TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2014) 44: 540-545
© TÜBİTAK
doi:10.3906/sag-1302-32

Comparison of intraarticular bupivacaine and levobupivacaine injection in rat synovial inflammation

Bekir Suat KÜRKÇÜOĞLU[1,*], Melehat DÖNMEZ[2], Seher ALTINEL[3], Seda Banu AKINCI[4], Fatma SARICAOĞLU[4], Ülkü AYPAR[4]

[1]Department of Anesthesia and Reanimation, Atatürk Education and Research Hospital, Ankara, Turkey
[2]Department of Pathology, Ankara Education and Research Hospital, Ankara, Turkey
[3]Department of Anesthesia and Reanimation, Keçiören Education and Research Hospital, Ankara, Turkey
[4]Department of Anesthesia and Reanimation, Faculty of Medicine, Hacettepe University, Ankara, Turkey

Received: 09.02.2013 • **Accepted:** 23.09.2013 • **Published Online:** 27.05.2014 • **Printed:** 26.06.2014

Background/aim: Both bupivacaine and levobupivacaine are local anesthetics with strong analgesic efficacy that can be used intraarticularly. The aim of this study was to compare the effects of intraarticular bupivacaine and levobupivacaine injection on inflammation in articular cartilage and the synovium of the rat knee joint.

Materials and methods: Twenty Sprague-Dawley rats were injected in the right knee joint with 0.2 mL of bupivacaine, while 0.2 mL of levobupivacaine was injected into the left knee joint. Groups of 5 were sacrificed on days 1, 7, 14, and 21 after bupivacaine and levobupivacaine administration and knee joints were examined for subintimal fibrosis, synovial hyperplasia, chronic inflammation, neutrophil infiltration, edema, and synovial and periarticular congestion by microscopy. Alterations in the articular cartilage structure were evaluated using Mankin scoring.

Results: We found that both drugs have similar effects on synovial and articular cartilage resulting in mild to moderate congestion, edema, neutrophil infiltration, chronic inflammation, and synovial hyperplasia, which diminished gradually. However, increases in fibrosis were also seen to varying degrees. Thus, the use of these drugs intraarticularly can be recommended.

Conclusion: Careful usage of bupivacaine and levobupivacaine is recommended in intraarticular applications since they cause inflammation shortly after injection and fibrosis at later time points.

Key words: Bupivacaine, levobupivacaine, intraarticular, rat, articular cartilage, histopathological changes

1. Introduction

Arthroscopic surgical interventions for diagnosis and treatment are prevalent in surgical practices. Local anesthetics are used alone or administered as an intraarticular treatment during arthroscopic surgical interventions to provide postoperative pain control (1,2). Bupivacaine amide is a local anesthetic (LA) with long-lasting efficacy (3) lasting approximately 5 h, and its duration of efficacy can be extended to 10 h with the addition of adrenaline (4). Levobupivacaine is a long-lasting LA with an S(-) isomer of bupivacaine and an amide structure. As with all LA agents, levobupivacaine functions by altering voltage-sensitive channels in neural membranes (3). Understanding the histopathological effects of intraarticularly injected LAs is critical as they may alter the joint cartilage and the synovium (5).

The purpose of our study is to compare the histopathological effects of bupivacaine and levobupivacaine on joint cartilage and the synovium after intraarticular injection into rat knee joints.

2. Materials and methods

This study was carried out in cooperation with the Hacettepe University Faculty of Medicine Anesthesiology and Reanimation Department, the Hacettepe University Faculty of Medicine Experimental Animals Laboratory, and the Ankara Education and Research Hospital Pathology Clinic after the approval of the Hacettepe University Experimental Animals Ethics Committee. All surgical operations were completed in accordance with the Declaration of Helsinki on animal rights.

We used 20 male Sprague-Dawley rats weighing 270–320 g, housed together in the Hacettepe University

* Correspondence: bekirsuat@gmail.com

Experimental Animals Laboratory. During the experiment, 5 rats were kept per cage, were fed with standard laboratory food, and had unrestricted access to water and food.

The rats were anesthetized with 0.4 mg kg^{-1} midazolam injected intraperitoneally. Under aseptic conditions, 0.2 mL of bupivacaine (5 mg mL^{-1}) was injected in the right knee joint and 0.2 mL of levobupivacaine (5 mg mL^{-1}) in the left knee joint of each rat. The rats were sacrificed with 30 mg kg^{-1} (a lethal dose) intraperitoneal ketamine (50 mg mL^{-1}) on days 1, 7, 14, and 21 after intraarticular injection. The area was then cleaned and arthrotomy was performed on both knees of rats with a medial parapatellar incision. After the distal femur anterior surface was opened by moving the patella laterally, the incision was extended distally to the tibial anterior surface. We separated all soft tissues from the femur and tibia and the intact knee joints were removed. After macroscopically examining whether there was hematoma, the right (R) and left (L) knees from each group (group 1: day 1, group 2: day 7, group 3: day 14, and group 4: day 21) were labeled and prepared for histology.

Tissue samples were fixed at room temperature in 10% formalin for 2 weeks and then decalcified with De Castro solution for 4 weeks. During the decalcification process, tissues were checked every 3 days and the De Castro solution was replaced. After tissues were decalcified and fixed in 10% formalin buffer, they were monitored by light microscopy. Serial sections of 5 μm were cut from tissue samples and stained using standard protocols for hematoxylin and eosin and Masson's trichrome staining. Stained sections were examined and photographed by 2 histologists using a Leica DM 6000B (Wetzlar, Germany) microscope connected to a DC490 digital camera (Leica).

The knee joint, peripheral areas, and synovium were examined for inflammation. Inflammatory changes were evaluated according to a 4-parameter scoring system (Table 1). Changes that occurred in the joint cartilage structure were evaluated using a modified Mankin score with 4 different parameters (Table 2).

2.1. Statistical analysis

Statistical analysis was completed using SPSS 12.0 (SPSS Inc., Chicago, IL, United States). Mann–Whitney U tests were used to compare the severity of inflammatory changes between 2 groups. Results were presented as medians (min–max). A chi-square test (Pearson chi-square or Fisher exact chi-square test) was used in group comparisons for categorical changes (inflammation degree). Results were considered statistically significant at $P < 0.05$.

3. Results

No macroscopic hematomas were observed in any of the 40 knee joints examined. No severe congestion or edema was observed in either group (R or L); however, slight congestion and edema was observed in the rat knees on day 7. When any 2 groups were internally examined, the difference between them was not statistically significant (day 1: $P = 0.262$; day 7: $P = 1$; day 14: $P = 0.50$; day 21: $P = 0.262$).

Rat knee joints were examined for neutrophil infiltration and a slight level infiltration was seen on day 1 ($P = 0.262$). No neutrophil infiltration was detected in any of the knees on the subsequent days.

Table 1. Grading system for histopathological inflammatory changes in rats injected with bupivacaine and levobupivacaine.

Histological feature	Score			
	0	1	2	3
Congestion, edema	Normal	Mild	Moderate	Severe
Neutrophil infiltration	Normal	Mild	Moderate	Severe
Chronic inflammation	Normal	Mild	Moderate	Severe
Synovial hyperplasia*	0	1	2	3
Subintimal fibrosis**	10% ↓	10-30%	30-50%	50% ↑

*: Synovial hyperplasia: Scored according to ranking of synovitis. 0 points: Single layer of synovitis, 1 point: 2 layers of synovitis, 2 points: 3 layers of synovitis, 3 points: 4 layers of synovitis.

**: Subintimal fibrosis: Percentage of fibrotic tissue in the loose joint tissue during synovitis in Masson's trichrome-stained sections.

Table 2. Modified Mankin histological scoring system.

Histologic feature	0	1	2	3	4	5	6
Cartilage structure	Normal	Surface irregularities	Pannus and surface irregularities	Clefts to transitional zone	Clefts to radial zone	Clefts to calcified zone	Complete disorganization
Cartilage cells	Normal	Pyknosis, lipid degeneration, hypercellularity	Clusters	Hypocellularity			
Staining with Masson's trichrome	Normal	Slight reduction	Moderate reduction	Severe reduction	No staining		
Tidemark integrity	Intact	Destroyed					

When joints were examined for chronic inflammation, severe levels of chronic inflammation were observed in only 1 rat, which had been given levobupivacaine and was sacrificed on day 7, while low or moderate levels of chronic inflammation were observed in all groups. Differences in chronic inflammation were not significant between the groups (Table 3).

Severe synovial hyperplasia was not observed in the joints. Low to moderate hyperplasia was observed on days 1, 7, and 14 and slight hyperplasia was observed in both groups on day 21. When both groups were compared, no statistical significance was detected.

While subintimal fibrosis percentages were below 50% in the knees, it was noted that recovery resulted in fibrosis at various levels for both groups on days 1 (P = 0.262), 7 (P = 0.668), 14 (P = 0.50), and 21 (P = 0.577).

Table 3. Chronic inflammation scores for each group.

Chronic inflammation	0	1	2	3	P
Day 1 (group R)	0	4	1	0	0.778
Day 1 (group L)	0	4	1	0	
Day 7 (group R)	0	3	2	0	0.160
Day 7 (group L)	0	1	3	1	
Day 14 (group R)	0	4	1	0	0.500
Day 14 (group L)	0	3	2	0	
Day 21 (group R)	0	4	1	0	0.500
Day 21 (group L)	0	5	0	0	

No statistical difference was found between the 2 groups when histological features were subjected to modified Mankin scoring, as the Mankin score was below 4 in all rat knees (day 1: P = 0.180; day 7: P = 0.095; day 14: P = 0.453; day 21: P = 0.738) (Figures 1a–1f).

4. Discussion

This study has determined that bupivacaine and levobupivacaine have similar effects on rat knee joints. Congestion, edema, neutrophil infiltration, chronic inflammation, and synovial hyperplasia were at low-to-moderate levels and decreased on day 21. Recovery of the joint was associated with fibrosis at various levels, proving that intraarticular usage of bupivacaine and levobupivacaine should be considered carefully.

Intraarticular treatment is widely used in arthroscopic procedures to provide preemptive and postoperative analgesia. The most commonly used medications are bupivacaine, NSAIDs, and morphine, whose intraarticular use has been well studied (2,6–11). However, few studies have examined the histopathological effects of intraarticular treatment in the joints (4,12,13).

In a study by Ozyuvaci et al. (14), tenoxicam was injected intraarticularly to the knee joints of 50 rats, which were then separated into groups of 10, and pathological changes in the joints were observed on days 2, 7, 14, and 21. No pathology was observed in the control group on days 1 and 2, while tissue loss and edema were observed in all rats treated with tenoxicam. No pathological changes were observed in either group on days 7, 14, and 21. In the bupivacaine and levobupivacaine groups, we observed statistically significant inflammation on day 1, while first- and second-degree inflammation was observed in a subset of the rats at the other time points. However, no significant difference was found when 2 groups were compared.

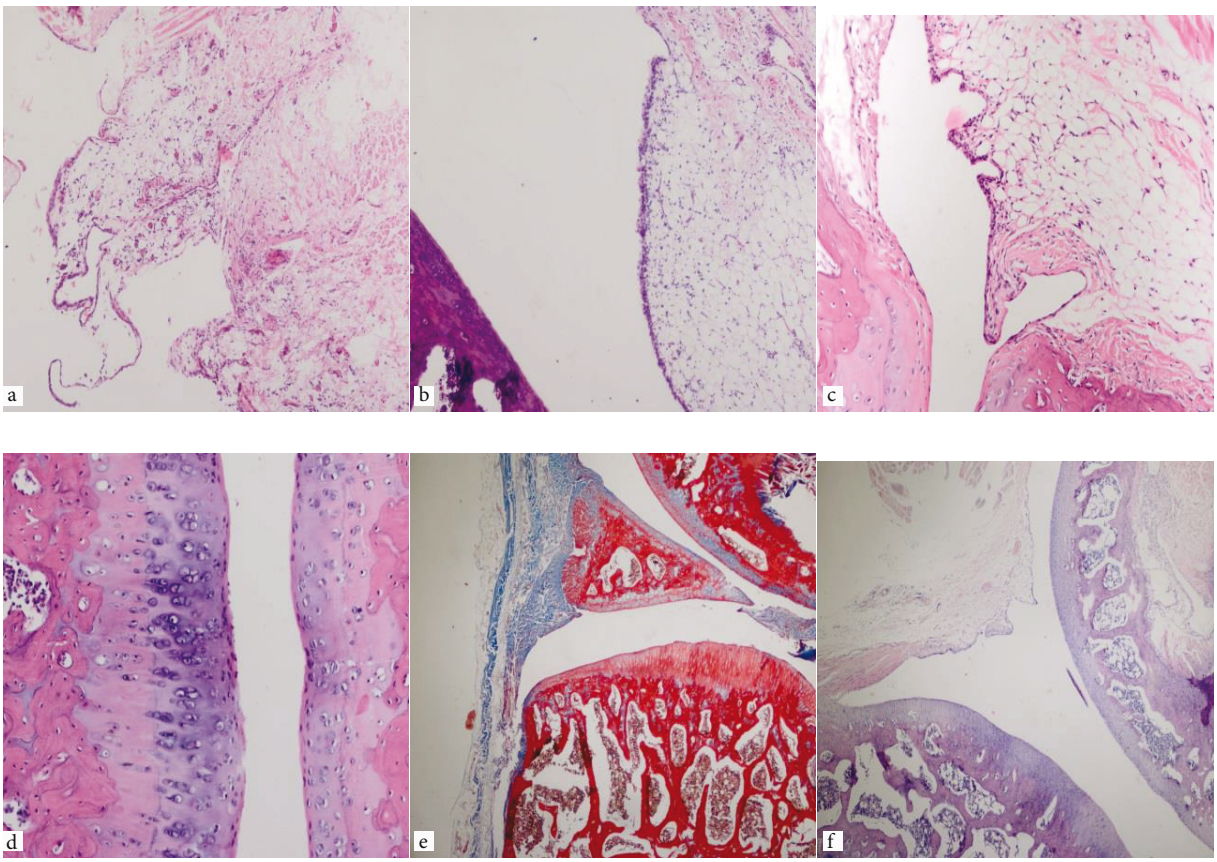

Figure 1. a) Representative day 1 tissues demonstrating congestion and edema observed in synovium of R and L group rats; b) representative day 1 tissues showing neutrophil infiltration and synovial hyperplasia observed in synovium in R and L group rats; c) representative day 7 tissues showing low levels of chronic inflammation with single layer of synovitis in R and L group rats; d) image of joint cartilage demonstrating clustering of chondrocytes and surface irregularity (on the left); e) representative day 7 tissues from R and L group rats stained with Masson's trichrome, demonstrating 30%–50% subintimal fibrosis; f) representative day 14 tissues from R and L groups showing low levels of chronic inflammation, single layer synovitis, and clusters of chondrocytes.

The effects of NSAIDs in intraarticular usage are most commonly studied. Studies by Fjuii et al. (12) on chondrocyte cultures and studies by David et al. (15) on human osteoarthritic cartilage in vitro showed that different NSAIDs had diverse effects on cartilage metabolism and proteoglycan synthesis. According to a study by Romsin et al. (16), NSAIDs, when injected locally, are able to inhibit proteoglycan synthesis and when injected systemically prevent chondrocyte metabolism.

In a study by Chu et al. (17), fresh bovine chondrocytes were treated with 0.5% bupivacaine or 0.9% saline and then examined by flow cytometry for apoptosis. Apoptosis was found in 20% of chondrocytes exposed to saline, while this rate was over 99% in the chondrocytes exposed to bupivacaine. Furthermore, it was shown that in vitro bupivacaine treatment was toxic to cartilage and chondrocytes and that intraarticular treatment with bupivacaine also had a similar effect on joint cartilage and chondrocytes.

In another study, the histopathological effects of intraarticular lornoxicam injections were examined in the knee joints of 25 rats on days 1, 2, 7, 14, and 21. On day 1, congestion, edema, and low neutrophil infiltration were detected in only 1 of the knees treated with lornoxicam, while no changes were found at other time points. Thus, intraarticular lornoxicam had no significant inflammatory effect on joints.

In a study carried out by Dogan et al. on rabbit knees (19), saline, bupivacaine, or neostigmine were injected intraarticularly and knee joints were histopathologically examined 1, 2, and 10 days after injection. Although findings were more severe with neostigmine treatment, bupivacaine injection also caused more increased inflammation, inflammatory cell infiltration, synovial hyperplasia, and hypertrophy than saline in joint cartilage and the synovium (19).

Gomoll et al. (20) developed an empirical model using 30 rabbits divided into 3 groups treated with saline

as a continuous infusion, bupivacaine as a continuous infusion, or bupivacaine and epinephrine as a continuous infusion into shoulder joints for 48 h. When osteochondral and synovial samples from the glenohumeral joint were examined by measuring metabolic sulfate acquisition after 1 week, sulfate was decreased by 50% and 56%, respectively, in bupivacaine and bupivacaine and epinephrine continuous-infusion groups compared with the saline continuous-infusion group. When cell survival rates were measured, the decrease was 32% and 20%, respectively. Significantly lower inflammation scores were noted during histological analysis in the bupivacaine and epinephrine ($P = 0.004$) and bupivacaine ($P = 0.02$) groups.

In a study by Erden et al., histopathological examination was done on days 1, 7, 14, and 21 after saline or levobupivacaine was injected into the rat knee joint. No inflammatory changes were observed in the saline group while minimal inflammation was observed in 4 rats from the levobupivacaine group (21).

Anakwenze et al. (22) reported 2 cases where young patients had glenohumeral chondrolysis, which developed after treatment with bupivacaine intraarticular pain pumps. Studies on the effects of bupivacaine in the joint have shown that the consequences of intraarticular usage differ depending on the model used. Results from these studies indicate that multiple treatments with intraarticular bupivacaine or continuous infusion of bupivacaine damages the chondrocytes and the synovium.

In this study, we observed that a single dose of bupivacaine or levobupivacaine resulted in similar effects on joint cartilage and the synovium. Usage of these medications is recommended since congestion, chronic inflammation and synovitis, and hyperplasia were at low-to-moderate levels and gradually decreased. However, the fibrosis that develops during recovery indicates that intraarticular injection of bupivacaine or levobupivacaine should be used cautiously. On the other hand, seeing chronic inflammation in only 1 rat that was sacrificed on day 7 is remarkable. There may be different results in studies implemented with greater numbers of rats.

References

1. Denti M, Randelli P, Bigoni M, Vitale G, Marino MR, Fraschini N. Pre- and postoperative intra-articular analgesia for arthroscopic surgery of the knee and arthroscopy-assisted anterior cruciate ligament reconstruction. A double-blind randomized, prospective study. Knee Surg Sports Traumatol Arthrosc 1997; 5: 206–212.
2. Calmet J, Esteve C, Boada S, Gine J. Analgesic effect of intra-articular ketorolac in knee arthroscopy: comparison of morphine and bupivacaine. Knee Surg Sports Traumatol Arthrosc 2004; 12: 552–555.
3. Gristwood RW, Greaves JL. Levobupivacaine: a new safer long acting local anaesthetic agent. Expert Opin Invest Drugs 1999; 8: 861–876.
4. Erdine S. Santral Sinir Blokları. Rejyonel Anestezi. İstanbul, Turkey: Nobel Tıp Kitabevleri; 2005 (book in Turkish).
5. Irwin MG, Cheung KMC, 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: 837–839.
6. Badner NH, Bourne RB, Rorabeck CH, MacDonald SJ, Doyle JA. Intra-articular injection of bupivacaine in knee-replacement operations. Results of use for analgesia and for preemptive blockade. J Bone Surg 1996; 78: 734–738.
7. Moiniche S, Mikkelsen S, Wetterslev J, Dahl JB. A systematic review of intra-articular local anesthesia for postoperative pain relief after arthroscopic knee surgery. Reg Anesth Pain Med 1999; 24: 430–437.
8. Huey-Ping NG, Nordström U, Axelsson K. Efficacy of intra-articular bupivacaine, ropivacaine, or a combination of ropivacaine, morphine and ketorolac on postoperative. Reg Anesth Pain Med 2006; 31: 26–33.
9. Choyce A, Peng P. A systematic review of adjuncts for intravenous regional anesthesia for surgical procedures. Can J Anaesth 2002; 49: 32–45.
10. Rosseland LA, Stubhaug A, Skoglund A, Breivik H. Intra-articular morphine for pain relief after knee arthroscopy. Acta Anaesthesiol Scand 1999; 43: 252–257.
11. Rueben SS, Connelly NR. Postoperative analgesia for outpatient arthroscopic knee surgery with intra-articular bupivacaine and ketorolac. Anesth Analg 1995; 80: 1154–1157.
12. Fjuii K, Tajiri K, Kajiwara T, Tanaka T, Murota K. Effects of NSAIDs on collagen and proteoglycan synthesis of cultured chondrocytes. J Rheumatol 1989; 18: 28–31.
13. Balabanova RM, Fedina TP, Tsurko VV, Mach ES, Khitrov NA, Agapova LA. Dynamic changes in synovitis activity after intra-articular administration of xefocam in patients with rheumatoid arthritis (according to clinical and device examinations). Ter Arkh 2003; 75: 33–35 (article in Russian with abstract in English).
14. Ozyuvaci H, Bilgic B, Ozyuvaci E, Altan A, Altug T, Karaca C. Intra-articular injection of tenoxicam in rats: assessment of the local effects on the articular cartilage and synovium. J Int Med Res 2004; 32: 312–316.
15. David MJ, Vignon E, Peschard MJ, Broquet P, Loisot P, Richard M. Effect of NSAIDs on glycosyltransferase activity from human osteoarthritic cartilage. Br J Rheumatol 1992; 31: 13–17.

16. Romsing J, Moiniche S, Ostergaard D, Dahl JB. Local infiltration with NSAIDs for postoperative analgesia: evidence for a peripheral analgesic action. Acta Anaesthesiol Scand 2000: 44: 672–683.

17. Chu CR, Izzo NJ, Papas NE, Fu FH. In vitro exposure to 0.5% bupivacaine is cytotoxic to bovine articular chondrocytes. Arthroscopy 2006; 22: 693–699.

18. Sarıcaoğlu F, Dal D, Atilla P, İskit AB, Tarhan Ö, Asan E, Aypar U. Effect of intra-articular injection of lornoxicam on the articular cartilage & synovium in rat. Indian J Med Res 2008; 127: 362–365.

19. Dogan N, Erdem AF, Erman Z, Kizilkaya M. The effects of bupivacaine and neostigmine on articular cartilage and synovium in the rabbit knee joint. J Int Med Res 2004; 32: 513–519.

20. Gomoll AH, Kang RW, Williams JM, Bach BR, Cole BJ. Chondrolysis after continuous intra-articular bupivacaine infusion: an experimental model investigating chondrotoxicity in the rabbit shoulder. Arthroscopy 2006; 22: 813–819.

21. Erden IA, Altinel S, Saricaoglu F, Zeybek ND, Akinci SB, Asan E, Aypar U. Effect of intra-articular injection of levobupivacaine on articular cartilage and synovium in rats. Anaesthesist 2012; 61: 420–423.

22. Anakwenze OA, Hosalkar H, Huffman GR. Case reports: Two cases of glenohumeral chondrolysis after intra-articular pain pumps. Clin Orthop Relat Res 2010; 468 : 2545–2549.