The Effect of NSAIDs on the Bone Healing Process in Rats

The Center for Advanced Studies and Research

Word count: 4102 (without citations)

Keywords: *NSAIDs, bone healing, osteoblasts, bone mechanical testing, cyclooxygenase enzymes, non-specific NSAIDs, indomethacin, side-effects of NSAIDs, prostaglandins, OTC drugs,*

Abstract

Non-steroidal anti-inflammatory drugs (NSAIDs) are anti-inflammatory pain relievers and are the most common pain relievers consumed. Most NSAIDS can be bought over-the-counter (OTC) and can be taken with minimal side effects. These most commonly include include aspirin, ibuprofen (Advil or Motrin), and naproxen (Aleve). Along with OTC NSAIDs, doctors can prescribe stronger forms of NSAIDs for chronic pain and excessively painful injuries. However, these drugs come with stronger side effects. Patients are aware of the side effects that come with the prescription forms of drugs because doctors are required to inform their patients. However, those who buy OTC drugs are not usually aware of the effects because there is no medical advisor present to inform them. Consumers must rely on the packaging of OTC drugs to be informed on the side effects. One side effect that is not currently relayed, is the adverse effect of NSAIDs on the bone healing process. This study evaluates the mechanisms and roles that NSAIDs take on during the stages of the bone healing process after an injury and their correlation, by analyzing the pathway of NSAIDs in the body. It also investigates the other side effects of NSAIDs and how they are caused. Through investigation and statistical analysis of many peer-reviewed academic papers, this study concludes NSAIDs slow down the bone healing process and warrants further research in this field.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most common pain relievers consumed, as many can be bought over-the-counter (OTC). The most common OTC NSAIDs

include aspirin, ibuprofen (Advil or Motrin), and naproxen (Aleve). NSAIDs are used to suppress pain and bring down inflammation from ailments including muscular and skeletal injuries, osteoarthritis (the degeneration of cartilage between two joints), rheumatoid arthritis (inflammation and deformity of joints), menstrual cramps, and acute pain (headaches, sprains, bruising, etc.). They can be taken with minimal short-term side effects, which include upset stomach or allergic reaction symptoms. Acute gastric side effects can be avoided by taking NSAIDs with food, and minimal allergic reaction side effects most often diminish on their own (Bjarnason & Rainsford, 2011). Doctors can prescribe stronger forms of NSAIDs for chronic pain and for excessively painful injuries, but those medications can come with stronger side effects such as the commonly known gastrointestinal toxicity and bleeding (Marnett, Rowlinson, Goodwin, Kalgutkar & Lonzo, 1999). While patients are aware of the side effects that come along with the prescription forms of any drugs because doctors are required to inform their patients of the side effects of any drug they prescribe, those who buy OTC drugs are not usually aware of the said minimal effects. In fact, in a 2005 study on public perception of anti-inflammatory drugs, 60% of OTC ibuprofen users were neither aware of nor believed that they were at risk for side effects from an NSAID (Wilcox, Cryer, & Triadafilopoulos). Similarly, in a National Consumer League (NCL) study in 2002, 83% had used an OTC NSAID in the past year, with 15% reporting daily use, and 49% unconcerned about potential side effects.

NSAIDs bring down inflammation, and therefore pain, by blocking the production of cyclooxygenase enzymes (COX) (Figure 1). Arachidonic acid, a polyunsaturated fatty acid, is made in the body from shorter omega-6 fatty acids found in vegetable oils or found in the diet in eggs, poultry and meats. It is responsible for the production of various forms of the hormone-like

substances called prostaglandins (PGs). COX enzymes catalyze the production of PGs from arachidonic acid after passing through a cell's phospholipid bilayer membrane when the enzyme phospholipase A2 is present (Radi & Kahn, 2005). PGs are responsible for the modulation of inflammation throughout the body. One form of PGs, called PGE2, is a type that is most abundantly required for the production of bone cells. There are four types of bone cells: osteoblasts, osteoclasts, osteocytes, and osteoprogenitor cells. PGE2 relates with osteoblasts and osteoclasts. PGE2 also plays a role in fevers, the production of gastric mucus, and in uterine contractions during childbirth. PGE2 has four types of receptors in all cells; EP1R, EP2R, EP3R, and EP4R, all encoded in the PTGER gene (Katoh, 2002). When EP2R and EP4R are activated by PGE2, osteoblast formation and osteoclast resorption occur. Osteoblasts differentiate from mesenchymal progenitor cells and are involved in bone formation by synthesizing bone matrix proteins into Ca2+ (Tanaka, Nakayamada & Okada, 2005). Osteoclasts are a type of

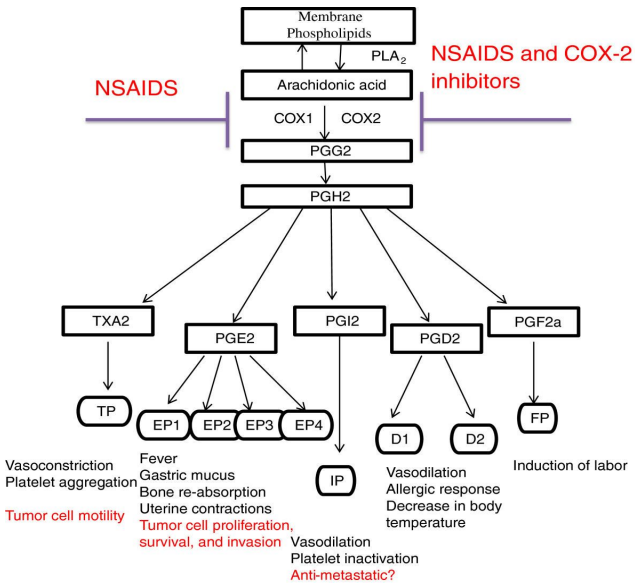
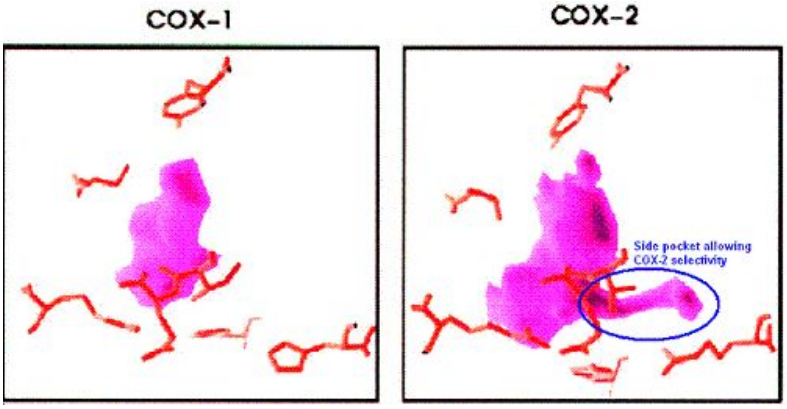


Figure 1: *Cell & Bioscience*, 2013- The molecular pathway of NSAIDs, prostaglandins, and the cyclooxygenase enzymes.



specialized polykaryon macrophage differentiated from hematopoietic progenitor cells, that break down bone cells and returns the Ca2+ to the bloodstream (Teitelbaum, 2000). Both of these cells are what maintain bone homeostasis, the balance between osteoblasts and osteoclasts (Shin & Cho, 2005). The COX enzymes that catalyze the transformation of arachidonic acid into PGs can be found in two isoforms: COX-1 and COX-2 (see Figure 2) (Marnett, Rowlinson, Goodwin, Kalgutkar & Lonzo, 1999). The crystal structures of COX-1 and COX-2 have shown to be the same in humans, rats, mice, and sheep (Marnett, Rowlinson, Goodwin, Kalgutkar & Lonzo, 1999). Rats have shown to be the subjects of the highest quantity of studies relating NSAIDs and the bone healing process.

Figure 2: T. Williams, 2007- This drawing is a representation of the difference between the crystal structures of COX-1 and COX-2. They have the same core structures except for a side pocket that indicates the COX-2 selectivity.

COX-1 is invariably expressed by the body and is found in endothelium, the stomach, and kidneys since it is required for systemic inflammation. On the contrary, COX-2 must be activated by an offending factor in order to be induced for production. It is produced by proinflammatory cytokines and at the sites of inflammation (Warner, et.al., 1999). Cytokines are small proteins secreted by cells that act as means of interactions and communication between cells. They are also released when tissue damage is present and contribute to the inflammation process (Zhand & An, 2009). Likewise, COX-2 is produced more abundantly during initial bone repair after an injury as tissue damage is present and therefore more cytokines are present. There are two types of cytokines: proinflammatory and anti-inflammatory. They are in a constant homeostasis in order to prevent over-inflammation (lasting cell damage) and under-inflammation

(failure to heal), as excessive inflammation can result in lasting cell damage and too little can result in a failure of healing. Certain NSAIDs are non-selective, in which COX-1 and COX-2 production is inhibited for the purpose of reducing inflammation whereas other selective NSAIDs only inhibit COX-2. The type of NSAID is determined by its own specific chemical makeup, but they all have the same purpose of reducing pain and inflammation. Since NSAIDs reduce inflammation by blocking the production of COX enzymes, the process of turning arachidonic acid into PGE2 is inhibited. With the consumption of selective NSAIDs, this process is able to continue, as COX-1 is still present. However, when non-selective NSAIDs are consumed for the purpose of reducing inflammation due to a bone deformation, the entire cycle of PG production needed for bone resorption is stopped. Many consumers are advised to take NSAIDs after a skeletal injury, however, they are not aware that COX-2 specific NSAIDs may possibly have underlying effects on bone healing rate, as they take them for pain and inflammation, not healing.

Background.

In 1979, the field of trauma and orthopedic surgery observed that NSAIDs can delay fracture healing in animals, and then in humans in 1996 (Geusens et al., 2013). Since consumers often use a decrease in their physical pain and inflammation as a marker for the healing of an injury, the incident of the non-union and incomplete healing of bone tissue frequently goes unnoticed. With the early intervention of competent bone healing, non-unions (the failure of two ends of a bone to unite) of bone fracture and prolonged healing can occur (Giannoudis, et al., 2000). Researchers from Trauma Centre Murnau and the Institute of Orthopedic Research and

Biomechanics of Ulm, Helmholtzstr, Germany (2007), tested the NSAID diclofenac on the implication of osteoblasts at an early stage of bone healing in rats. 20 male rats were divided in half with 10 in a placebo group and 10 in a drug group. Bone defects were created at the lateral side of the distal femur on the left leg. The placebo and diclofenac were distributed in pellets orally. After euthanization, radiographic, computed tomography, and histology tests were all performed. The counts of osteoblasts in newly formed bone tissue were significantly reduced in the drug tested group with a median of 73.5 cells on a slide of a sample compared to the placebo group with a median of 171.5 cells per slide (Krischak, et al., 2007).

Another similar study examines the effects of different NSAIDs on fracture healing sites in rats (Meade, et. al, 1993) . The groups included in the study were a control (placebo) group and groups given the NSAIDs ketorolac and parecoxib in high and low dosages. The rats had fractures created in the femur by inserting an intramedullary pin. The rats healed, then were euthanized. Their femurs were harvested and underwent processes including decalcification, torsion testing, pulverization, and RNA analysis. Before euthanasia, the rats were given 42 days to heal and were given different dosages of the different NSAIDs. The combined results concluded that the high-dose parecoxib rat group showed a failure to unite their fracture sites.

In another study by Stuart Goodman and his team of researchers (2002), various surgeries on white rabbits to mimic bone deformities in their femurs were performed. They had a placebo-treated group, a naproxen treated group, and a rofecoxib treated group. There was no statistical difference in bone regrowth when naproxen or rofecoxib treatments were compared ($p = 0.706$). This is because rabbits have different tissue differentiation and bone ingrowth processes, and the COX enzymes have no effect on their PGE2 production. However, this study

did provide insight and helped investigate specific variables important to osseointegration (the direct structural and functional connection between living bone and the surface of a load-bearing artificial implant) for joint replacement and fracture healing (2002).

In 2007, a team of researchers from Finland wrote a review and stated that the gastrointestinal and cardiovascular adverse effects of NSAIDs are acknowledged, but their effects on bone are not as known. They also stated that in some clinical trials, NSAIDs are used to prevent an ectopic bone formation after hip and joint replacement surgeries. An ectopic bone formation is a disposition of calcium salts in tissues after exposure to new tissue. It can lead to the calcification of body tissues and form into abnormal bone growths. Considering that professionals use NSAIDs to suppress the formation of bone tissue and it has proven to be successful, they conclude that NSAIDs play a role in the delay and incompletion of bone healing.

Another factor to take into consideration in this study is the widespread acceptance of the gastrointestinal adverse effects that NSAIDs have on the body. Prostaglandin production is also essential in the production of gastric mucus (see Figure 1). With the inhibition of the COX enzymes required for the formation of PGE2, gastric mucus is unable to be produced, leading to an exposure of the stomach lining to gastric acid. Stomach ulcers are a common side effect of NSAIDs, as well as stomach bleeding, which are caused from an exposure of the stomach lining to the acidic gastric acid and bile. Gastric acid has a pH ranging from 1.5 to 3.5, so its acidic nature has detrimental effects on stomach function (Rodriguez & Jick, 1994). In a 2005 study on Japanese patients hospitalized with stomach ulcers and bleeding, 28.8% had a history of NSAID consumption. Stomach ulcers are always caused by an infection with the bacterium *Helicobacter*

pylori, by frequent use of NSAIDs, or Zollinger-Ellison syndrome (the increase in a body's production of acid). Out of the 238 patients admitted with stomach ulcers, a large group of them acquired the ulcers from the using of NSAIDs (Nakashima, et. al.). With a diminishment of gastric mucus protecting the stomach lining from gastric acid due to the inhibition of the COX enzymes, users are often at risk for stomach ulcers and bleeding. When COX enzymes are not present, PGs are unable to form and obstruct the production of the needed gastric mucus.

Purpose

The purpose of this study is to better understand the effects of specific COX-inhibiting drugs on the bone healing process. OTC NSAIDs are most often consumed to decrease pain and inflammation that occur due to many different factors in the body. Inflammation is regulated by means of hormone-like substances called PGs. NSAIDs work by inhibiting the processes required for the production of prostaglandins. However, a form of prostaglandins called PGE2 is not only used in the modulation of inflammation, but also in the formation of osteoblast cells (Radi & Kahn, 2005). When a bone is fractured, more osteoblasts are required for the formation and healing of new bone. So, when COX-inhibiting drugs are consumed after a fracture, healing may be prolonged, without regards to the other side effects of NSAIDs including gastrointestinal ulcers and bleeding (Cottrell & O'Connor, 2010). It is known that there is animal and human evidence that NSAIDs prolong initial healing of fractures when taken in the early stages of healing. However, this effect is not clearly understood by consumers, and most data and conclusions available supporting this concept is conveyed through evidence on animals. If

patients are unaware of a major effect that OTC pain-relievers have on the healing of their injury, they are not being sufficiently informed. This can lead to prolonged healing of a fracture, and could even lead to the nonunion of a fracture if taken without precautions and restrictions (Giannoudis et. al, 2000).

Hypothesis

Alternate Hypothesis: COX-2 inhibiting NSAIDs slow down the bone healing process in rats. The information collected can be applied to humans as well, with the consideration that the structures involved in these processes are the same in both humans and rats (Marnett, Rowlinson, Goodwin, Kalgutkar & Lonzo, 1999).

Null hypothesis:There is no indication that COX-2 inhibiting drugs have any effect on the maintenance of bone homeostasis in rats.

Methods

This research was conducted through systematic literature review of many peer-reviewed journals and articles. Literature was retrieved through online databases including PubMed, Medline, PLOS, Journal of Biological Chemistry, Journal of Bone and Mineral Research, MDPI, Journal of Dental Research, Sports Sciences Commons, Wiley Online Library, Medicinal and Pharmaceutical Chemistry Commons, Elsevier, NIH, Springer-Verlag, The Physician, and SportsMedicine. Many of these databases were accessed through searches on Google Scholar,

Google Search, and EBSCOhost. Access to literature from these databases that require subscriptions or memberships was granted through Thousand Oaks High School, mentors with Ph.D. level education in the orthopedic and pharmacologic fields, and the student library at California State University Channel Islands. When searching for studies to analyze, the keywords included: NSAIDs, bone healing, osteoblasts, ibuprofen, bone mechanical testing, cyclooxygenase enzymes, non-specific NSAIDs, indomethacin, side-effects of NSAIDs, rats and humans, prostaglandins, OTC drugs, and bone healing and rats. When searching "NSAIDs" on Google Scholar, 311,000 results occurred. When narrowing down the range of results by changing the search to "NSAIDs and the bone healing process," there were 30,200 results. When relating the studies with this research and by changing the search to "NSAIDs and bone healing in rats," 23,400 results occurred. Of these results, academic, peer-reviewed papers were read, annotated, summarized, and contributed to this research.

Literature review commenced from August 2017 through December 2017. Submission of the research proposal to a panel of the International Review Board (IRB) took two attempts and was finally accepted in November 2017. After the collection of literature, data sets were collected from the academic, peer-reviewed journals. Data sets were analyzed and input into the Microsoft Excel software and graphed. A Chi-square test using the p-values of multiple studies was conducted for each set of data and graph.

Results

A: P. E. Persson, G. Sisask, and O. Nilsson, 2005
B: E. Sudmann and G. Bang, 1979.
C: H. L. Allen, A. Wase, and W. T. Bear, 1980
D: T. Karachalios, L. Boursinos, L. Poultsides, L. Khaldi, and K. N. Malizos, 2007
E: K. D. Riew, J. Long, J. Rhee et al., 2003

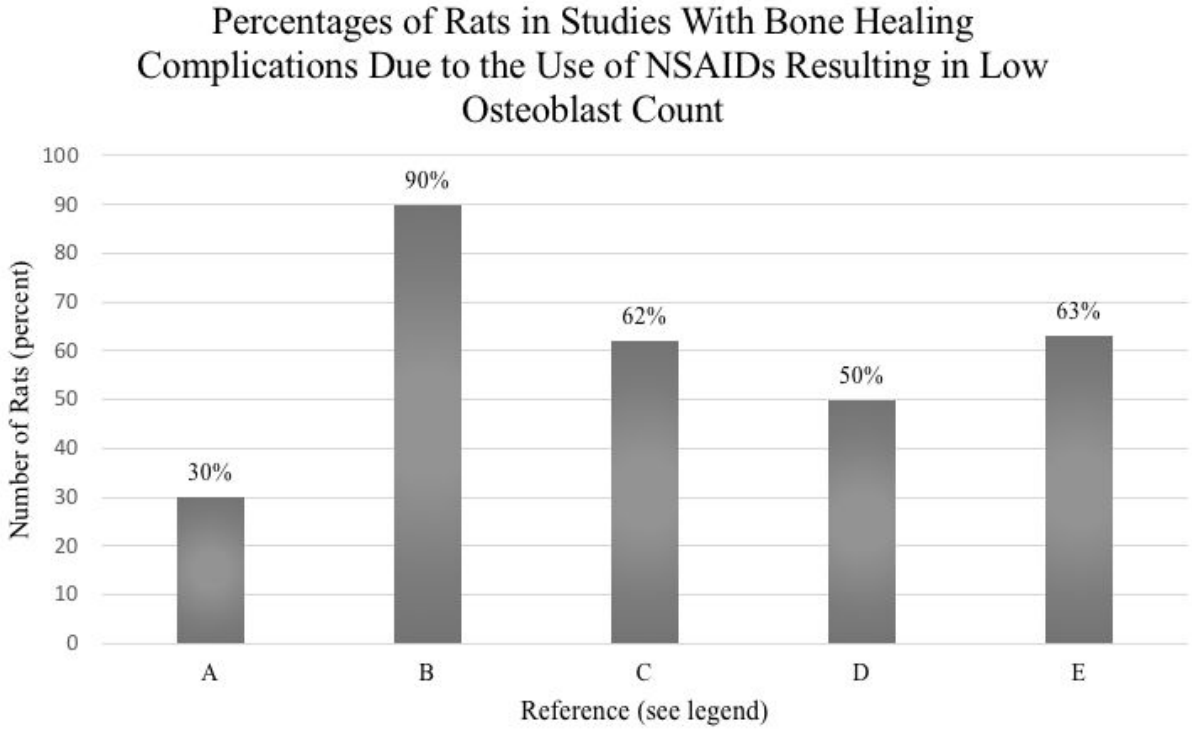


Figure 3: The average percentages of rats out of the studies referenced in the legend are displayed. The percentages compare rats with bone healing complications due to low osteoblast counts in different studies. The studies each compared osteoblast counts during healing in rats treated with NSAIDs and a placebo.

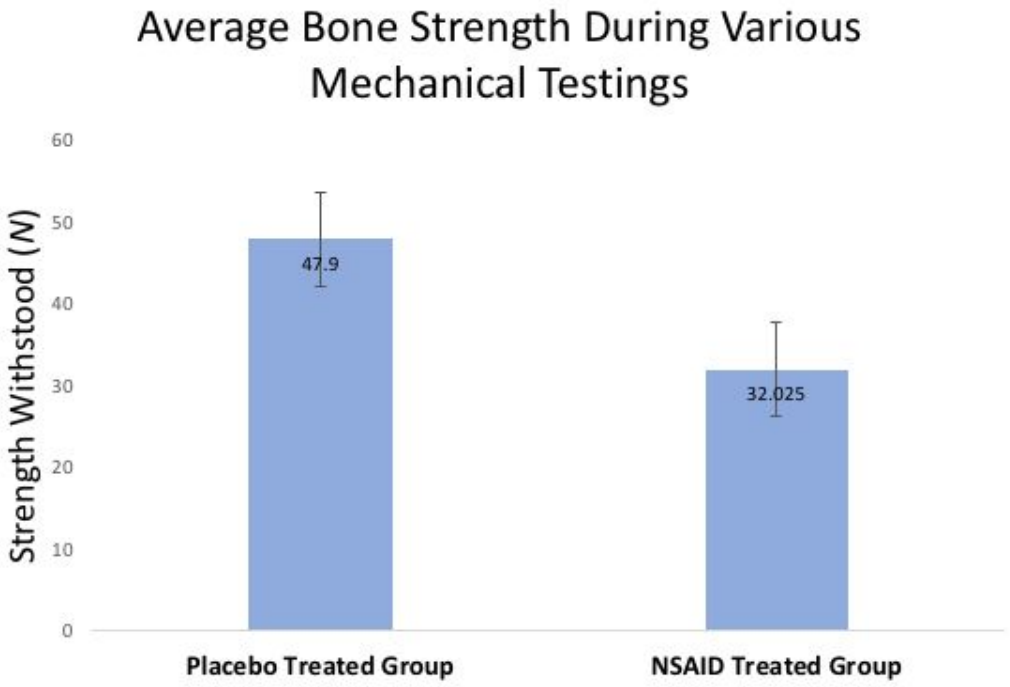


Figure 4: p=0.01; Averages of bone strength withstood during various types of mechanical testing are shown (in newtons) on the y-axis, and the placebo and NSAID treated rat groups are displayed on the x-axis.

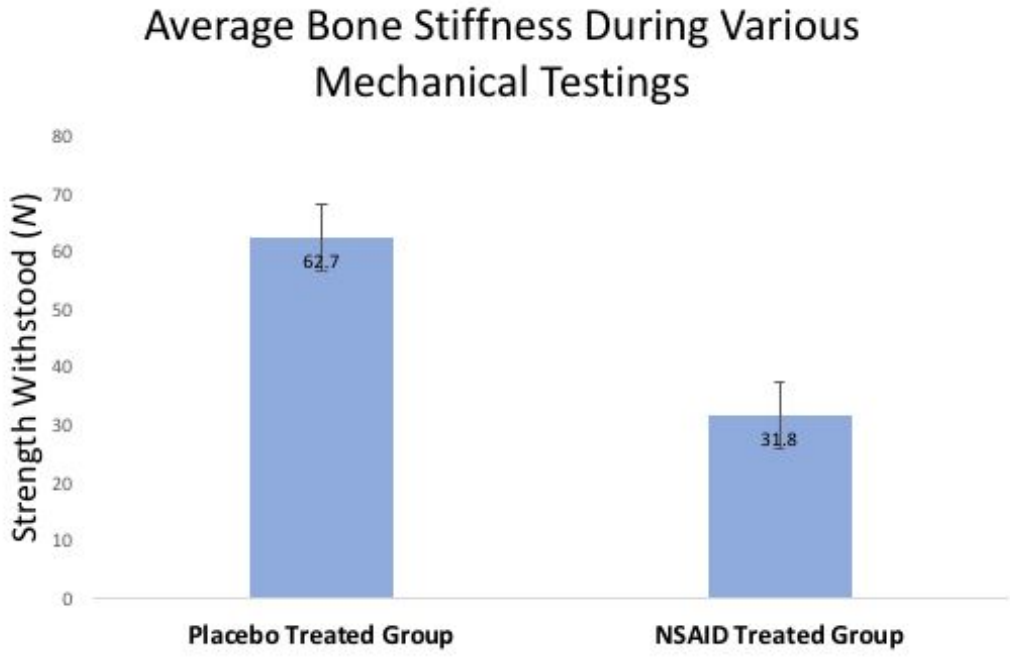


Figure 5: p=0.01; Averages of bone stiffness during various types of mechanical testing are shown (in newtons) on the y-axis, and the placebo and NSAID treated rat groups are displayed on the x-axis.

Discussion

In Figure 3, the references for studies that tested the effects of the NSAID indomethacin on rats are given. These studies all tested placebos and indomethacin on various bone deformities and were given their results through means of osteoblast count. After euthanization of rats, slides of each specimen were taken. Osteoblast counts were taken of each slide. The average osteoblast count among all five studies was ≈180 cells per slide. Among the five indomethacin groups, an average of 74.75 osteoblasts was counted on each slide. In the placebo groups, an average of 172.75 osteoblasts was counted per slide. The one study to take into account that was the outlier of the five analyzed studies is Reference 1. The percentage of rats in the study with bone healing complications was 30% which is a low number supporting the insignificant importance of indomethacin on osteoblast count. However, considering that there were still some rats in the study that had healing complications due to an intake of indomethacin as opposed to the placebo group, this data can still support this study's hypothesis. Osteoblast count was lower in the indomethacin-treated groups due to the inhibition of COX enzymes. Without the presence of these COX enzymes, there is no pathway for the conversion of arachidonic acid into PGs. PGE2 is necessary for the production of osteoblasts, so deprivation of this PG can result in the failure of a bone to heal after an injury.

Another way of analyzing the effects of drugs on bone healing is through means of mechanical testing. Mechanical testing includes numerous tests such as a three-point flexure test, or angulation measurement with a protractor. In the studies analyzed, the three-point flexure test was used to test the strength and stiffness of the femurs of rats who were given different

NSAIDs. After euthanization of the rats in the multiple studies evaluated, their femurs were used to test the durability of the bone. As can be viewed in Figure 5, the average bone stiffness of rats treated with a placebo and with an NSAID from 10 different academic, peer-reviewed papers is shown. The different studies had a variety of units of measurement, so each result was converted to newtons (*N*). By means of angulation measurement with a protractor, the placebo-treated group had an average of 62.7 *N* withstood. The NSAID treated group had an average of 31.8 *N* withstood. The Chi-squared value for this set of data is p=0.01. By measuring the angulation of the bone, stiffness can be assumed. The farther a bone can bend, the less dense it is. Bone density is crucial for adequate strength and function. A decrease in bone density is a sign of incomplete healing, ultimately relating back to a bone's osteoblast count. In Figure 4, a similar analysis was conducted. The average values of bone strength from 10 academic, peer-reviewed journals are presented. Bone strength is similar to bone stiffness but is tested by means of the three-point flexure test. It again is measured in *N*. The average strength of the placebo-treated group was 47.9 *N* and the average of the NSAID treated rats was 32.03 *N*. The chi-squared value of this data is p=0.01. The strength of a bone is also determined by osteoblast quantity. The higher the density, the more weight that can be withstood. With the inhibition of COX enzymes, bone resorption and homeostasis are unable to be maintained. These

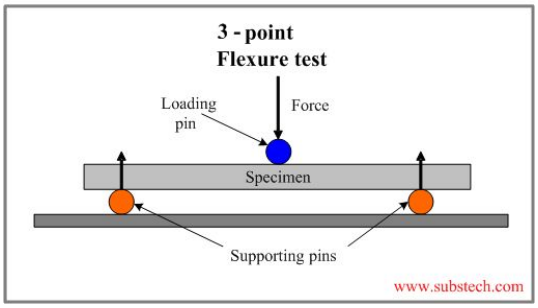


Figure 6: A three-point flexure test diagram is shown. In a three-point flexure test, force is applied with loading to an object to test its strength and ability to withstand force and weight.

data sets show significant differentiation between placebo-treated rat groups and NSAID treated rat groups.

Conclusion

In animals, there is evidence that the NSAIDs aspirin, celecoxib, diclofenac, etodolac, ibuprofen, indomethacin, ketoprofen, meloxicam, naproxen, parecoxib, rofecoxib, tenoxicam, and valdecoxib all impair the healing and regeneration of bone, with indomethacin having the largest evidence. This study looked into the effects of aspirin, celecoxib, ibuprofen, naproxen, and indomethacin in rats, as the data can be applied to humans, and because these listed NSAIDs are the only ones available in the United States. While there have also been reports in which the drugs have no effect, there is greater evidence and support that there is impairment of bone healing. This analysis had a specific focus on NSAID use in rats. However, the structures of the COX enzymes that are inhibited by the of NSAIDs and subsidize this effect, are the same in both humans and rats. For this reason, this study can also be applied to humans.

As can be assumed through the molecular pathway of arachidonic acid (see Figure 1), the COX-1 and COX-2 enzymes are essential for the formation of prostaglandins. PGs give way to the pathways of the more specialized PGs that have essential receptors in all cells. Specifically, PGE2 binds to the EP2R and EP4R receptors which signal bone resorption to occur. Bone resorption is the homeostasis of bone, where osteoclasts break down bone cells into Ca2+ and return the mineral to the bloodstream and osteoblasts absorb the Ca2+ and contribute to the formation of bone matrices. When this process is inhibited due to an absence of COX enzymes,

osteoclasts will continue to break down and absorb the calcium needed for the formation of new osteoblasts. This results in a lesser number of osteoblasts and therefore a decrease in bone density. This interpretation is related to the consumption of NSAIDs for the purpose of reducing inflammation and pain after a bone injury such as a complete breakage or fracture. New osteoblasts are required for the formation of a new bone matrix after an injury, in order to reunite the broken bone tissue. Since NSAIDs inhibit the COX enzymes, osteoblast formation is also hindered. In many studies that have investigated this performance and course, NSAIDs used for the purpose of reducing inflammation and pain have shown to have detriments on the bone healing process.

Through the analysis of the data above, it can be concluded that NSAIDs do in fact have adverse effects on bone healing. In Figure 3, the percentages of rats with decreased osteoblast counts and given NSAIDs in contrast with those given a placebo were very significant. The decrease in bone strength (Figure 4) and decrease in bone density (Figure 5) was also significant in the NSAID treated groups as opposed to those treated with a placebo. Each of these data sets supports that there is a decrease in osteoblast count, considering that bone strength and density can be attributed with osteoblast count. The alternative hypothesis of this study can be accepted.

Positive Social Change Implications

COX-inhibiting NSAIDs have the capability to slow down the rate of initial fracture healing. While they reduce inflammation, they suppress the COX enzymes which are required for the production of prostaglandins. The prostaglandin named PGE2 is required for new

osteoblast formation, especially after a bone fracture, and therefore the suppression of the COX-2 enzymes reduces the speed of bone healing. People who consume prescription and OTC NSAIDs are often unaware and misinformed by medical professionals that these drugs can have an effect of the rate of healing of the injury that they are trying to reduce the inflammation and pain of. While the knowledge of the gastrointestinal side effects of these drugs is widespread, the concept of NSAIDs having negative effects on the bone healing process is not. By looking further into the defect that NSAIDs can cause during the bone healing process, a more widespread understanding of the issue can come about and there will be a better social awareness of this issue.

Further Work

There are many aspects to take into account that contribute to this field of study. One aspect that can be further analyzed is the time that complete bone healing takes in regards to NSAID consumption. When non-selective NSAIDs are taken on a regular basis, the production of PGs for osteoblast and osteoclast formation is very minimal. However, selective NSAIDs only block the production of either COX-1 or COX-2, allowing one or the other to continue to be expressed. Many already-published studies have taken into account the time it takes for a complete bone healing cycle to occur.

Another aspect of the issues relating NSAIDs and bones is research on arthritis patients. Many studies have looked into this, while this study did not. However, in regards to Figure 4 and Figure 5, NSAIDs reduced the stiffness and strength of bones and therefore the density, due to

low osteoblast counts. Arthritis patients are most commonly prescribed NSAIDs for the purpose of reducing inflammation and pain in joints and are the highest population of users of NSAIDs. Arthritis on its own is caused by the degeneration of bone and cartilage tissue and eventually can lead to the grinding of bone on bone. Without an adequate understanding of the effects of NSAIDs on bone tissue and cells, this issue could become even more painful for arthritis patients. With further investigation of the effects of these drugs on arthritis patients, an alternative solution and exact answers of the correlation would be found.

Acknowledgements

I would like to acknowledge everyone who helped me throughout the extent of this research: Dr. Nikki Malhotra, Tasha Beaudoin, Dr. Robert Deamer, Dr. Kimberley Caputo, the CSUCI library, the staff at Thousand Oaks High School, and the Center for Advanced Studies and Research Parent Network.

References

Allen, H. L., Wase, A., & Bear, W. T. (1980, 01). Indomethacin and Aspirin: Effect of Nonsteroidal Anti-Inflammatory Agents on the Rate of Fracture Repair in the Rat. *Acta Orthopaedica Scandinavica, 51*(1-6), 595-600. doi:10.3109/17453678008990848

Beck, A., Krischak, G., Sorg, T., Augat, P., Farker, K., Merkel, U., . . . Claes, L. (2003, 09). Influence of diclofenac (group of nonsteroidal anti-inflammatory drugs) on fracture healing. *Archives of Orthopaedic and Trauma Surgery, 123*(7), 327-332. doi:10.1007/s00402-003-0537-5

Dimar, J. R., Ante, W. A., Zhang, Y. P., & Glassman, S. D. (1996, 08). The Effects of Nonsteroidal Anti-inflammatory Drugs on Posterior Spinal Fusions in the Rat. *Spine, 21*(16), 1870-1876. doi:10.1097/00007632-199608150-00006

Endo, K., Sairyo, K., Komatsubara, S., Sasa, T., Egawa, H., Yonekura, D., . . . Yasui, N. (2002). Cyclooxygenase-2 Inhibitor Inhibits the Fracture Healing. *Journal of PHYSIOLOGICAL ANTHROPOLOGY and Applied Human Science, 21*(5), 235-238. doi:10.2114/jpa.21.235

Engesæter, L. B., Sudmann, B., & Sudmann, E. (1992, 01). Fracture healing in rats inhibited by locally administered indomethacin. *Acta Orthopaedica Scandinavica, 63*(3), 330-333. doi:10.3109/17453679209154794

Karachalios, T., Boursinos, L., Poultsides, L., Khaldi, L., & Malizos, K. N. (2007, 09). The effects of the short-term administration of low therapeutic doses of anti-COX-2 agents on the healing of fractures. *The Journal of Bone and Joint Surgery. British Volume, 89-B*(9), 1253-1260. doi:10.1302/0301-620x.89b9.19050

Katoh, Masaru. “GIPC Gene Family (Review).” *International Journal of Molecular Medicine*,

Jan. 2002, doi:10.3892/ijmm.9.6.585.

Krischak, G. D., Augat, P., Blakytny, R., Claes, L., Kinzl, L., & Beck, A. (2007, 01). The non-steroidal anti-inflammatory drug diclofenac reduces appearance of osteoblasts in bone defect healing in rats. *Archives of Orthopaedic and Trauma Surgery, 127*(6), 453-458. doi:10.1007/s00402-007-0288-9

Marnett, L. J., Rowlinson, S. W., Goodwin, D. C., Kalgutkar, A. S., & Lanzo, C. A. (1999, 08). Arachidonic Acid Oxygenation by COX-1 and COX-2. *Journal of Biological Chemistry, 274*(33), 22903-22906. doi:10.1074/jbc.274.33.22903

Nakashima, S., et al. "A Clinical Study of Japanese Patients with Ulcer Induced by Low‐Dose Aspirin and Other Non‐Steroidal Anti‐Inflammatory Drugs." *Alimentary Pharmacology & Therapeutics*, Wiley/Blackwell (10.1111), 7 June 2005, onlinelibrary.wiley.com/doi/full/10.1111/j.1365-2036.2005.02476.x.

Persson, P., Sisask, G., & Nilsson, O. (2005, 01). Indomethacin inhibits bone formation in inductive allografts but not in autografts. *Acta Orthopaedica, 76*(4), 465-469. doi:10.1080/17453670510041420

Radi, Z. A., & Khan, N. K. (2005, 09). Effects of cyclooxygenase inhibition on bone, tendon, and ligament healing. *Inflammation Research, 54*(9), 358-366. doi:10.1007/s00011-005-1367-4

Rainsford, Kim D., and Ingvar Bjarnason. "NSAIDs: Take with Food or after Fasting?" *Journal of Pharmacy and Pharmacology*, vol. 64, no. 4, 2011, pp. 465–469., doi:10.1111/j.2042-7158.2011.01406.x.

Riew, K. D., Long, J., Rhee, J., Lewis, S., Kuklo, T., Kim, Y. J., Zhu, Y. (2003, 04).

Time-Dependent Inhibitory Effects Of Indomethacin On Spinal Fusion. *The Journal of Bone and Joint Surgery-American Volume, 85*(4), 632-634. doi:10.2106/00004623-200304000-00007

Shin, Chan Soo, and Hwa Young Cho. "Bone Remodeling and Mineralization." *Journal of Korean Society of Endocrinology*, vol. 20, no. 6, 2005, p. 543., doi:10.3803/jkes.2005.20.6.543.

Simon, A. M., Manigrasso, M. B., & O'connor, J. P. (2002, 06). Cyclo-Oxygenase 2 Function Is Essential for Bone Fracture Healing. *Journal of Bone and Mineral Research, 17*(6), 963-976. doi:10.1359/jbmr.2002.17.6.963

Tanaka, Y., Nakayamada, S., & Okada, Y. (2005, 06). Osteoblasts and Osteoclasts in Bone Remodeling and Inflammation. *Current Drug Target -Inflammation & Allergy, 4*(3), 325-328. doi:10.2174/1568010054022015

Teitelbaum, S. L. (2000, 09). Bone Resorption by Osteoclasts. *Science, 289*(5484), 1504-1508. doi:10.1126/science.289.5484.1504

Warner, T. D., et al. "Nonsteroid Drug Selectivities for Cyclo-Oxygenase-1 Rather than Cyclo-Oxygenase-2 Are Associated with Human Gastrointestinal Toxicity: A Full in Vitro Analysis." *Proceedings of the National Academy of Sciences*, vol. 96, no. 13, 1999, pp. 7563–7568., doi:10.1073/pnas.96.13.7563.

Wilcox, M. C., Cryer, B., & Triadafilopoulos, G. (2005). Patterns of use and public perception of over-the-counter pain relievers: Focus on nonsteroidal antiinflammatory drugs. *The Journal of Rheumatology, 32*(11), 2218-2224.

Zhang, Jun-Ming, and Jianxiong An. "Cytokines, Inflammation, and Pain." *International*

Anesthesiology Clinics, vol. 45, no. 2, 2007, pp. 27–37.,

doi:10.1097/aia.0b013e318034194e.