Effect of intraarticular injection of lornoxicam on the articular cartilage & synovium in rat

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Background & objectives: Intraarticular (i.a) drug application is consider to be a new therapeutic approach for the treatment of postoperative pain after arthroscopic knee surgery without any systemic adverse effects. Lornoxicam, a nonsteroid anti-inflammatory drug is a short acting agent, and its anti-inflammatory and analgesic activity may be effective in the postoperative pain management in minor surgery. In this study, the effects of intraarticular administration of lornoxicam on the synovium and articular cartilage in the rat knee joint were investigated.

Methods: Lornoxicam (0.25 ml) was given as an injection into the right knee joint and 0.25 ml of 0.9 per cent saline solution by injection into the left knee joint as a control in 25 rats. Groups of five rats were sacrificed by a lethal injection of ketamine 1st, 2nd, 7th, 14th and 21st days after lornoxicam administration. Knee joints were detached, fixed in 10 per cent buffered formalin and decalcified. Serial sections of 5 μm were stained with haematoxylin-eosin and evaluated for the presence of inflammation in the articular, periarticular regions and synovium. Inflammatory changes in the joints were graded according to a five-point scale, histologically.

Results: There were no significant differences in inflammation and cartilage degeneration, between control and lornoxicam applied knees. Grade 3 inflammatory changes occurred only in one knee in lornoxicam group, at 24 h after injection. No pathological changes were observed in both groups at any time point.

Interpretation & conclusions: Lornoxicam did not show significant effect on inflammation on rat synovia in knee joint. Further studies including in human need to be done before any recommendations are made for i.a. administration of lornoxicam.

Key words Intraarticular injection - lornoxicam - nonsteroidal anti-inflammatory drugs (NSAIDs) - postoperative analgesia - rat

Postoperative pain is the most common form of acute pain, which affects 50 to 70 per cent of patients undergoing surgery1. The most commonly used agents for the control of postoperative pain are parenteral opioid analgesics and nonsteroid anti-inflammatory drugs (NSAIDs)2-3. Although opioid analgesics are potent and extremely effective, fears about addiction and respiratory and cardiovascular depression can result in a reluctance
to administer sufficient amounts of medication and may lead to inadequate management of postoperative pain. NSAIDs have also many adverse effects (gastrointestinal and coagulation complications) when systemically used. Postoperative pain after arthroscopic knee surgery is the major impediment to early discharge and rehabilitation. Intraarticular (i.a.) drug application may be a new therapeutic approach for the treatment of postoperative pain after arthroscopic knee surgery without any systemic adverse effects. It has been shown that intraarticular NSAIDs such as tenoxicam, provides superior postoperative analgesia and reduces postoperative analgesic requirements compared with intravenous tenoxicam. Thus i.a. injection of local anesthetic drugs or opioids or both is commonly used to manage pain after arthroscopic knee surgery.

Lornoxicam is a new NSAID, a thienothiazine derivative of the “oxicam” class of NSAIDs, that has been shown to be effective and well tolerated in the treatment of pain associated with a variety of conditions, including rheumatoid arthritis, osteoarthritis, ankylosing spondylitis and postoperative dental pain. There are however several well-documented side effects associated with the use of NSAIDs, particularly at higher dosages. Although it is approved for intravenous, intramuscular and oral use, no toxicological data are available regarding its i.a. administration. The principal effects of NSAIDs are peripheral, and therefore, local application to the site of injury should produce analgesia while minimizing systemic side effects. In animal studies lornoxicam has shown anti-inflammatory (carrageenan oedema) and analgesic activity about 10 times greater than that of tenoxicam. Although lornoxicam is a short acting agent, its effective anti-inflammatory and analgesic activity makes it attractive for the postoperative pain management in minor surgery.

We therefore carried out this study to investigate the possible inflammatory effects of lornoxicam on the synovium and articular cartilage after direct injection into the knee joint of rats.

**Material & Methods**

Approval for this study protocol was obtained from the ethics committee of the Hacettepe University, Ankara, Turkey and experiments were performed in accordance with guidelines on the use of live animals in teaching and research centers (European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, Strasbourg, 18 March 1986, No: 2004/49-5).

Twenty five adult Sprague-Dawley rats (from the Animal Laboratory of Hacettepe University) weighing 250-300 g, were anaesthetized with 6-8 mg/kg intramuscular ketamine. Under aseptic conditions, 0.25 ml of standard preparation of lornoxicam (4 mg/ml) which also contained trometamol (6 mg/ml), mannitol (50 mg/ml) and disodium edetat (0.1 mg/ml) and water as vehicle, was injected into the right knee joint while 0.25 ml of vehicle was injected into the left as control at the same time. The animals were then returned to their cages. Groups of five rats were sacrificed by a lethal injection of ketamine (30 mg/kg) at 1st, 2nd, 7th, 14th and 21st days after the administration. The knee joints were detached and examined for gross signs of haematoma. The joints were labeled A, as left or B as right and the time of death was noted. The detached knee joints were fixed in 10 per cent buffered formalin for 2 wk at room temperature and decalcified in “De Castro” solution consisting of 300 ml absolute alcohol, 670 ml distilled water, 30 ml 70 per cent nitric acid and 50 g chloral hydrate. Decalcification procedure lasted for four weeks at room temperature. Decalcified tissue specimens were dehydrated in ascending degrees of ethanol, cleared and embedded in paraffin. Serial sections of 5 μm were stained with haematoxylin-eosin and were evaluated with Leica DM 600B light microscope.

The knee joint samples were evaluated for the presence of inflammation in the articular and periarticular regions and synovium. Two histologists who were blinded to the treatment examined all the sections. Inflammatory changes in the joints were graded according to a five-point scale. Grade 1- no inflammation, Grade 2- minimal inflammation: mild congestion and oedema, Grade 3- mild inflammation: erosion of joint surface, congestion and oedema, small number of neutrophils), Grade 4- moderate inflammation: neutrophils and macrophages, synoviocyte hyperplasia, and Grade 5- severe inflammation: neutrophils and macrophages, synoviocyte hyperplasia, fibrin exudation.

**Statistical analysis:** The Fischer Exact test was used to compare differences between the experimental and control groups at 1st, 2nd, 7th, 14th and 21st days. The differences within groups were not calculated because all the groups were sacrificed at the determined time intervals. P<0.05 was taken as significant.

**Results & Discussion**

The degree of histological inflammatory changes in each group is shown in the Table. In the specimens...
of control group, there was a mild dilatation of some blood vessels with a few erythrocytes. Articular cartilages had a normal histological structure in the control group. In the synovial tissue, the dense connective tissue under the synoviocytes was composed of collagen fibers scattered randomly and rich in blood vessels (Fig. 1). In lornoxicam group, there was only Grade 3 inflammation, 24 h after lornoxicam administration (Fig. 2). There was no significant difference in inflammation and cartilage degeneration between control and lornoxicam groups at any time intervals.

Lornoxicam is a new compound combining the potency of the “oxicams” with reduced risk of side effects. In comparison with other oxicams, lornoxicam has a relatively short elimination half-life (3-5 h) and good intestinal tolerability. In vitro studies have shown that different NSAIDs have different effects on cartilage metabolism and proteoglycan synthesis. Lornoxicam is not licensed by Food and Drug Administration (FDA) and Turkish Ministry of Health for intraarticular use in humans and the manufacturer was unable to provide any animal toxicological data regarding such administration. Several NSAIDs have effects on joint cartilage even when given systemically, due to their ability to disrupt chondrocyte metabolism and to inhibit proteoglycan synthesis. These effects may be more pronounced in patients with osteoarthritis and vary between different NSAIDs. We could find only one clinical trial from Russia that examined the anti-inflammatory and analgesic activity of xefocam by i.a. application to the knees. In this study, Balabanova et al., applied xefocam injection into the knee joints of 58 patients with rheumatoid arthritis once a week for 3 wk in a dose of 8 mg. The treatment efficacy was evaluated by changes in the severity of arthralgias, pain in the joints at palpation, circumference of the knee joints at the level of the upper edge of the patella, ultrasound and thermography of the knee joints. Xefocam relieved arthralgia (in 44 patients at least by 30%), pain in the joints at palpation and joint circumference. Ultrasound investigation revealed a significant thinning of the synovial membrane and decrease in the amount of exudates. The investigators concluded that, if local steroid therapy is not indicated, intraarticular administration of xefocam can effectively

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Fig. 1. Control (vehicle treated) group: Intact articular cartilage surface (C) and a few dilated blood vessels with erythrocytes (arrow head) are seen in the synovial tissue (S). Haematoxylin-eosin x100.

Fig. 2. Lornoxicam Group (Grade 3): synovial tissue (S) is oedematous and a few inflammatory cells are observed, cartilage surface (C). Haematoxylin-eosin x100.
be used for suppression of moderate inflammation in the joints in rheumatoid arthritis patients.

In this study, we did not find any significant differences between lornoxicam and control groups, but our results revealed that lornoxicam caused Grade 3 severe synovial inflammation only in one rat after the 24 h. Based to this result one cannot speculate that lornoxicam can be used safely through i.a. route without any adverse effect. Also in this study, lornoxicam was not used chronically and we did not use the preservative free form of the lornoxicam. Further studies including electron microscopic examination of the tissue specimens and the use of preservative free lornoxicam may yield more information about the possible tissue damage caused by chronic i.a. administration of lornoxicam in knee joint.

In conclusion, lornoxicam did not have significant anti-inflammatory effect on rat synovia. Our preliminary results indicated that though no side effects of i.a. administration of lornoxicam were detected in animal study, attention should be paid to the toxicology of intraarticular administration of drugs before its widespread clinical usage.

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References


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