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## SCIENTIFIC ARTICLE

# The chondrotoxic and apoptotic effects of levobupivacaine and bupivacaine on the rabbit knee joint<sup>☆</sup>



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### KEYWORDS

Apoptosis;  
Bupivacaine;  
Chondrotoxicity;  
Levobupivacaine;  
Local anesthetic

### Abstract

**Background:** A single dose injection or continuous infusion of local anesthetics into the joint space is considered to be a well-defined analgesia technique. The aim of this study was to investigate the chondrotoxic and apoptotic effects of single-dose intra-articular injection of levobupivacaine and bupivacaine on rabbit knee joint tissues.

**Materials and methods:** The animals were allocated into two groups each containing 20 rabbits. 0.5% levobupivacaine (Group L) and 0.5% bupivacaine (Group B) were applied intra-articularly to the left posterior joints of rabbits. At the same time, normal saline was applied to the right posterior leg knee joints of rabbits in both groups and used as a control (Group S). At the end of the 7th and 28th days after the intra-articular injections, ten randomly chosen rabbits in each group were killed by applying intraperitoneal thiopental. Sections of cartilage tissue samples were stained for light microscopic examinations and the TUNEL method was used to investigate apoptotic cells.

**Results:** As a result of immunofluorescence microscopic examination, the number of apoptotic cells in Group B at day 7 and day 28 were both significantly higher than Group L and S ( $p < 0.05$ ). Also, the number of apoptotic cells in Group L at day 7 and day 28 were both significantly higher than Group S ( $p < 0.05$ ).

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**PALAVRAS-CHAVE**

Apoptose;  
Bupivacaína;  
Condrototoxicidade;  
Levobupivacaína;  
Anestésico local

**Conclusions:** We found that bupivacaine is more chondrotoxic than other anesthetic agent and increases the number of apoptotic cells. These results indicated that bupivacaine caused high chondrotoxic damage and it led to more apoptotic activation than levobupivacaine.

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### Os efeitos condrotóxicos e apoptóticos de levobupivacaína e bupivacaína na articulação do joelho de coelhos

#### Resumo

**Justificativa:** Uma injeção em dose única ou infusão contínua de anestésicos locais no espaço articular é considerada uma técnica de analgesia bem definida. O objetivo deste estudo foi investigar os efeitos condrotóxicos e apoptóticos da injeção intra-articular com dose única de levobupivacaína e bupivacaína em tecidos articulares do joelho de coelho.

**Material e métodos:** Os animais foram alocados em dois grupos, cada um contendo 20 coelhos. Levobupivacaína a 0,5% (Grupo L) e bupivacaína a 0,5% (Grupo B) foram aplicadas intra-articularmente nas articulações posteriores esquerdas de coelhos. Ao mesmo tempo, solução salina normal foi aplicada nas articulações do joelho da perna posterior direita de coelhos em ambos os grupos e usada como controle (Grupo S). Ao final do 7° e 28° dias após as injeções intra-articulares, 10 coelhos escolhidos aleatoriamente em cada grupo foram mortos por aplicação de tiopental intraperitoneal. Seções de amostras de tecido cartilaginoso foram coradas para exames de microscopia de luz, e o método TUNEL foi usado para investigar células apoptóticas.

**Resultados:** Como resultado do exame microscópico de imunofluorescência nos dias 7 e 28, o número de células apoptóticas no Grupo B foi significativamente maior que nos grupos L e S ( $p < 0,05$ ). Além disso, o número de células apoptóticas nos dias 7 e 28 foi significativamente maior no Grupo L que no Grupo S ( $p < 0,05$ ).

**Conclusões:** Demonstramos que a bupivacaína é mais condrotóxica que o outro agente anestésico e aumenta o número de células apoptóticas. Esses resultados indicaram que a bupivacaína causou intensa lesão condrotóxica e levou a uma ativação apoptótica maior que a levobupivacaína.

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## Introduction

Postoperative pain is an extremely important problem for patients. One of the safe and effective methods to control postoperative pain is treatment with regional local anesthetic agents. In recent years, together with the development of regional anesthesia methods, the use of local anesthetics for postoperative analgesia is becoming increasingly widespread. Nowadays, administration of single-dose injection or continuous infusion of local anesthetic into the joint space is considered to be a well-defined analgesia technique for perioperative analgesia, especially in shoulder and knee arthroscopy.<sup>1</sup>

However, in recent years, we have encountered a growing number of researches and case reports about long-term exposure to local anesthetics alone or in combination with other drugs, which causes damage to chondrocytes. This situation causes the appearance of concerns about these techniques.<sup>2</sup>

Due to the effect of high potency and long action, bupivacaine is the preferred agent in clinical use. Also,

it is known that bupivacaine is a local anesthetic which exhibits high potential for cardiotoxic, myotoxic and neurotoxic effects.<sup>3-5</sup> Levobupivacaine is an enantiomer of pure bupivacaine S (–). The cardiotoxic effects of racemic bupivacaine were shown to be related to more of the R (+) enantiomer; as a result, levobupivacaine was developed in order to obtain a less cardiotoxic agent.<sup>6</sup>

Although there are some studies about the chondrotoxic effects of bupivacaine and levobupivacaine in the literature,<sup>7-9</sup> there is no sufficient number of study comparing the potential apoptotic activities of levobupivacaine and bupivacaine in chondrocytes. In our study, we aim to investigate and compare the potential apoptotic effects of bupivacaine and levobupivacaine on chondrocytes as an indicator of chondrotoxicity.

## Materials and method

This study was performed at the Experimental and Clinical Research Centre (DEKAM) and Pathology Laboratories

of Erciyes University. All applications were realised under veterinarian control in accordance with the Universal Declaration of International Animal Rights, after receiving approval from the Erciyes University Experimental Animal Ethics Committee (date 08.02.2012 n° 12/30).

### Experimental design

Forty New Zealand female rabbits ( $2.0 \pm 0.2$  kg) were randomly divided into two groups, each containing 20 rabbits. After 6 h of fasting, intramuscular injection of anesthetic agent ketamine hydrochloride ( $10 \text{ mg} \cdot \text{kg}^{-1}$ ) was applied and then intra-articular injection was performed by using a 38G insulin syringe. The first group (Group L), (Chirocaine® 0.5%; Nycomed Pharma AS, Elverum, Norway) was injected with 0.5% concentration and 0.25 mL volume of levobupivacaine into the left posterior knee joints. The second group (Group B), (Marcaine Spinal Heavy 0.5%; AstraZeneca, Istanbul, Turkey) was injected with the same concentration and volume of bupivacaine to the left posterior knee joints. In the same way Normal saline (0.25 mL, 0.9% concentration) was injected into the right posterior knee joints of rabbits in each group and used as a control (Group S). After the injections, rabbits of each group were placed in cages and housed in the same room with the same experimental conditions of 12 h/12 h light/dark cycle, temperature and diet to achieve optimum living conditions. At the end of the 7th and 28th days after the intra-articular injections, ten randomly chosen rabbits in each group were sacrificed by applying intra-peritoneal thiopental ( $150 \text{ mg} \cdot \text{kg}^{-1}$ ). Knee joints were taken out under aseptic conditions without damaging the joint capsule. Then, a 1 cm length piece of tissue was taken from the articular cartilage of the joint using a scalpel. For light and immunofluorescence microscopic examinations, cartilage tissue samples were fixed in 10% neutral formalin solution for 48 h. After fixation, tissues were kept in molten paraffin at  $60^\circ\text{C}$  overnight. Sections ( $5 \mu\text{m}$  thickness) were taken from paraffin-embedded cartilage tissue pieces. For light microscopic examination, sections were stained with Haematoxylin and Eosin (H&E). Besides that, sections were stained using a Millipore TUNEL Apoptosis Detection Kit to determine apoptotic activation and were examined by using an immunofluorescence microscope.

### Light microscopic examinations

After fixation in 10% neutral formalin for 48 h, tissue samples were washed under tap water and then dehydrated with an alcohol series (respectively 70%, 80%, 90%, 96%, and 100%). Then, tissues were kept in xylene for 1 h in three separate containers at the same concentration. Liquid paraffin at  $60^\circ\text{C}$  was added to a mould of tissue pieces and then allowed to solidify at room temperature overnight. Sections ( $5 \mu\text{m}$  thickness) of paraffin-embedded tissue were obtained by using a microtome; tissue sections were kept in xylene twice each for 20 min, and then hydrated with 90%, 80% and 70% alcohol concentrations for 10 min, respectively. Then, sections were kept in distilled water for 5 min and stained with haematoxylin for 8 min. Afterwards, sections were washed in acid-alcohol solutions and stained

with eosin for 4 min. Tissue sections were dehydrated with 70%, 80% and 90% alcohol concentrations and kept in xylene for 10 min, respectively. Tissue sections were covered with a coverslip after dripping a drop of Entellan onto the tissue and examined under a light microscope (Olympus BX51, Japan).

### Immunofluorescence microscopic examinations

Five micron thickness sections were placed on Polysine slides (Thermo Scientific) and kept in an incubator at  $60^\circ\text{C}$  for 1 h and then submerged in xylene twice for 10 min. After the sections were immersed in 99%, 96%, 70% and 50% ethanol for 5 min, respectively, they were washed in PBS for 5 min. Proteinase-K was dripped onto sections and, after waiting 15 min, sections were washed in distilled water twice for 2 min. Afterwards, sections were put in 3%  $\text{H}_2\text{O}_2$  and then washed in distilled water twice for 5 min to remove endogenous peroxidase. Sections were treated with the Equilibration Buffer, TdT Enzyme Stop/Wash Buffer and Anti-Digoxigenin Conjugate, respectively. After contrast staining, sections were covered with a covering medium.

Nuclear condensation and fragmentation were considered as characteristics of apoptosis in H&E-stained sections under light microscopic examination. Apoptotic nuclei, which were marked as positive by the TUNEL method at  $\times 40$  magnification in a fluorescent microscope, were counted in 10 randomly-selected different areas by a blinded pathologist. The number of apoptotic cells was determined in the displayed fields.<sup>10</sup>

### Statistical analysis

The average, standard deviation, the median 1Q–3Q, frequency and ratio values of the data were used as descriptive statistics. The distribution of versions was controlled by the Shapiro–Wilk test. The Kruskal–Wallis test was used for the analysis of quantitative data and the Mann–Whitney *U*-test was used for subanalysis. For repeated measures, the Wilcoxon test was used. All analyses were performed using SPSS 22.0 and the value of  $p < 0.05$  was accepted as statistically significant.

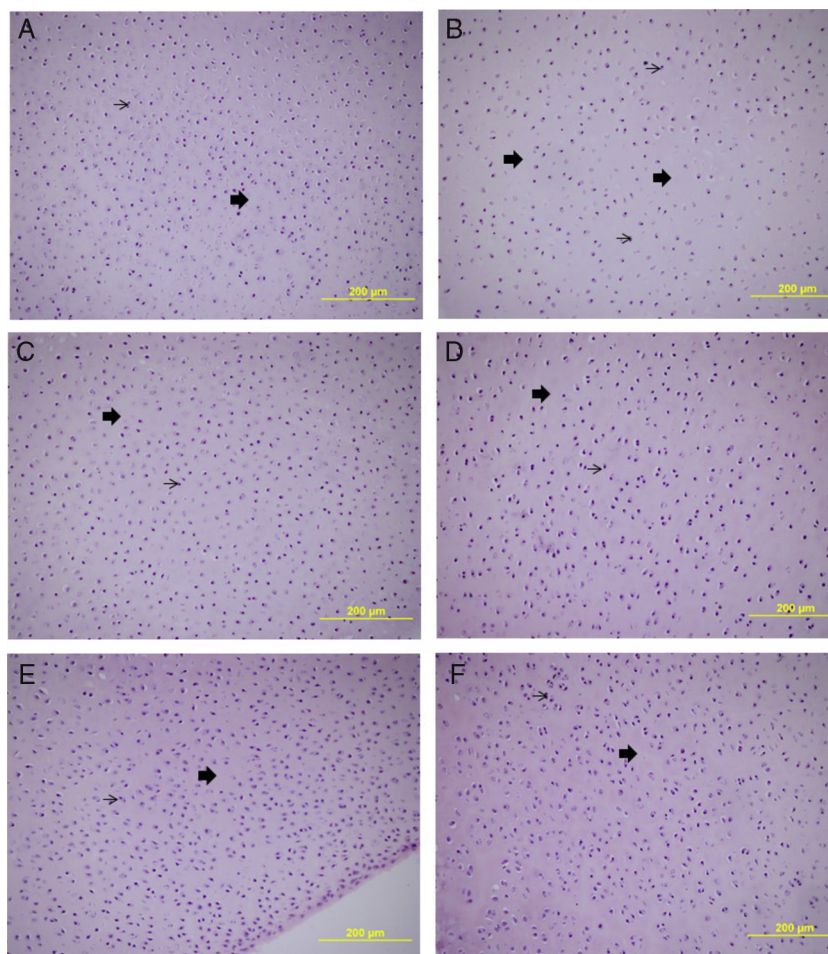
## Results

### Light microscopic findings

No inflammatory cell infiltration was observed in the chondrocytes and their interterritorial matrix (Fig. 1A–F).

### Immunofluorescence microscopic findings

As a result of immunofluorescent microscopic examination, apoptotic chondrocytes, which have an average and standard deviation per unit of  $1.9 \pm 1.6$  and  $2.0 \pm 1.7$ , respectively, were observed in the cartilage tissue sections of the 0.9% normal saline group (Group S) after normal saline injection on day 7 and day 28 (Table 1) (Fig. 2A and B). Apoptotic chondrocytes, which have an average and standard deviation per unit of  $5.0 \pm 3.1$  and  $5.2 \pm 3.5$ , respectively, were observed in cartilage tissue sections of



**Figure 1** Light microscopic view of cartilage tissue samples taken 7 days (A) and 28 days (B) after 0.9% normal saline injection. Light microscopic view of cartilage tissue samples taken 7 days (C) and 28 days (D) after 0.5% bupivacaine injection. Light microscopic view of cartilage tissue samples taken 7 days (E) and 28 days (F) after 0.5% levobupivacaine injection (H&E,  $\times 200$ ). Arrow: Chondrocytes, Thick arrow: Interterritorial matrix.

**Table 1** The rate of changes in the apoptotic cell numbers according to the groups in fluorescence microscopy.

		Comparison of fluorescence microscopic results			
		Group B	Group L	Group S	<i>p</i>
Day 7	Mean $\pm$ SD	5.0 $\pm$ 3.1	2.7 $\pm$ 1.9 <sup>a</sup>	1.9 $\pm$ 1.6 <sup>a</sup>	0.000 <sup>b</sup>
	Median (1Q–3Q)	5 (3–7)	3 (1–4)	2 (1–3)	
Day 28	Mean $\pm$ SD	5.2 $\pm$ 3.5	3.3 $\pm$ 2.3 <sup>a</sup>	2.0 $\pm$ 1.7 <sup>a</sup>	0.000 <sup>b</sup>
	Median (1Q–3Q)	5 (3–7)	3 (1–5)	2 (1–3)	
Difference	Mean $\pm$ SD	0.2 $\pm$ 4.7	0.6 $\pm$ 2.9	0.1 $\pm$ 2.5	0.499 <sup>b</sup>
	Median (1Q–3Q)	0 (–3 to 4)	2 (1–3)	0 (–1 to 3)	
<i>p</i>		0.769 <sup>c</sup>	0.051 <sup>c</sup>	0.476 <sup>c</sup>	

<sup>a</sup> Difference with Group B/Difference with Group L.

<sup>b</sup> Kruskal–Wallis (Mann–Whitney *U*-test).

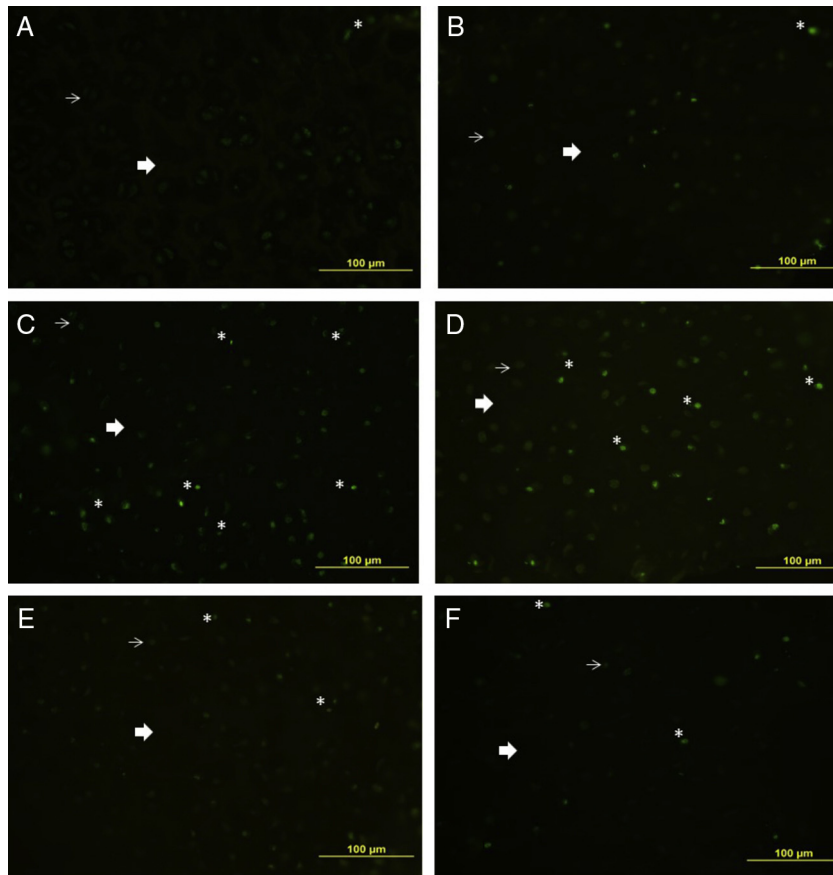
<sup>c</sup> Wilcoxon test.

the bupivacaine group (Group B) after bupivacaine injection on day 7 and day 28 (Table 1) (Fig. 2C and D). Apoptotic chondrocytes, which have an average and standard deviation per unit of 2.7  $\pm$  1.9 and 3.3  $\pm$  2.3, respectively, were found in the cartilage tissue sections of the levobupivacaine

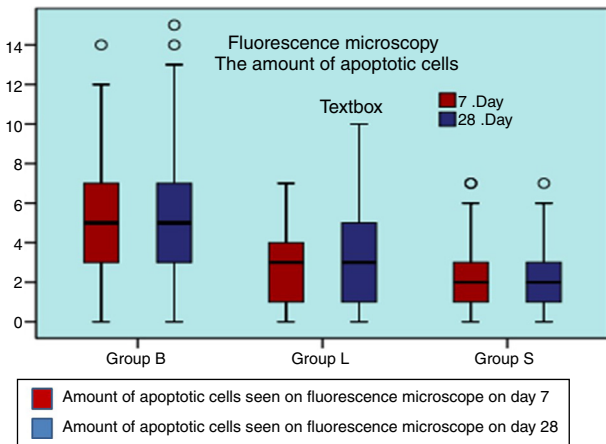
group (Group L), after levobupivacaine injection on day 7 and day 28 (Table 1) (Fig. 2E and F).

The number of apoptotic cells in Group B at day 7 and day 28 were both significantly higher than Group L and S ( $p < 0.05$ ) (Table 1) (Fig. 3). Also, the number of apoptotic





**Figure 2** Immunofluorescence microscopic views of cartilage tissue samples taken 7 days (A) and 28 days (B) after 0.9% normal saline injection. Immunofluorescence microscopic view of cartilage tissue samples taken 7 days (C) and 28 days (D) after 0.5% bupivacaine injection. Immunofluorescence microscopic view of cartilage tissue samples taken 7 days (E) and 28 days (F) after 0.5% levobupivacaine injection (TUNEL  $\times$  40, bar = 100  $\mu$ m). Arrow, Chondrocytes; Thick arrow, Interterritorial matrix; Star, Apoptotic cells.



**Figure 3** Graphic of changes in the apoptotic cell numbers according to fluorescence microscopic examination.

cells in Group L at day 7 and day 28 were both significantly higher than Group S ( $p < 0.05$ ) (Table 1) (Fig. 3). The number of apoptotic cells in Group B, L and S at days 7 and 28 showed no differences ( $p > 0.05$ ) (Table 1).

### Discussion

A single-dose injection or continuous infusion of local anesthetic into the joint space is considered to be a well-defined analgesia technique, especially in shoulder and knee arthroscopy, in order to provide perioperative analgesia.<sup>1</sup> Although bupivacaine is the preferred agent in clinical use due to its high potency and long-acting capacity, it is a local anesthetic known to have the highest potential effect of cardiotoxicity, neurotoxicity and myotoxicity.<sup>4,5</sup>

It has been reported in recent studies that the chondrotoxic effect of bupivacaine is related to dose and duration of application and is more effective when used with epinephrine.<sup>11-13</sup> Levobupivacaine is a new local anesthetic which is an enantiomer of pure bupivacaine S (-). After showing that the cardiotoxic effect of racemic bupivacaine was more related to R (+) isomers, levobupivacaine was developed in order to achieve a less cardiotoxic agent.<sup>4,6</sup>

In our study, we compared to chondrotoxic and apoptotic effects of levobupivacaine and bupivacaine, which are widely used in arthroscopic surgery and known as chondrotoxic agents. Adverse cardiac effects of bupivacaine depend on interaction between Ca<sup>++</sup> channels, flow of intracellular Ca<sup>++</sup> and ATP synthesis in mitochondria. Due to the

moderative effect on intracellular Ca<sup>++</sup> balance, levobupivacaine was defended as a less cardiotoxic and myotoxic agent.<sup>4,14</sup>

At the same time, impairment in intracellular Ca<sup>++</sup> balance has been known to have a role in activation of apoptotic mechanisms.<sup>4</sup> Based on these results, mitochondrial dysfunction and activation of apoptosis are held responsible for chondrotoxicity, resulting in chondrolysis and necrosis.<sup>15</sup> Unlike necrosis, apoptosis, which is initiated and regulated by the cell itself, is the most common form of physiological cell death. The apoptotic process is initiated via stress, heat, hypoxia, ionised radiation and extracellular chemicals, and causes cell death by the activation of an intracellular signal cascade.<sup>7</sup>

The role of intracellular Ca<sup>++</sup> balance became prominent in the identification of the cardiotoxic and mycotoxic properties of levobupivacaine. At the same time, this factor has been known to have a role in the mechanism of apoptotic activation. So we approve to evaluate the apoptotic activation and number of apoptotic cells caused by these two local anesthetic agents in.<sup>5,16</sup> Many studies that examined the effects of local anesthetics on apoptotic activity can be found in the literature.<sup>15,17</sup>

Local anesthetics have been shown to cause apoptosis in neural tissues and skeletal muscle tissues as well as in renal cells, chondrocytes and corneal endothelial cells in these studies.<sup>7,8,17</sup>

Bupivacaine is held most responsible for apoptosis which is induced by local anesthetics. Bupivacaine triggers apoptosis by increasing intracellular Ca<sup>++</sup> levels and decimating the oxidative phosphorylation step in the mitochondria under in vivo conditions. Many researchers think that bupivacaine causes apoptotic activation due to its lipophilic nature.<sup>5,7,15,16</sup>

Park et al.<sup>6</sup> reported that bupivacaine induces apoptosis by increasing the production of Reactive Oxygen Species (ROS) and caspase-3 activation and reducing Poly-ADP-Ribose Polymerase (PARP) in Schwann cells. The only research in the literature which investigated apoptotic activation caused by levobupivacaine was done by Borazan et al.<sup>7</sup> They applied lidocaine, ropivacaine and levobupivacaine to rabbits by intracameral injection and examined the number of TUNEL-positive apoptotic cells at days 1 and 7. These researchers reported that all three agents triggered the apoptotic process in the anterior chamber and caused morphological changes in the corneal endothelial cells, but thought that this effect was temporary and completely terminated after 7 days. Nauette-Gaulain et al.<sup>14</sup> reported that levobupivacaine, which has the same lipophilic properties as bupivacaine, activated RyR and the Ca-ATPase pump in the sarcoplasmic reticulum to increase the amount of intracellular Ca<sup>++</sup>. These researchers reported that, although bupivacaine has the same lipophilic character as levobupivacaine, it did not show the same effect. They explained this as due to the stereospecific properties of these two agents. In addition to this, it is not clear that the factors that determine the effect of local anesthetics on intracellular Ca<sup>++</sup> are linked to their lipophilic or stereoselective properties.

Rabbit knee joints are described as a good model in scientific studies related to joint structures, so we used both right and left posterior knee joints of rabbits in our study, like other similar studies. The volume and concentration

of both local anesthetics and saline were adapted from intra-articular bupivacaine doses used in humans (0.25 mL, 0.5%).<sup>18</sup> Monoiodoacetate-injected samples of joint surfaces were observed as stable for 12 weeks in macroscopic examination. Lysis of superficial chondrocytes was determined in one week and lysis of chondrocytes in all layers was identified in four weeks in fluorescence and light microscopic examination. The optimal detection time of chondrocyte apoptosis after injury was days 7–10.<sup>19,20</sup> Dogan et al.<sup>18</sup> reported that histologic changes in chondrocytes were minimal in the first 24 h, but then these changes significantly increased by 48 h and the 10th day after injection. According to this information, we decided to take cartilage tissues in the first and fourth weeks after injection in terms of TUNEL positive apoptotic cell number.

To determine apoptosis in chondrocytes, conventional light microscopy might be suggested because it is the best way to define the typical histological changes of apoptosis, while fluorescence microscopy is a highly specific and sensitive method for apoptosis identification, and TUNEL staining increases the sensitivity of fluorescence microscopy by making pre-apoptotic cells visible.<sup>21,22</sup> Although Transmission Electron Microscopy (TEM) is sensitive for detecting the cellular changes in apoptosis, it is an expensive process which has achievement limitations.<sup>23</sup> Apoptotic changes in cartilage tissue were evaluated by using H&E staining in light microscopy; on the other hand, the TUNEL staining technique, which allowed us to assess the evaluation of pre-apoptotic cells, was used for fluorescence microscopic examination. Inflammatory effects of these agents on cartilage tissue were not evaluated. Webb et al.<sup>24</sup> investigated studies related to intra-articular chondrotoxic effects of bupivacaine, and reported that continuous postoperative intra-articular analgesia of bupivacaine was applied in 25 of 27 post-arthroscopic glenohumeral chondrolysis cases.

Chu et al.<sup>13</sup> studied the in vitro effects of bupivacaine on cartilage tissue both in cell culture and osteochondral explants. Gomoll et al.<sup>11</sup> investigated the effects of bupivacaine on cartilage tissue by applying an infusion of bupivacaine for 48 h via a catheter inserted into the glenohumeral joints of a rabbit shoulder model. These researchers demonstrated the presence of chondrotoxicity which was characterised by chondrocyte death and a decrease in chondrocyte metabolism in all three studies. Dragoo et al.<sup>12</sup> examined the effects of bupivacaine and lidocaine containing epinephrine on chondrocyte viability, applied via pain pump to human articular cartilage samples of total knee replacement surgeries. These researchers determined a significant decrease of chondrocyte viability in cultures which were exposed to local anesthetics combined with epinephrine. Also, the same study showed that bupivacaine had minimal effect on the viability of chondrocytes in the first 48 h and caused significant cell death in 72 h when combined with epinephrine.

In another in vivo study, temporary suppression of proteoglycan synthesis in porcine articular cartilage was reported after the application of 0.5% bupivacaine for 3 days.<sup>25</sup> No permanent changes were reported in the cartilage function in another in vivo study which investigated long-term results of chondrotoxicity for 3 months after

bupivacaine fusion in the rabbit shoulder model. However, an 'increase in anabolic activity', which was characterised by increased sulphate intake and high proteoglycan content in the cartilage exposed to bupivacaine, was determined in the same study. These contradictory results suggested that the function of cartilage was disrupted temporarily after bupivacaine exposure in experimental models. One week after bupivacaine exposure, cartilage metabolism was reduced, but 3 months after bupivacaine exposure, cartilage metabolism was improved and anabolic activity was increased. This increase in anabolic activity and proteoglycan synthesis in the late period can be evaluated as a reparative response of cartilage tissue against harmful stimuli.<sup>26</sup> Dogan et al.<sup>18</sup> investigated the effects of bupivacaine and neostigmine in articular cartilage tissue and synovium tissue of the rabbit knee joint in their study. Bupivacaine and neostigmine were shown to cause inflammation in cartilage tissue and synovial membrane and also led to structural changes in chondrocytes in animal model studies which used the rabbit posterior leg knee joint. In this study, it was observed that bupivacaine caused minimal inflammation in 24 h and 48 h but caused moderate inflammation in the articular cartilage tissue on day 10. Gomoll et al.<sup>11</sup> reported that intra-articular concentration of bupivacaine decreased after a single-dose injection, because of its rapid absorption and contribution to the systemic circulation. So, they hypothesised that continuous infusion of bupivacaine may cause death of more chondrocytes.

In the study which is about the effects of bupivacaine, levobupivacaine and tramadol on articular cartilage texture in rat knee joint, Beyzadeoğlu et al.<sup>9</sup> reported that the chondrotoxicity of bupivacaine was less harmful than levobupivacaine and tramadol, in addition they suggested that all three local anesthetics negatively affect articular cartilage and chondrocytes. In a study conducted by Gomoll et al.,<sup>11</sup> continuous infusion of bupivacaine was reported to be more chondrotoxic than single-dose bupivacaine, Although Beyzadeoğlu et al.<sup>9</sup> suggested that levobupivacaine is more chondrotoxic than bupivacaine in their studies.

Unlike these researchers, as in the study of Doğan et al.<sup>18</sup> investigating the effects of single doses of bupivacaine and neostigmine on inflammatory changes in rabbit knee joint cartilage, we applied the TUNEL assay method<sup>7,22</sup> which is frequently preferred in studies to show apoptotic cells in our study when we used a single dose intra-articular injection technique.

Also, we used a single-dose intra-articular injection technique in our study and observed more apoptotic activation than the saline group on both days 7 and 28. On the other hand, we also observed that levobupivacaine had lower apoptotic activation than bupivacaine. The low apoptotic activation of levobupivacaine showed that levobupivacaine was less chondrotoxic than bupivacaine. The number of apoptotic cells in either local anesthetic agents at day 7 and day 28 was observed to be significantly higher than Group SF under fluorescent microscopic examination.

Moreover, apoptotic activation of bupivacaine on cartilage tissue on day 7 was seen at the same level as apoptotic activation on day 28. On the other hand, apoptotic activation caused by levobupivacaine was at the minimum level on day 7. Apoptotic activation of levobupivacaine increased on day 28, even though it was less than the

apoptotic activation of bupivacaine on day 28. These results showed that the effects of apoptotic activation caused by levobupivacaine were lower than the effects of apoptotic activation caused by bupivacaine, and it started more slowly.

We did not observe any differences when we compared the number of apoptotic cells in the between levobupivacaine group and Group SF on day 7 and day 28 under fluorescence microscopic examination. Fragmented nuclei were marked with the TUNEL staining method to be visible specifically under fluorescence microscopy. Therefore, we believe that the differences between the light and fluorescent microscopic results derived from methodological differences in both investigations.

In conclusion, in this study, we determined that levobupivacaine causes less and slower apoptotic activation than bupivacaine. Although our study needs to be supported with further research, we suggest that levobupivacaine, which has a less chondrotoxic effect, could be preferred for intra-articular local anesthetic injection for postoperative analgesia in patients undergoing arthroscopic surgery.

## Funding

All applications were realised under the veterinarian control in accordance with the Universal Declaration of International Animal Rights, after receiving approval of Erciyes University Experimental Animal Ethics Committee (Date 10.02.2010 No. 10/22).

## Conflicts of interest

The authors declare no conflicts of interest.

## References

1. Zeidan A, Kassem R, Nahleh N, et al. Intraarticular tramadol bupivacaine combination prolongs the duration of postoperative analgesia after outpatient arthroscopic knee surgery. *Anesth Analg.* 2008;107:292–9.
2. Fester EW, Noyes FR. Postoperative chondrolysis of the knee: 3 case reports and a review of the literature. *Am J Sports Med.* 2009;37:1848–54.
3. Chu CR, Izzo NJ, Coyle CH, et al. The in vitro effects of bupivacaine on articular chondrocytes. *J Bone Jt Surg Br.* 2008;90:814–20.
4. Casati A, Putzu M. Bupivacaine, levobupivacaine and ropivacaine: are they clinically different? *Best Pract Res Clin Anaesthesiol.* 2005;19:247–68.
5. Zink W, Seif C, Bohl JRE, et al. The acute myotoxic effects of bupivacaine and ropivacaine after continuous peripheral nerve blockades. *Anesth Analg.* 2003;97:1173–9.
6. Park CJ, Park SA, Yoon TG, et al. Bupivacaine induces apoptosis via ROS in the Schwann cell line. *J Dent Res.* 2005;84:852–7.
7. Borazan M, Karalezli A, Oto S, et al. Induction of apoptosis of rabbit corneal endothelial cells by preservative-free lidocaine hydrochloride 2%, ropivacaine 1%, or levobupivacaine 0.75%. *J Cataract Refract Surg.* 2009;35:753–8.
8. Grishko V, Xu M, Wilson G, et al. Apoptosis and mitochondrial dysfunction in human chondrocytes following exposure to lidocaine, bupivacaine, and ropivacaine. *J Bone Joint Surg Am.* 2010;92:609–18.

9. Beyzadeoğlu T, Torun Köse G, Ekinci ID, et al. Cytotoxicity of local anesthetics to rats' articular cartilage: an experimental study. *Acta Orthop Traumatol Turc.* 2012;46:201-7.
10. Natori S, Selzner M, Valentino KL, et al. Apoptosis of sinusoidal endothelial cells occurs during liver preservation injury by a caspase-dependent mechanism. *Transplantation.* 1999;68:89-96.
11. Gomoll AH, Kang RW, Williams JM, et al. Chondrolysis after continuous intra-articular bupivacaine infusion: an experimental model investigating chondrotoxicity in the rabbit shoulder. *Arthroscopy.* 2006;22:813-9.
12. Dragoo JL, Korotkova T, Kanwar R, et al. The effect of local anesthetic administered via pain pump on chondrocyte viability. *Am J Sports Med.* 2008;36:1484-8.
13. Chu CR, Izzo NJ, Papas NE, et al. In vitro exposure to 0.5% bupivacaine is cytotoxic to bovine articular chondrocytes. *Arthroscopy.* 2006;22:693-9.
14. Nouette-Gaulain K, Sirvent P, Canal-Raffin M, et al. Effects of intermittent femoral nerve injections of bupivacaine, levobupivacaine and ropivacaine on mitochondrial energy metabolism and intracellular calcium homeostasis in rat psoas muscle. *Anesthesiology.* 2007;106:1026-34.
15. Boselli E, Duflo F, Debon R, et al. The induction of apoptosis by local anesthetics: a comparison between lidocaine and ropivacaine. *Anesth Analg.* 2003;96:755-6.
16. Unami A, Shinohara Y, Ichikawa T, et al. Biochemical and microarray analyses of bupivacaine induced apoptosis. *J Toxicol Sci.* 2003;28:77-94.
17. Lee HT, Xu H, Siegel CD, et al. Local anesthetics induce human renal cell apoptosis. *Am J Nephrol.* 2003;23:129-39.
18. Doğan N, Erdem AF, Erman Z, et al. The effects of bupivacaine and neostigmine on articular cartilage and synovium in the rabbit knee joint. *J Int Med Res.* 2004;32:513-9.
19. Kuwata K, Sato S, Era S, et al. Cross relaxation times of normal and biochemically induced osteoarthritic rabbit knee cartilages. *Jpn J Physiol.* 1997;47:291-7.
20. Kockx MM, Muhring J, Knaapen MW, et al. RNA synthesis and splicing interferes with DNA in situ end labeling techniques used to detect apoptosis. *Am J Pathol.* 1998;152:885-8.
21. Mountz JD, Zhou T. Apoptosis and autoimmunity. In: Koopman WJ, editor. *A textbook of rheumatology: arthritis and allied conditions.* Philadelphia: Lippincott Williams & Wilkins; 2001.
22. Amaniti E, Drampa F, Kouzi-Koliakos K, et al. Ropivacaine myotoxicity after single intramuscular injection in rats. *Eur J Anesth.* 2006;23:130-5.
23. Stahelin BJ, Marti U, Solioz M, et al. False positive staining in the TUNEL assay to detect apoptosis in liver and intestine is caused by endogenous nucleases and inhibited by diethylpyrocarbonate. *Mol Pathol.* 1998;51:204-8.
24. Webb ST, Ghosh S. Intraarticular bupivacaine: potentially chondrotoxic? *Br J Anaesth.* 2009;102:439-41.
25. Fulkerson JP, Damiano P. Effect of prostaglandin E2 on adult pig articular cartilage slices in culture. *Clin Orthop Relat Res.* 1983;179:266-9.
26. Gomoll AH, Yanke AB, Kang RW, et al. Long-term effects of bupivacaine on cartilage in a rabbit shoulder model. *Am J Sports Med.* 2009;37:72-7.