In the quest for the etiology of the delayed union of fractures: the inhibitory role of non-steroidal anti-inflammatory drugs - a complex pharmacological phenomenon?

Charalampos Dokos, Maria Mironidou-Tzouveleki

A΄ Laboratory of Pharmacology, Medical School, Aristotle University of Thessaloniki, Greece

ABSTRACT: Failure of fracture healing is one of the problems that clinical orthopaedics face in practice. This review will examine the role of non-steroidal anti-inflammatory drugs in fracture healing. It is believed that they are inhibitors of the early stages of fracture healing process, according to experimental models. Clinical studies in this area are few in number and without clear evidence regarding the inhibitory effect of non-steroidal anti-inflammatory drugs. Surprisingly the new substances of this class of drugs (COX-2 inhibitors) have the same action in fracture healing. Despite the possible adverse effects of non-steroidal anti-inflammatory drugs in gastrointestinal, cardiovascular systems and fracture healing, they are widely used for post-surgery orthopaedic pain and inflammation.

Key Words: Non-steroidal anti-inflammatory drugs, Fracture healing, Coxibs, Non-union fracture.

INTRODUCTION

Fractures are indeed a vital cause of death, particularly in aging populations, combined with cardiovascular and respiratory disorders. The repair of bone fracture is a slow and complex procedure that involves a regeneration of new bone and absorption of old bone scaffolds. Surprisingly from the early stages of this process, many cell types are involved in this bone remodeling process. From the early callus tissue until the healed new bone, systemic and non-systemic factors take place in this orchestra of bone regeneration. Differentiation and proliferation of many cellular types like chondrocytes, osteoblasts e.t.c are influenced by genetic background, systemic hormones, sensitivity to mechanical input, drug administration, other disorders, cytokines and many more factors. Consequently fracture healing is declined by aging, so delayed union of fractures is most common in elderly patients (Figure 1).

Fractures are thoroughly classified according to the external force that induces the damage of bone continuity. They can be due to external violence and pressure on the bone, continuous pressures in the area, while a very unique form of fractures is caused by pathologic conditions such as osteoporosis. Most of the times the process of bone healing has no distinct phases. In the early stages there is an increased blood supply causing a hematoma in the area of fracture. This is observed in experimental models of rodents fracture and it is the very first stage of bone healing. Simultaneously there is an increased surrounding by macrophages and the broken ends become necrotic. Osteoclasts then remove the dead bone and osteoblasts produce new bone, parallel with fibroblasts that produce fibrous tissue in soft tissue injuries of the area. Therefore a growing firm mass, or callus, is created and that is the early beginning of ossification of the osteoid. Bone remodeling has begun, and two levels of growth callus are observed: subperiosteal callus that forms the outside of injured bone and endosteal callus. The pH in this stage is increased and calcium hydroxapatite crystals are deposited. After 6 weeks (in normal conditions) the gaps are filled and bone regains mechanical strength, but only after 1 year does bone remodeling finish with normal bone microarchitecture to be restored[1-6].

Corresponding author: Dr. Maria Mironidou-Tzouveleki, Anaesthesiologist, Associate Professor of Pharmacology, Komninon 4, 57010, Filyro, Tel.: +30 2310 999345, Fax: +30 2310 999345, Thessaloniki, Greece, mmyronid@auth.gr

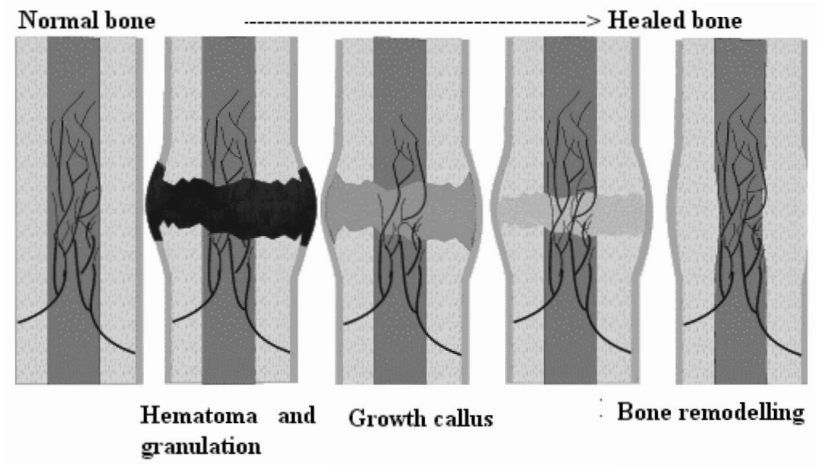


Figure 1. Bone healing stages are not always distinct. Bone remodeling process is an established model regulated by systemic and non-systemic factors.

Bone gradually gains its strength, density and architecture, ideally in a smooth but complicated procedure. Unfortunately the stimuli for each stage are still unknown and affected by several external and internal factors. At the end bone may be joined by a mat of fibrous tissue or a false joint called pseudarthrosis. Through observations, non-steroidal anti-inflammatory drugs (NSAIDs) seem to have an inhibitory role in the whole bone healing process, from the early stages of angiogenesis in bone fracture until the bone remodeling final stages. This review examines and analyzes the experimental and clinical data from the use of NSAIDs in bone fractures.

PHARMACOLOGY OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

Back in Hippocrates' times, the cortex of *Salix alba* tree was used for its antipyretic property. This was due to the large quantities of salicylic acid in the cortex of the tree. In the Roman times Celsius used an extract of *Salix alba*'s leaves as analgesic. Many years passed until in the 18th century, Edward Stone presented the first clinical study of 50 patients cured from malaria's fever with the use of *Salix alba*'s leaves. In 1763, he sent his results in a letter to the president of the Royal Society of London. From that point scientists tried to isolate and produce the pure substance of *Salix alba*'s leaves. Leroux in 1829 managed to isolate the pure form of salicylic acid and proved that it has antipyretic properties. Finally in 1860 for the first time it was synthetically produced in Germany, but it was tasteless. After many unsuccessful tries, Bayer was the first pharmaceutical company that produced aspirin. Felix Hoffman, a young ambitious chemist, managed to produce aspirin and experiment on mouse arthritis models. For the very first time the name aspirin was given by Dresser in 1899. It is believed that the name was taken by the name of the plant *Spiraea*, from which aspirin was first produced[2,6,7].

In 1938 the Lancet journal (see study from *Douthwaite AH, Lindott GAM. Gastroscopic observation of the effect of aspirin in certain other substances in the stomach. Lancet 1938; 2:1222-1225*) published on the front page one of the most unwanted effects of aspirin administration, peptic ulcers. Then a new group of NSAIDs was developed with indomethacin as a prototype but it had the same unwanted effects. In 1971 for the very first time, after long research by Sir John Vane, the scientific community realized the mechanism of action of NSAIDs. Until nowadays NSAIDs are the number one on the list of prescribed drugs in the USA and Europe with many unwanted effects[2,7].

NSAIDs is a class of drugs with anti-inflammatory action, used widely by physicians for its other two properties: analgesic and antipyretic. According to studies NSAIDs must be prescribed only if there is an emergency and even then unwanted effects must be considered. The main unwanted effects involve peptic ulcers, nausea, diarrhea, stomach ache, gastric tract

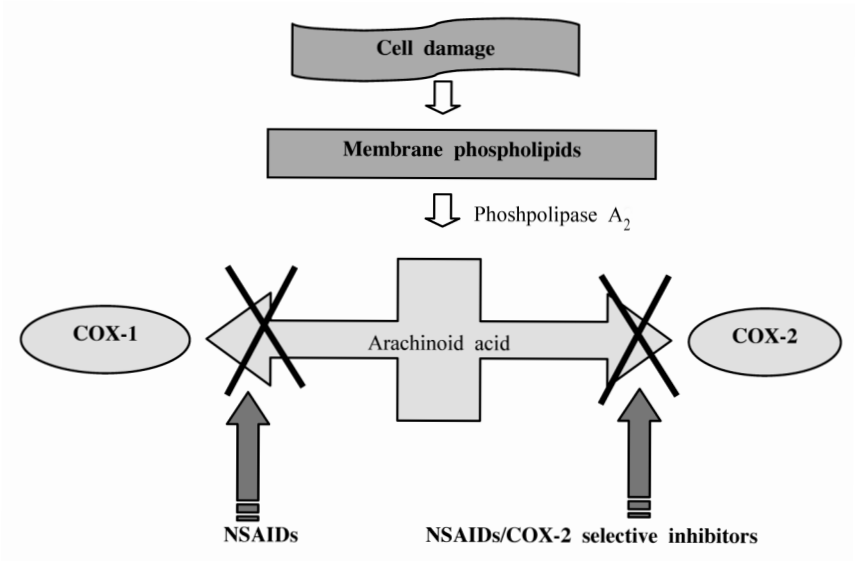


Figure 2. The characteristic pathways of prostaglandins synthesis. Non-steroidal anti-inflammatory drugs inhibit both the isoforms of cyclooxygenase enzyme.

bleeding, sensitivity reactions, cephalalgia. Aging population is a group of high risk of unwanted effects, even the slightest and easy going effects like nausea, diarrhea and cephalalgia. According to NICE, people over 65 years old and especially women, persons with peptic ulcers in the past/present or consequences of it, are among the high risk group for unwanted effects from administration of NSAIDs. Additionally careful attention must be paid to patients that receive medication known to increase the severity of gastric tract conditions and also patients with long-term intake of NSAIDs. With any NSAID administration patients must follow a gastric protection treatment scheme. In the current medical bibliography there are many case reports and clinical studies of gastric bleeding and ulcers. Many times these cases are accompanied by anaemia and hypoproteinaemia symptoms[7].

The mechanisms by which NSAIDs cause gastric ulcers and bleeding are under investigation. Proposed mechanisms involve inhibition of prostaglandins synthesis, reduction of immunity defenses, gastric mucosa protection and production of cytokines. Possibly there is a dysfunction of mitochondria in intestine cells resulting in an increased permeability of intestine membrane and invasion of bacteria. In the area of injury, neutrophils concentrate and free radicals are produced. For this reason patients that take a small amount of aspirin for cardiovascular problems must have gastric protection medication, for example a proton pump inhibitor. Gastric protection is not necessary for young patients. In extreme cases, it will be useful to have a microbial test for the presence of helicobacter of pylori (*H. pylori*). This microorganism may cause gastric ulcer and bleeding according to several studies[7-11].

NSAIDs are a group of substances that inhibits the enzyme cyclooxygenase (COX) for non-synthesis of prostaglandins by arachidonic acid (Figure 2). The arachidonic acid (cellular membrane phospholipids derivative) is synthesized by enzyme phospholipase A_2. So NSAIDs have a special distinct characteristic, they selectively inhibit each of the two isoforms, either COX-1 or COX-2. In the past there was a theory that the COX was a whole enzyme. Following research it seems that there are two isoforms of the enzyme and probably a third isoform. COX-1 is responsible for the production of prostaglandins that protect gastric mucosa, induce kidneys and platelets normal function (production of thromboxane A_2). COX-2 involves the production of prostaglandins that take part in inflammatory processes, pain and renal function (rennin production). Although COX-2 seems to be a sound non-fatal enzyme to be inhibited, the use of NSAIDs COX-2 inhibitors is unwanted in patients

with ischemic cardiovascular conditions or history of heart attacks. So physicians must prescribe this type of NSAIDs for small term therapies[7,12-14]. It seems that COX-2 has also a physiological role.

In normal conditions, COX-1 is activated in all tissues and it is the consistent morph of COX enzyme. COX-2 the «inducible» form of COX is expressed in some tissues. The «constitutive» form exists in central nervous system, kidneys, testicles and epithelium of trachea. Crystallographic studies in the two isoforms showed that the presence of each form of enzyme is due to few morphologic changes in the structure. The important difference is in the active centre of COX-2 which is a little bit larger and has a second internal junction position for heavier substances in molecular weight. Therefore selective NSAIDs with different molecular weights may be conjugated time-dependently and non-reversibly with COX-1 or COX-2. Big molecular weight non-selective NSAIDs are conjugated with both the two isoforms of COX enzyme in instantly reversible way[7,12-14].

NSAIDs have three distinct features: analgesic, antipyretic and anti-inflammatory. The most important NSAIDs are shown on Table I. In the family of propionic acid, the prototype drug is ibuprofen. It was the first drug commercialized in USA and then it was followed by naproxen, fenoprofen, ketoprofen, flurbiprofen and last oxaprozin. Another family is indolonic acids (indomethacin, sulindac and etodolac). Oxicam derivatives were used extensively when released, before their use was stopped because of the unwanted effects. Meloxicam is the latest that had fewer unwanted effects. Phenylbutazon is a pyrazol product with anti-inflammatory action but weak analgesic and antipyretic properties. Diclofenac, ketorolac, tolmentin and nabumetone are good analgesic agents. Last but not least coxib family with celecoxib, rofecoxib, eterocoxib is used under supervision for their side effects in cardiovascular and gastric system[5,6,8,15-17].

Just like all agents, NSAIDs have interactions with other drugs shown on Table II**.**

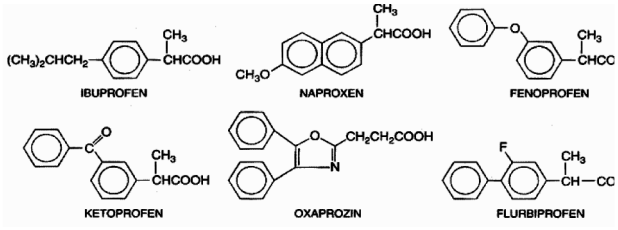
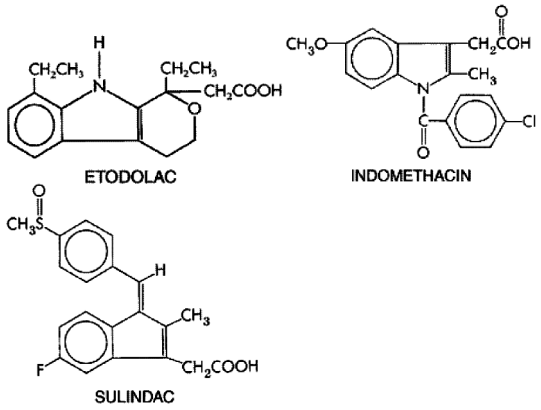
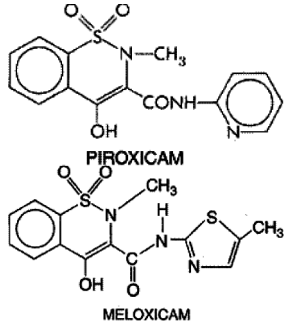
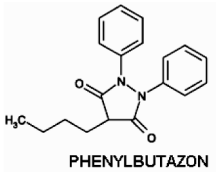
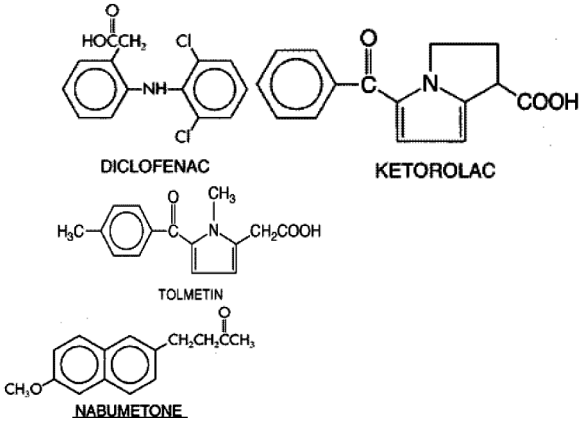
IN THE QUEST FOR THE ANSWER

Animal studies are directed to one preferable conclusion: Conventional non-specific NSAIDs delay healing in complete fractures. The best documented substances were indomethacin, diclofenac, naproxen, ketorolac, ibuprofen and naproxen. These drugs caused slower fracture healing in early stages[2,14,18-22]. Fractures may vary including rat forelimb, vertebra or femur. Notice that the doses of NSAIDs used in animal fracture models that show an obvious delayed bone union (according to histological, radiological and biochemical testings), are larger than the typical doses of administration in humans[35]. Many substances must reach toxicity levels in experimental animal models so as to have fracture delayed union. A possible cause of this fact, that is less known by the researchers, is that COX inhibitors are metabolized very quickly by hepatic enzymes (first route of metabolism), so they present low final serum concentrations relevant to bone healing fracture[6,8,23].

Indomethacin followed by ketorolac both appeared to have an important inhibitory action in bone healing process. Experimental models indicate that there is poor mineralization of demineralized bone matrix grafts and Harvesian remodeling. These agents delay bone union process but they do not inhibit it[2,8,14,24,32]. Studies in knockout mice (COX-1 and COX-2 null) have very impressive results. COX-2$^{-/-}$ mice's endochondral ossification was delayed compared with COX-1 knockout mice. It seems that COX-2 selective inhibitors delay bone formation more than COX-1 inhibitors. In Saos-2 osteoprogenitor cell lines, after treatment with celecoxib, a selective COX-2 inhibitor, there was a decrease in osteogenesis potential. It seems that COX-2 inhibitors like celecoxib and rofecoxib inhibit bone regeneration. The question is in what phase do NSAIDs inhibit bone formation?[2,19,25-28]

It is believed that COX inhibitors act in the processes downstream of the initial inflammatory response at fracture sites. By using knockout COX-2 mice Zhang et al. showed that there was a reduction in expression levels of bone morphogenetic protein - 2 (BMP-2), osteogenic differentiation transcription core binding factor (cbf-α1) and osterix. BMP-2 is a powerful osteoblastic growth factor, expressed in the early stages of bone union. It is a member of TGF-β superfamily, crucial for bone formation. One proposed mechanism indicates the inhibition caused by COX inhibitors of the expression of growth factors such as interleukine -1,-6 (IL-1,-6), tumor necrosis factor - α (TNF-α) and others. It is known that these factors play vital role in the initiation of inflammatory processes and vasculo-

Table 1. Major groups of non-steroidal anti-inflammatory drugs[34,35].

Propionic acid derivatives family	
Indolonic acid derivatives family	
Oxicam derivatives family	
Phenylbutazon	
Diclofenac, ketorolac, tolmetin and nabumetone family	

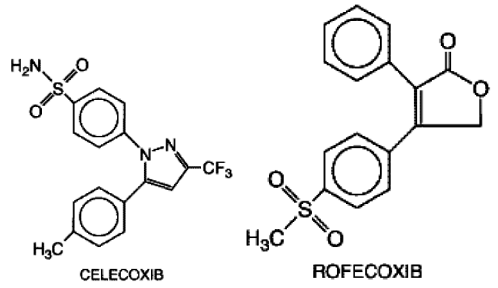
Coxib family	

Table 2. Important drug reactions with non-steroidal anti-inflammatory drugs[34,35].

Aminoglycosides	Reduction of clearance levels of aminoglycosides through kidney with induced toxicity.
Anticoagulants	Hypoprothrombithemia, low platelet concentration with high risk of gastric bleeding.
Antihypertensitives (α-blockers, β-blockers, diuretics, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors)	With the co-administration of NSAIDs there is an inhibition of antihypertensitives action, ion and water retention by pyrazole derivatives.
Corticosteroids	Increased risk for peptic ulcer .
Lithium	Decreased clearance of lithium through kidneys.
Cyclosporine	Increased nephrotoxicity.
Methotrexate	Decreased clearance of methotrexate in kidney and increased levels of methotrexate in serum - toxicity.

genic proliferation at the early stages of bone healing[14,25-29]. Another mechanism that may involve COX-2 and bone repair indicates that COX-2 maintains a population of mesenchymal stem cells in pre-osteoblast state, so during injury cases like bone fractures, COX-2 may affect through regulation of cbfa1 and osterix in an upstream of reactions with osteoblast differentiation to mature osteocytes. COX-2 inhibitors delay this procedure by reducing BMP-2 levels as previously reported[21].

In clinical level, NSAID seems to inhibit heterotopic bone formation after hip arthroplasty, according to several studies. This is an advantage in these situations, but brings us in front of a debate whether NSAIDs inhibit fracture healing. Nevertheless there is not yet a defined answer whether NSAIDs inhibit bone healing process. Risk factors that adversely may inhibit fracture healing include smoking, diabetes mellitus, peripheral arterial occlusive disease and others[30,31]. Some researchers believe that administration of NSAIDs in late phase of fracture healing is associated with non-union cases[18,30]. Most studies found no significant differences in fracture risk and use of NSAIDs[30-32]. Despite the fact that most cases found no association between NSAIDs and bone mal-union, there is a lack of evidence in these studies. No clinical trials are accomplished with specific protocols of exclusion of NSAIDs use in other treatments. Notice that NSAIDs are widely used in today clinical practice for musculoskeletal conditions. Most of the studies were retrospective and not randomized-controlled trials with lack of methodology. This is an issue of an Orthopaedics clinic whether or not to use NSAIDs in post-operation pain and inflammation. Scientifically

established results come only from animal studies so there is a need for more studies in clinical level.

NEW TRENDS IN THE AREA OF NSAIDS AND FRACTURE NON-UNION

In the search for the answer to this major problem, we must consider that mal-union of fractures is not just a topic of NSAIDs administration. Genetic background of patients is an important factor of non-union and its key role should be considered. Despite the fact that there is no clear evidence for the use of NSAIDs in post-surgical pain and inflammation during orthopaedic operation, NSAIDs are widely used for these purposes. There is need for more research in both experimental and clinical level for the safe use of NSAIDs in bone fracture. There is a precautionary sense about the use of NSAIDs because of their inhibitory role in bone fracture, therefore orthopaedists must be very careful in the use of NSAIDs in these cases. Maybe new technologies must be innovated so as to implement NSAIDs in topical form by using nanomolecules[33].

Αναζητώντας την αιτιοπαθογένεια καθυστερημένης πώρωσης των καταγμάτων. Ο ανασταλτικός ρόλος των μη στεροειδών αντιφλεγμονωδών φαρμάκων είναι ένα σύνθετο φαρμακολογικό φαινόμενο;

Χαράλαμπος Δόκος, Μαρία Μυρωνίδου-Τζουβελέκη

Α΄ Εργαστήριο Φαρμακολογίας, Ιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Ελλάς

ΠΕΡΙΛΗΨΗ: Η καθυστερημένη πώρωση του κατάγματος αποτελεί σημαντικό πρόβλημα στην Ορθοπαιδική. Στην ανασκόπηση αυτή προσεγγίζεται ο ρόλος των μη στεροειδών αντιφλεγμονωδών φαρμάκων (ΜΣΑΦ) στην πώρωση των καταγμάτων. Σύμφωνα με εργαστηριακές μελέτες, τα ΜΣΑΦ αναστέλλουν την πώρωση των καταγμάτων στα πρώτα στάδια της διαδικασίας. Εντούτοις ελάχιστες είναι οι κλινικές μελέτες που έχουν γίνει μέχρι σήμερα. Οι περισσότερες δεν παρέχουν σαφή απάντηση ως προς την ανασταλτική δράση των ουσιών αυτών. Οι αναστολείς COX-2 φαίνεται ότι ασκούν παρόμοια δράση με τα κλασικά ΜΣΑΦ. Παρ' όλες τις ανεπιθύμητες ενέργειές τους στο γαστρεντερικό, καρδιαγγειακό σύστημα και στην πώρωση του κατάγματος, χρησιμοποιούνται ευρέως στον μετεγχειρητικό πόνο μετά από ορθοπαιδικές επεμβάσεις και σε φλεγμονώδεις καταστάσεις.

Λέξεις Κλειδιά: Μη στεροειδή αντιφλεγμονώδη φάρμακα, Πώρωση καταγμάτων, Κοξίμπες, ψευδάρθρωση.

REFERENCES

1. Dandy JD, Edwards JD. Essential Orthopaedics and Trauma. Churchill Livingstone, 2003.
2. Dokos Ch, Mironidou-Tzouveleki M. The inhibitory effect of non-steroidal anti-inflammatory drugs in fracture healing. Epitheorese Klinikes Farmakologias kai Farmakokinetikes 2007; 25(1):38-39.
3. McRae R. Pocketbook of Orthopaedics and Fractures. Churchill Livingstone, 2006.
4. Pournaras DI. Orthopaedic Surgery, Codex Publications, Thessaloniki, 2006.
5. Skinner BH. Current Diagnosis & Treatment in Orthopedics. Lange medical book, McGraw - Hill, 2006.
6. Brunton L, Lazo J, Parker K. Goodman and Gillman's: the pharmacological basis of therapeutics. Chapter 27: Analgesic-Antipyretic and Anti-inflammatory Agents and Drugs Employed in the Treatment of Gout. McGraw-Hill, 2005.
7. Hawkey CJ, Wight NJ. Clinician's manual on NSAIDs and Gastrointestinal Complications. Science Press, 2004.
8. Kaskani E. The effect of anti-rheumatic drugs in bone metabolism. Skeletiki Ygeia (Greek Ed) 2006; 5(3): 89-91.
9. McCarthy MD. Occult GI bleeding in NSAID users-the base of the iceberg! Clin Gastr & Hep 2005; 3:1071-1074.
10. Menozzi A, Pozzoli C, Giovanni E et al. Intestinal effects of nonselective and selective cyclooxygenase inhibitors in the rat. Eur J Pharm 2006; 552:143-150.
11. Raidi AZ, Khan KN. Effects of cyclooxygenase inhibition on the gastrointestinal tract. Experimental and Toxicologic Pathology 2006; 26:163-173.
12. Berg MJ, Tymoczko LJ, Stryer L. Biochemistry. 5th Edition, W.H Freeman and Company, 2002.
13. Botting MR. Cyclooxygenase: past, present and future. A tribute to John R. Vane (1927-2004). J Therm Biol 2006; 31:208-219.
14. Seidenberg BA, An HY. Is there an inhibitory effect of COX-2 inhibitors on bone healing? Pharmacological Research 2004; 50:151-156.
15. Basbaum IA, Julius D. Toward better pain control. Sci Am 2006; 294(6):51-57.
16. Harvey AR, Champe CP. Lippincott's Illustrated Reviews: Pharmacology. JB Lippincott 2000.
17. Katzung GB. Basic & Clinical Pharmacology. Chapter 36: «Nonsteoidal anti-inflammatory drugs, disease-modifying antirheumatic drugs, nonopioid analgesics and drugs used in gout» Lange medical book, McGraw - Hill, 2004.
18. Goodman S, Ma T, Trindade M et al. COX-2 selective NSAIDs decreases bone ingrowth in vivo. J Orthop Res 2002; 20(6):1164-69.
19. Leonelli SM, Goldberg BA, Safanda J et al. Effects of cyclooxygenase-2 inhibitor (rofecoxib) on bone healing. Am J Orthop 2006; 35(2):79-84.
20. Mullis HB, Copland TS, Weinhold SP et al. Effects of COX-2 inhibitors and non-steroidal anti-inflammatory drugs on a mouse fracture model. Injury 2006; 37: 827-837.
21. Puzas EJ, O'Keefe JR, Schwarz ME et al. Pharmacologic modulators of fracture healing: the role of cyclooxygenase inhibition. J Musculoskel Neuron Interact 2003; 3(4):308-312.
22. Wheeler P, Batt EM. Do non-steroidal anti-inflammatory drugs adversely effect stress fracture healing? A short review. Br J Sports Med 2005; 39:65-69.
23. Aspenberg P. Drugs and fracture repair. Acta Orthopaedica 2005; 76(6):741-748.
24. Nazon D, Abergel G, Hatem MC. Critical care in orthopedic and spine surgery. Crit Care Clin 2003; 19:33-53.
25. Arasapam G, Scherer M, Cool CJ et al. Roles of COX-2 and iNOS in the bony repair of the injured growth plate cartilage. J Cell Biochem 2006; 99:450-461.
26. Daluiski A, Ramsey KE, Shi Y et al. Cyclooxygenase-2 inhibitors in human skeletal fracture healing. Orthopedics 2006; 29(3):259-61.
27. Einhorn AT. The role of cyclooxygenase-2 in bone repair. Arthritis Res Ther 2003; 5:5-7.
28. Endo K, Sairyo K, Komatsubara S et al. Cyclooxygenase-2 inhibitor delays fracture healing in rats. Acta Orthopaedica 2005; 76(4):470-474.
29. Murnaghan M, Li G, Marsh RD. Nonsteroidal anti-inflammatory drug-induced fracture nonunion: an inhibition of angiogenesis? J Bone Joint Surg 2006; 88:140-147.
30. Beck A, Salem K, Krischak G et al. Nonsteroidal anti-inflammatory drugs (NSAIDs) in the perioperative phase in traumatology and orthopedics effects on bone healing. Oper Orthop Traumatol 2005; 17(6):569-78.
31. Bhattacharyaa T, Levin R, Vrahas SR et al. Nonsteroidal anti-inflammatory drugs and nonunion of humeral shaft fractures. Arthr & Rheum 2005; 53(3):364-367.
32. Van Staa PT, Leufkens MGH, Cooper C. Use of non-steroidal anti-inflammatory drugs and risk of fractures. Bone 2000; 27(4):563-568.
33. Dokos C, Mironidou-Tzouveleki M. New trends and prospects of nanotechnology in drug administration. Epitheorese Klinikes Farmakologias kai Farmakokinetikes 2006; 24(3):165-172.
34. Paradellis A. Drug Interactions. University Studio Press. 1985.
35. Ellsworth JA, Witt MD, Dugdale CD et al. Mosby's 2006 Medical Drug Reference, Elsevier Mosby Company, St. Louis, 2006.