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Original Article
Orijinal Araştırma

Sıçan Diz Ekleminde Kıkırdak ve Sinovyum Üzerine Ketorolak Trometamin'in İntraarti küler Enjeksiyonunun Etkileri

The Effects Of Intraarticular Injection Of Ketorolac Tromethamine On Cartilage And Synovium In Rat Knee Joint

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ABSTRACT

Background: This study histopathologically investigated the local applicability of ketorolac tromethamine injected into knee joints of rats and its effects on cartilage and synovial tissue of the knees.

Methods: The study involved 30 Sprague-Dowley rats with a weight of 250-300 gr. After halotan anesthesia, all the rats were injected 0.25 ml ketorolac tromethamine prepared with 0.9% serum physiologic at a total of 10 mg/ml into their right knee joints, comprising the study (ketorolac) groups.

The left knee joints of all the rats were injected 0.25 ml 0.9 % serum physiologic and formed the control groups of the study. All the rats were sacrificed by phenobarbital in the following order: Group I (n=10) 24 hours after intra-articular (i.a.) injection, Group II (n=10) 48 hours after the injection, Group III (n=10) rats 5 days after the injection. The samples were then paraffin embedded and histopathologically examined. For inflammatory and degenerative changes, the parameters such as congestion, edema, fibrin exudation, synovial hyperplasia, neutrophil increase, macrophage increase, macrophage like cells (type A cells) and fibroblast like cells (type A cells) were studied.

Results: In Ketorolac group, the rats which were sacrificed at the 24th and 48th hour had superficial erosion and decreased synoviocytes in the subsynovial layer of the inner surface of the joint capsule and synovial epithelium (P=0.127, P=0.042).

Particularly, in Group III, which was sacrificed on the 5th day, congestion, edema, neutrophile and macrophage increase in the subsynovial connective tissue, type B cell increase, and lymphocyte increase in the deeper sections of synovial membrane were detected in the joint capsules (P=0.000).

None of the groups presented synovial membrane hyperplasia and fibrin exudation that could suggest severe inflammation.

Conclusion: The long-term i.a. application of ketorolac should be avoided. On the other hand, since pure ketorolac is known to cause less inflammation than stabilized ketorolac, agents which will inhibit inflammation should be the choice of preference.

Key words: Ketorolac, Analgesic, Intra-articular, Synovium, Rat knee joint.

ÖZET

Amaç: Bu çalışmada, sıçan diz eklem içine lokal ketorolak tromethamine enjeksiyonunun dizin kıkırdak ve sinovyal dokuları üzerindeki etkilerini histopatolojik olarak araştırdık.

Gereç ve yöntemler: Çalışma 250-300 gr ağırlığında 30 adet Sprague-Dowley dahil edildi. Halotan anestezisi sonrası, tüm sıçanların sağ diz eklem içine toplam 10 mg olmak üzere %0.9 serum fizyolojik ile hazırlanan 0.25 ml ketorolak trometamin enjekte edildi. Tüm sıçanların sol diz eklemleri 0.25 ml %0.9'luk serum fizyolojik enjekte edildi ve çalışmanın kontrol gruplarını oluşturuldu. Bütün sıçanlar aşağıdaki sırayla fenobarbital ile sakrifiye edildi: Grup I (n = 10) intra-artiküler (ia) enjeksiyondan 24 saat sonra, Grup II (n = 10) enjeksiyondan 48 saat sonra, Grup III (n = 10) enjeksiyondan 5 gün sonra. Örnekler parafine gömüldü sonra histopatolojik olarak incelendi. Enflamatuvar ve dejeneratif değişiklikler için, konjesyon, ödem, fibrin eksüdasyonu, sinovyal hiperplazi, nötrofil artışı, makrofaj artışı, makrofaj benzeri (A tipi hücreler) fibroblast benzeri hücreler (A tipi hücreler) gibi parametreler çalışıldı.

Sonuçlar: Ketorolak grubunda, 24 ve 48. saatte sakrifiye edilen sıçanlar yüzeysel erozyon ve eklem kapsülü iç yüzeyinin subsynovial tabakasında ve sinovyal epitelinde ki sinoviyositleri azaldı (p = 0.127, p = 0.042). Özellikle 5. gün kurban edilen Grup III'de, eklem kapsülü içinde sinovyal membranın derin bölümlerinde subsynovial bağ dokusunda konjesyon, ödem, nötrofil ve makrofaj artışı, B tipi hücre artışı ve lenfosit artışı tespit edildi (P = 0.000). Grupların hiçbirinde ciddi iltihab olan fibrin eksüdasyonu ve sinovyal membran hiperplazisi izlenmedi.

Karar: Uzun vadeli hidrotik ketorolak uygulanmasının kaçınılmalıdır. Diğer taraftan, Saf ketorolak stabilize ketorolaktan daha az inflamasyona neden olduğu bilinmektedir. İnflamasyonu inhibe edecek ajanlar tercih tercih edilmelidir.

Anahtar Kelimeler: Ketorolac, analjezi, intra-artiküler, sinoviyum, sıçan diz eklemi.

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INTRODUCTION

Systemic nonsteroidal anti-inflammatories (NSAIDs), physical therapy, and local corticosteroid injections all are used as nonoperative treatments of these conditions. Each of these current treatment modalities has limitations (1). Ketorolac is a strong nonsteroidal anti-inflammatory agent, has been considered a powerful alternative to other NSAIDs and opioids in postoperative pain treatment and following knee arthroscopic surgery. It is also promising for the treatments of renal colic, acute muscle and bone pains, and ocular inflammation (2,5,7).

The standard preparation of ketorolac is in the form of tromethamine salt and in clinical studies, it is used as intramuscular, intravenous and local ophtalmic solution. It inhibits prostaglandin synthesis and creates an effective analgesic and moderate degree of anti-inflammatory effect. Its local anesthetic effect has also been studied. Its 30 mg. doses create an analgesia equivalent to that of 12 mg morphine, and its superiority over 6 mg. of morphine within five-day period has also been shown (8,9).

Following the reports of renal deficiency, gastrointestinal hemorrhage, and death from anaphylactic shock among the patients using ketorolac tromethamine in the years 1990-1992, Syntex Pharmaceuticals, the manufacturer of ketorolac, recommended a lower daily dose of the drug, i.e. 90 mg/day instead of 120 mg/day while the number of days for use of the drug was also limited to three days. However, alternative use of ketorolac to opioids has become a common practice. Therefore, in this study we studied 5- day exposure with a suggested 1-2-day exposure despite 5 days was not suggested. Ketorolac does not have any opioid like effects on central nervous system. Ketorolac supportive morphine or only ketorolac use remains as a current practice due to the inability to use sufficient amount of morphine for its side-effects on respiratory and circulatory systems (10).

Intravenous or i.a. injection of ketorolac in arthroscopic knee surgery has been reported to provide comfort by reducing the postoperative pain while causing a tissue damage; thus, leading to an apprehensive approach to its use (11,12). Therefore, this study aimed at histopathologically investigating whether ketorolac tromethamine injected into rat knee joint could be used locally with regard to its effects on knee cartilage and synovium tissue.

MATERIAL AND METHOD

After the approval of research proposal by Animal Ethics Committee, 30 Sprague-Dowley rats, weighing 250-300 gr., comprised the subjects of the study in Gülhane Medical Faculty, Research Animals Lab.

The rats were anesthetized with inhaled 2% halotan, 50% 0_2, and 50% N_2O. Pure ketorolac was prepared to be 10 mg/ml with 0.9% of serum physiologic. Under aseptic conditions, 25 ml of the standard solution with no ethanol was injected into the right knee joints of the rats. The left knee joints were injected with 25 ml of 0.9% serum physiologic, and control groups were formed. The rats were then allowed to awaken and were returned to their cages.

All the rats were sacrificed by a lethal injection of phenobarbital in the following order: Group I (n=10) 24 hours, Group II (n=10) 48 hours, Group III (n=10) rats 5 days after i.a. injection. The knee joints were detached and evaluated for gross signs of haematoma and histopathologically studied. The tissue samples obtained from and labeled for left and right knees were placed in 10% neutral buffered formalin for two weeks. The samples which were then decalcified in Decastro solution (300 ml absolute ethanol, 50 gr chloralhydrate, 670 ml distilled water, 30 ml nitric oxide) for another two weeks and embedded into paraffin. Sections of 6-10 micrometer were prepared and stained with haematoxylin and eosin method.

Preparations were evaluated for histopathological, inflammatory, and degenerative changes. Congestion, edema, synovial hyperplasia, neutrophils and macrophage increase, plasma cell increase, and type A and type B cells were studied.

The inflammatory changes in the joint concerned were classified as follows: (3)

1. No inflammation 2. Minimal inflammation (minimal congestion, edema and superficial erosion on synovium cell layer) 3. Mild inflammation (congestion and edema, increased number of neutrophils) 4. Severe inflammation (congestion, edema, neutrophil and macrophage increase in subsynovial tissue) 5. Severe inflammation (congestion, edema, neutrophil and macrophage increase in subsynovial tissue and lymphocyte increase in deep synovial layer, decrease in the number of synoviocytes, synovial membrane lessening, fibrin accumulation)

The Mann-Whitney U test was used to calculate the probability of the differences between the study and control groups at 24 h, 48 h and 5 days being attributable to change. The value of P< 0.05 was taken as significant. The SPSS for windows (version 11.0) statistical package was used.

RESULTS

On gross examination, the incidence of haematoma over the joint capsule (control/ ketorolac) was 0/0 at 24 h, 0/0 at 48 h, and 0/1 at 5 days after injection. Haematoma were very small and the difference was not significant. Table I presents the degree of inflammatory change. Despite the incidence of minimal congestion, superficial edema of synovial epithelium, congestion and neutrophil increase in a limited number in all three control groups, the difference was not statistically significant. However, the degree of inflammation in the study groups with ketorolac injection was statistically compared to that of control groups (P=0.127, P=0.042). The fifth degree inflammation was observed only in the study groups 5 days after the injection and the difference between the 5th day control and study groups was significant (P=0.000).

In ketorolac injected groups, the joints which were sacrificed at 24 h, and 48 h, a superficial erosion characterized with distortion of consistency in intima and synovial lining layer, and a decreased number of synoviocytes in the synovial layer were striking. The groups with minimal inflammation also had light congestion (Figure 1). Mild inflammation was accompanied by neutrophil increase in the subsynovial ligament tissue in all three of the study groups (Figure 2a,b). Severe inflammation, however, was accompanied by increases of both neutrophils and macrophages in the subsynovial tissue (Figure 3a). Particularly in the joints sacrificed on the 5th day had lymphocyte increase in the deep synovial lining layer apart from all the other findings, which was considered as severe inflammation (Figure 3b). No statistically significant difference was found between groups with regard to type A cells; however, ketorolac group, especially on the 5th day, had slightly higher number of type B cells compared to that in control group (Figure 3c). There were no synovial membrane hyperplasia and fibrin exudation findings to be considered severe inflammation in any of the groups.

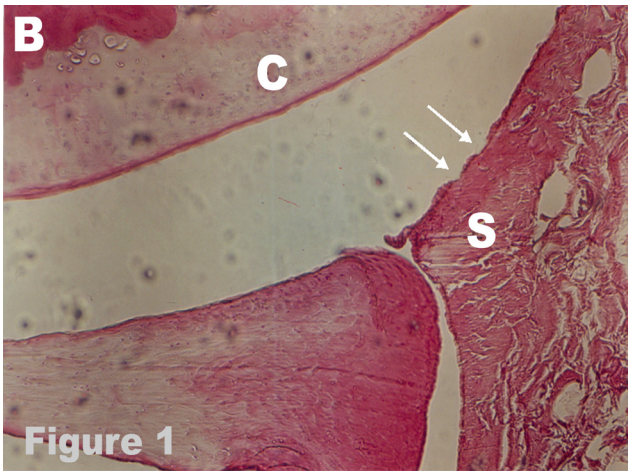

Table 1. The changes and the degrees of inflammation of knee joint of study and control groups.

	Control Group İnflammation					Ketorolac Group inflammation				
	1	2	3	4	5	1	2	3	4	5
Time after injection 24 h (P= 0,127)	8	2	0	0	0	5	3	2	0	0
48 h (P=0,042)	7	3	0	0	0	3	4	1	2	0
5 days (P=0,000)	7	2	1	0	0	0	0	1	2	7

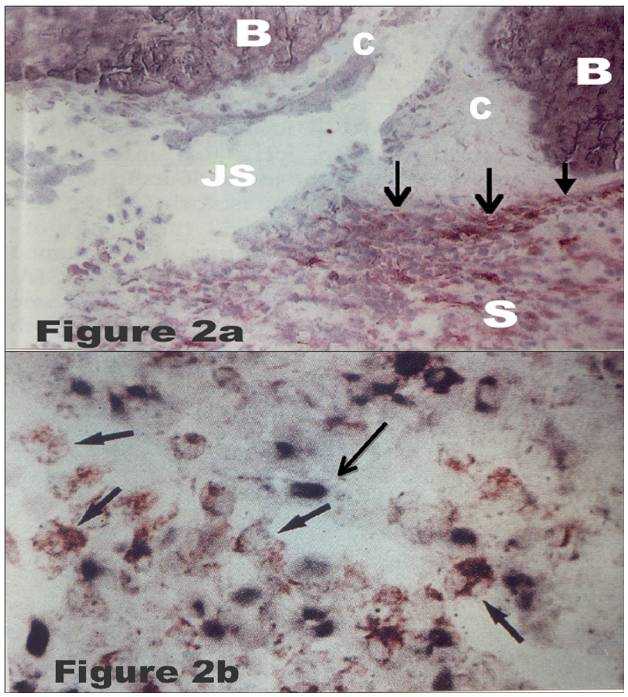

Figure 2a

Figure 2b

DISCUSSION

Despite the chemical properties and the effect pattern of NSAIDs are markedly varied, ketorolac is one of the three preparations licensed for intravenous administration. It is less irritating for tissues than other NSAIDs. The cartilage metabolism and proteoglycan synthesis in in vitro studies have shown different effects compared to those of other NSAIDs; thus, ketorolac has not been considered as an appropriate agent for i.a. administration. Cook et al. have emphasized the importance of clear identification of all the effects of i.a. NSAID drugs (13). However, there has been increasing interest in i.a. administration of these drugs. Monahan and colleagues compared 60 mg of ketorolac in 30 ml saline with 30 ml of 0.25% bupivacaine instilled into the knee joint after arthroscopy, and found that visual analogue pain scores were lower in the immediate period with bupivacaine but did not differ significantly between the groups over the following 5 days (5).

Furthermore, Reuben and Connelly concluded that i.a. ketorolac produces comparable analgesia to that achieved with i.a. morphine, with no benefit when given in combination 3). In another investigation by the same authors, it was shown that 60 mg of i.a. ketorolac improved comfort in the early postoperative period in patients undergoing knee arthroscopy, especially when combined with i.a. bupivacaine, and provided better analgesia compared with i.a. bupivacaine alone. They also asserted that this combination yielded better results than i.a. bupivacaine and intravenous ketorolac (60 mg, 2 ml) in this 80-subject study (11). Wilkinson DJ, on the other hand, emphasize that ketorolac is not licensed for i.a. application, and that there are no reliable data for its long and short-term

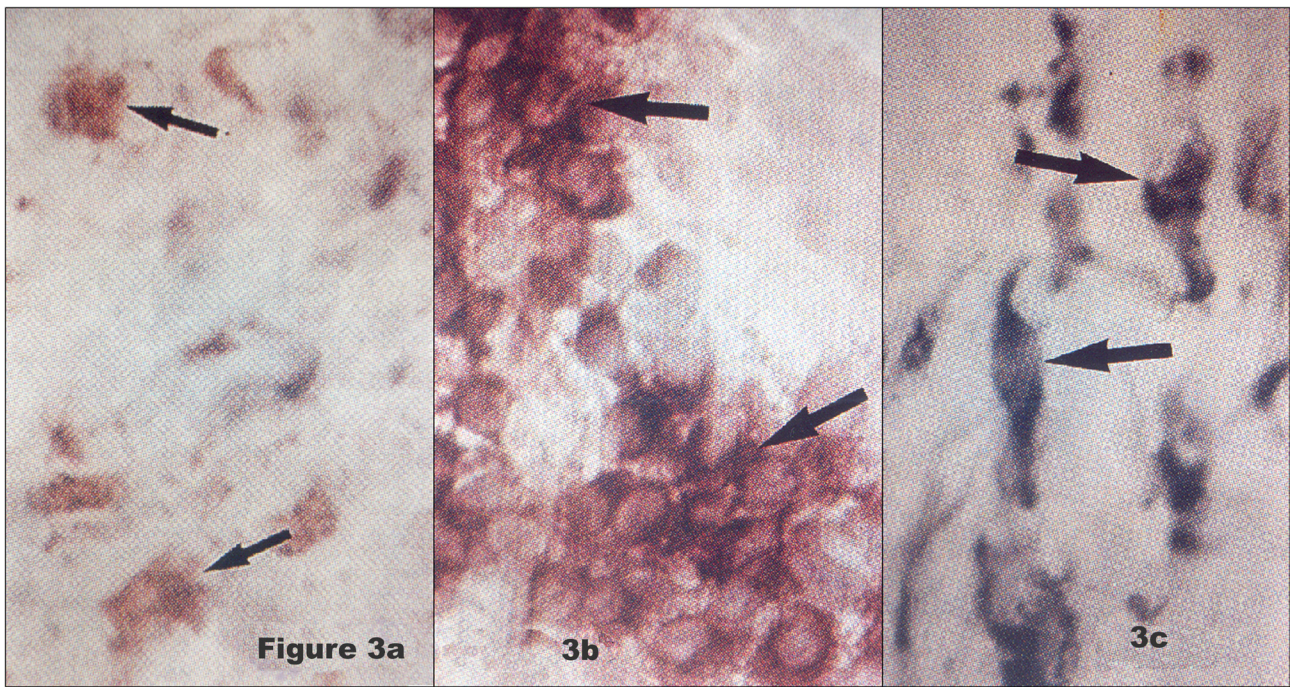
Figure 3a 3b 3c

toxicologic effects. Furthermore, he highlights the lack of studies on the long-term local effects of ketorolac on human and animal cartilage, bone and synovium (12).

Therefore, the i.a. use of ketorolac also portrays ethical concerns. The results of Gupta et al study are supportive of Reuben et al study. In a study of 100 patients (three groups) who had undergone knee arthroscopy and were treated for postoperative pain with the consent of hospital ethics committee, they report a better analgesic effect of 30 mg of ketorolac and 3 mg. of morphine, particularly in the first 4-48 after injection, in i.a. ketorolac (30 mg, 60 mg ve 30mg +3 mg morphine) administration. They further report on the synergistic effect of this combination (10). In another study involving 60 patients by Convery et al, a local injection of ketorolac was found to as equally effective as 10 mg systemic dose with regard to analgesia or postoperative visual analogue pain scores[2]. In addition, De Andres et al have reported higher success ratio in the group which received intramuscular ketorolac along with i.a. bupivacaine and morphine (14).

The histopathological effects of any drug administered locally are important. Irwin et al, in a similar study in which the rats were administered i.a. ketorolac which also contains ethanol, have reported inflammatory cell infiltration, hyperplasia, and hypertrophy in the synovial membrane cells, or inflammation of knee joint articular cartilage. However, they have found ethanol used for stabilization to cause the least amount of inflammation (3,4). Although the inflammation was not lower when concomitantly used with ketorolac, which did not support our study. Concomitant use of ethanol, however, seems to have caused inflammation. In this study, we did not detect any severe inflammation of joint tissues because of i.a. ketorolac administration. The agents used for ketorolac stabilization could be responsible for severe inflammation after commercial ketorolac administration, as was in Irwin et al study. Since no ethanol was used in the current study, there was lesser inflammation in both the control and study groups. Furthermore, there were no findings such as fibrinous exudate in pure ketorolac group. Therefore, we arrived at the conclusion that pure ketorolac leads to lesser inflammation than stabilized ketorolac does. Nevertheless, it is clear that it is not safe for long-term use.

In synovial inflammation typical histological changes and clinical manifestations have not been studied comprehensively through semiquantitative methods. The significance of coefficiency among T cells, adjacent macrophages, and fibroblast-like synoviocytes (f cells) in synovial inflammation has been shown (15). Synovial cells usually make up permanent, oval shaped cell layers with cytoplasma extension and no basal membrane. Macrophage like type A cells and fibroblast like type B cells can change their internal structure upon various stimulations and regulate their own functions. Synovial cells secrete hyaluronic acid, fibronektin and laminin, which provides adherence to matrix. There may be lymphocytes in synovial tissue. Decreasing number of synoviocytes in synovial tissue may lead to a decrease in synovial fluid. Similarly, thining of synovial membrane and lower number of synoviosits in subsynovial tissue may cause lesser secretions of fibronektin ve laminin glikoproteins. The inflammations encountered in this study had strikingly lower numbers of synoviosits, increased numbers of cells in subsynovial ligament tissue, and increased numbers of f cells. Literature presents studies confirming the prevention of synovial changes with Nitric Oxide administration (16).

We believe exploration of thinning synovial membrane and decreased number of synoviocytes after i.a. administration through immunohistochemical methods will prove beneficial. We may also emphasize the need to avoid long-term i.a. use of ketorolac. However, pure ketorolac can be said to cause lesser inflammation than stabilized ketorolac; thus, agents which will inhibit this inflammation should be used.

REFERENCES

1. Shapiro PS, Rohde RS, Froimson MI, Lash RH, Postak P, Greenwald AS. The Effect of Local Corticosteroid or Ketorolac Exposure on Histologic and Biomechanical Properties of Rabbit Tendon and Cartilage. Am Asso Hand Surg. 2007;2:165-172.
2. Convery PN, Milligan KR, Quinn P, Scott K, Clarke RC. Low-dose intra-articular ketorolac for pain relief following arthroscopy of the knee joint. Anaesthesia. 1998;53(11):1125-9.
3. Irwin MG, Cheung KM, Nicholls JM, Thompson N. Intra-articular injection of ketorolac in the rat knee joint: effect on articular cartilage and synovium. Br J Anaesth. 1998;80(6):837-9.
4. Dogan N, Erdem AF, Gundogdu C, Kursad H, Kizilkaya M. The effects of ketorolac and morphine on articular cartilage and synovium in the rabbit knee joint. Can J Physio Pharma 2004;502-5.
5. Reuben SS, Connelly NR. Postarthroscopic meniscus repair analgesia with intraarticular ketorolac or morphine. Anesth Analg. 1996; 82(5):1036-9.
6. Chow GK, Fabrizio MD, Steer T, Potter SR, Jarrett TW, Gelman S, Kavoussi LR. Prospective double-blind study of effect of ketorolac administration after laparoscopic urologic surgery. J Endourol. 2001; 15(2):171-4.
7. Ben-David B, Katz E, Gaitini L, Goldik Z. Comparison of i.m. and local infiltration of ketorolac with and without local anaesthetic. Br J Anaesth. 1995; 75(4): 409-12.
8. Avellaneda C, Gomez A, Martos F, Rubio M, Sarmiento J, de la Cuesta FS. The effect of a single intravenous dose of metamizol 2 g, ketorolac 30 mg and propacetamol 1 g on haemodynamic parameters and postoperative pain after heart surgery. Eur J Anaesthesiol. 2000;17(2):85-90.
9. Brocks DR, Jamali F. Clinical pharmacokinetics of ketorolac tromethamine. Clin Pharmacokinet. 1992;23(6):415-27.
10. Gupta A, Axelsson K, Allvin R, Liszka-Hackzell J, Rawal N, Althoff B, Augustini BG. Postoperative pain following knee arthroscopy: the effects of intra-articular ketorolac and/or morphine. Reg Anesth Pain Med. 1999;24(3):225-30.
11. Reuben SS, Connelly NR. Postoperative analgesia for outpatient arthroscopic knee sugery with intraarticular bupivacaine and ketorolac. Anesth Analg.1995;80(6):1154-7.
12. Wilkinson DJ. Intraarticular ketorolac (letter). Anesth Analg. 1996; 82(2): 426-39.
13. Cook TM, Nolan JP, Tuckey JP. Postarthroscopic meniscus repair analgesia with intraarticular ketorolac or morphine. Anesth Analg.1997;84(2):466-7.
14. De Andres J, Valia JC, Barrera L, Colomina R. Intra-articular analgesia after arthroscopic knee surgery: comparison of three different regimens. Eur J Anaesthesiol. 1998;15(1):10-5.
15. McInnes LB, Leung BP, Liew FY. Cell-cell interactions in synovitis, interactions between T lymphocytes and synovial cells (review). Arthritis Res.2000 2:374-8.
16. Granados-Soto V, Flores-Murrieta FJ, Castaneda-Hernandez G, Lopez-Munoz FJ. Evidence for the involvement of nitric oxide in the antinociceptive effect of ketorolac. Eur J Pharmacol. 1995;277(2-3):281-4.