NSAIDs may cause toxicity in the body. In order to reduce such adverse effects, local injections of diclofenac liposomal formulations are used. Administrating the drug locally will help provide the same treatment without the risk of damage to the conventional route followed by the drugs. Using live animal MRI, it studies the efficiency of such dosages against osteoarthritis. The carriers used are liposomes which are bio adhesive in nature. Bio adhesive liposomes were made by combining dipalmitoyl phosphatidylethanolamine and soybean phosphatidylcholine in the ratio of 5:95. Rats were

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injected with saline water in the left knee and osteoarthritis inducing drugs in the right knee, using a 30 gauge needle. The process was repeated 3 times every day. An MRI scan was done to verify that the rats had archived the required disease state. The animals were treated with the liposomal diclofenac and some were left with no treatment. MRI scans were done to ensure the inflammation on the knees. Over the span of 17 days, knee inflammation was reduced to 12.9 % from the initial inflammation. Even though the level of inflammation reduced only later, it is evident that the inflammation was completely cleared by day 17. From the study, it was revealed that a single dose of such intra-articular injections was efficient in reducing knee joint inflammation in osteoarthritis induced rats. This study can pave the way for the production of novel medicines that can treat osteoarthritis. The drug did not showcase any other adverse side effects during the study too.[22]

Overview and Characteristics of Ibuprofen

This is a review paper and goes through the general overview of ibuprofen and summarizes its main pharmacological effects, drug-drug interactions and its therapeutic applications. Ibuprofen is very popular over the counter medicine used as an analgesic and antipyretic drug for both adults and children. This safe drug's use has increased exponentially over the past 10 years. Ibuprofen is a propionic acid and was the first member of propionic acid derivatives to be introduced in 1969 as a better alternative to Aspirin (Bradbury F., 2004).

Its efficient effects are due to its inhibitory actions on a compound called cyclooxygenases, which are involved in the synthesis of prostaglandins. These Prostaglandins have a very important role in the production of pain, inflammation and fever (Wahbi A.A et al,2005) Ibuprofen is usually given at a dose of 400-800 mg about three times a day and its peak serum concentration is achieved after 1-2 hours. It can be effective for Rheumatoid and osteoarthritis, dental pain, cystic fibrosis, hypertension, Parkinson's disease, breast cancer Dysmenorrhea, fever and headache.

Even though ibuprofen is widely used, if taken inappropriately may lead to serious health problems. The most major adverse effect of the same would include gastrointestinal tract and kidney coagulation. This can lead to GI bleeding, omitting, gastric ulcers and renal failure. Some other uncommon effects include thrombocytopenia, rashes, headache, dizziness, blurred vision and in few cases toxic amblyopia, fluid retention and edema (Burke A, 2006). Nevertheless, studies have proved that ibuprofen may block the cardio protective effects of aspirin. Other drug interactions have also been mentioned. However, Ibuprofen is found to be suitable for self-medication in accordance with its relatively wide spectrum of indications, good tolerance and safety. [23]

Hydrolyzed Collagen Vs Glucosamine Sulphate Enzymatic Treatment of Knee Osteoarthrosis

Non-steroidal anti-inflammatory drugs are the preliminary treatment but have adverse side effects and may even worsen the disease. This study aims to find out if orally administered enzymatic hydrolyzed collagen (EHC) (Colatech®) and glucosamine sulphate (GS) have the same analgesic properties as NSAID. A hundred adults aged above 40 were selected for this test that spanned over three months. Patients were made to discontinue their ongoing NSAID therapy and made to demonstrate a pain flare. They were randomized to therapy at random and surreptitiously, and 93 of them finished the experiment, with 47 in the Colatech® group and 46 in the GS group. During the testing period, information on patient's the demographic data, medical history, medication history, physical assessment and vital signs, and functional impairment were all collected and statistically analyzed. After 2, 4, 8, and 12 weeks of treatment, Colatech® outperformed glucosamine sulphate in terms of clinical efficacy In the tests, the general areas were measured and the results are as follows: general health (Colatech® 3.7 vs. GS 2.9), vitality (Colatech® 4.1 vs. GS 3.4), social function (Colatech® 9.3 vs. GS 4.1), physical function (Colatech® 12.2 vs. GS 7.0), physical work (Colatech® 24.8 vs. GS 13.6), emotional function (Colatech® 6.8 vs. GS 2.2) and mental health Colatech® 3.5 vs. GS 1.7), and it is seen treatment with Colatech® always induced a better improvement in the various investigated parameters. From the results of the study, we can infer that Colatech® massively decreased pain and stiffness in the patients. Statistically, it exhibited better potency than Glucosamine sulphate. There are also no side effects in using this and hence is a safer and much more advantageous treatment for osteoarthritis than Glucosamine sulphate. [24]

Effect of Ibuprofen on the Urinary and Serum Levels in Patients with Osteoarthritis

The study investigated if ibuprofen and nimesulide could affect the levels of biochemical markers taking part in joint inflammation and collagen catabolism in patients that have a recent flare up of knee or hip osteoarthrosis. Ibuprofen may downregulate the synthesis of collagenases and reduce the release of pro-inflammatory cytokines. For this study, 90 patients were divided into two categories with 45 each and they were administered to either nimesulide or ibuprofen for a period of 4 weeks (1 month). Through ELISA, the study measured three things. The urinary levels of C-terminal cross-linking telopeptide of type II collagen (CTX-II) which is an important marker of collagen breakdown. Secondly, they measured the serum levels of a synovial inflammation marker called hyaluronan and lastly, they measured the circulating levels of collagenases. ELISA was followed by statistical analysis using ANOVA. Results obtained from this study were unsatisfactory as ibuprofen did not reduce urinary levels of CTX-100 ($p < 0.001$) neither did it reduce cartilage collagen breakdown ($p < 0.05$). Even though ibuprofen is effective in reducing pain in such osteoarthritic patients, the extent to which ibuprofen can affect joint metabolism and structure is unclear and need to be studied. However, another drug called nimesulide was able to reduce the blood serum levels of collagenase and also reduce the urinary levels of CTX-100. More studies are needed to conclude the accurate working mechanism of these drugs.[25]

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Evaluation of intra-articular collagenase injections as an alternative model to study nociception associated with osteoarthritis

Intra-articular collagenase injection mimics several of the key events linked with osteoarthritis. This includes articular degradation through the digestion of collagen from the cartilage of the body and also by causing instability in the arteries. This model is much more efficient than using animal models for studying osteoarthritis associated pain. Nociception is a signal that arrives at the central nervous system as a result of stimulation of specialized sensory receptors in the peripheral nervous system called nociceptors. These signals arise from actual tissue damage from sprains, fractures and inflammation. Osteoarthritis pain is considered to be a nociceptive pain arising from damaged joints in the body. The study assessed the efficacy of intra-articular collagenase injection as an alternate paradigm for studying nociception related with osteoarthritis. Along with this, the effects of anti-inflammatory drug diclofenac acting on the nociceptive change were also observed and studied. Two intra-articular injections of collagenase, 250U and 500U, were administered to the knees of mature male Wistar rats. These rats were from Spain and weighed around 230g. After 6 weeks, CatWalk tests and Knee-bend test were performed and induced nociception was assayed. The Knee-Bend test involves tracking the number of squeaks and/or struggle reactions to knee joint extensions conducted within the physiological limits of knee extension. The CatWalk test is performed by making the animal walk on a glass platform which is illuminated in such a way that only the points of contact of paw with the surface would result in a bright image. The effect of diclofenac was also evaluated in the animals. Diclofenac was only substantially effective at week 1, and its greatest impact came 30 minutes after injection, with a drop of 21 ± 3% in the Knee-Bend score ($P < 0.05$) and an increase of 22 ± 5% in the percent TIPPI in the CatWalk test ($P < 0.05$).This study concludes that intra-articular injection of collagenase in the knee of rats (500 U) can be a potential alternative model for the study of nociception associated with osteoarthritis as it induces significant nociceptive changed that associated with osteoarthritis. [26]

Collagen Sponges for Targeted Ibuprofen Delivery

Collagen sponges are made out of biomaterials that are homologous to extracellular matrices which can be used to target drug delivery. This paper tested the efficiency of the *in-vitro* release of ibuprofen with the help of collagen-based sponges. The study used pure collagen in the form of a gel that was extracted from bovine. The collagen-based biomaterial was prepared by freeze-drying as it is the easiest method to obtain porous sponges. Collagen gel with a Ph. of 7.4 was mixed with water solution f Ibuprofen and cross-linked with glutaraldehyde solution. Glutaraldehyde solution is used to increase resistance to enzymatic degradation and to ensure a systematic, moderate release rate of ibuprofen. The homogenous gel was then freeze-dried using a free-dryer to obtain disc-shaped porous collagen sponges. The presence of Ibuprofen was confirmed by Infrared spectroscopy. The incorporation of Ibuprofen inside the collagen matrix was determined based on the presence of different characteristic absorption spectrums and bands that are typical of ibuprofen and the hydrogen bonds between the collagen molecules. Kinetic profiles of the same were studied and the per cent of Ibuprofen released was maximum without glutaraldehyde (90.95%) and with an increase in glutaraldehyde, time taken to reach an equilibrium state is more (74.09%). This is due to the string cross-linking in the presence of glutaraldehyde. The results of this study conclude that ibuprofen can be successfully delivered to the target system with the use of collagen. Collagen being non-toxic, biocompatible and biodegradable makes it all the more ideal to be used as a drug delivery system. This could potentially be used for delivering analgesics to target sites.[27]

Diclofenac Vs Ketorolac Post operation Analgesic Effect

The objective of the study was to compare the analgesic effects of diclofenac vs ketorolac for patients diagnosed with keratoconus. Ketorolac is a nonsteroidal anti-inflammatory drug used to treat moderate to severe pain. The population was divided into two groups where Group A was given a single dose of topical diclofenac 0.1% and ketorolac 0.5% to Group-B 30 minutes before the corneal collagen cross linking (CXL) procedure. After the operation, the patients were asked the intensity of their pain and it was assessed with a visual analog scale where indicated no pain and 5 indicated the worst possible pain. This was measured 36 hours after the operation. A total of 60 eyes were tested out of which 16 were male and the rest were female patients. The patients ranged from 20 to 29 years old with a mean age of 24. After the analysis, the mean pain of Group A was 2.57 ± 0.67 while in Group-B it was 3.20 ± 0.61. Hence the study concluded that the topical diclofenac 0.1% is efficiently comparable to topical ketorolac 0.5% in reducing the severity of pain after corneal collagen cross linkage operation. [28]

2. CONCLUSION

This research review's purpose is to help the reader understand different aspects posed by the research on the therapeutic activity of collagen with analgesics. This is significant because it gives insights about usage of collagen with analgesics especially cox-inhibitors. There has been much research and discussion conducted on these opinions of usage and activity of both. Most of the research found, shows therapeutic activity of collagen with analgesics, including cox-inhibitors like celecoxib, etoricoxib, diclofenac and ibuprofen. More research and testing is required to gain a better understanding of the therapeutic activity of collagen with analgesics

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Human and Animal Rights

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The author confirms that the data supporting the findings of this research are available within the article.

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