https://doi.org/10.23934/2223-9022-2019-8-3-288-294

Double-Blind, Placebo-Controlled Study of Myotoxicity and Neurotoxicity of Bupivacaine in Rats

R.E. Lakhin*, I.A. Gemua, V.V. Shustrov

Department of Anesthesiology and Intensive Care
Federal State Budgetary Military Educational Institution of Higher Education S.M. Kirov Military Medical Academy of the Ministry of Defense of Russian Federation
4 Klinicheskaya St., St. Petersburg 194044, Russian Federation

*** Contacts:** Roman Y. Lakhin, Dr. Med. Sci., Docent, Professor of the Department of Anesthesiology and Intensive Care, S.M. Kirov Military Medical Academy of the Ministry of Defense of the Russian Federation. Email: doctor-lahin@yandex.ru

ABSTRACT When penetrating into the cell, local anesthetics affect some structures and processes, in addition to blocking sodium channels, leading to the development of cell damage. The aim of the article was to study the damaging effect of bupivacaine on the sciatic nerve and biceps femoris in rats.

The study is double-blind and placebo-controlled. We used 0.9% sodium chloride as the placebo. The studied concentrations of bupivacaine were 0.2%, 0.5%, 0.75%, and 1%. We performed perineural introduction of 0.2 ml into the sciatic nerve and administered 0.2 ml into the biceps femoris muscle under the ultrasound guidance. The samples were taken twice: 1 hour after administration, and over 14 days. Cell necrosis or apoptosis were not found in the muscle and nerve after the 0.9% sodium chloride administration; occasional inflammatory cells were detected. Introduction of all concentrations of bupivacaine induced damage and inflammatory infiltration of muscle tissue and neural structures compared with 0.9% sodium chloride solution. Dystrophic changes and neutrophilic infiltration were detected in nerve fibers. Perimuscular edema, apoptosis, polychromasia, necrosis, disappearance of cross-striation of muscles, clusters of inflammatory cells were found in the biceps femoris. Signs of damage and inflammatory infiltration decreased, but continued to persist over 14 days.

The study showed the presence of neurotoxicity and myotoxicity of all concentrations of bupivacaine compared to a 0.9% sodium chloride solution. It was revealed that signs of damage and inflammatory infiltration persisted 14 days after the administration of bupivacaine.

Keywords: albino rats, bupivacaine, local anesthetic, myotoxicity, neurotoxicity, skeletal muscle, sciatic nerve

For citation Lakhin RE, Gemua IA, Shustrov VV. Double-Blind, Placebo-Controlled Study of Myotoxicity and Neurotoxicity of Bupivacaine in Rats. *Russian Sklifosovsky Journal of Emergency Medical Care.* 2019;8(3):288–294. https://doi.org/10.23934/2223-9022-2019-8-3-288-294 (in Russ.)

Conflict of interest Authors declare lack of the conflicts of interests

Acknowledgments The study had no sponsorship

Affiliations

Roman Y. Lakhin	Dr. Med. Sci., Docent, Professor of the Department of Anesthesiology and Intensive Care, S.M. Kirov Military Medical Academy of the Ministry of Defense of the Russian Federation, https://orcid.org/0000-0001-6819-9691
Inal A. Gemua	Postgraduate of the Department of Anesthesiology and Intensive Care, S.M. Kirov Military Medical Academy of the Ministry of Defense of the Russian Federation
Vyacheslav V. Shustrov	Postgraduate of the Department of Anesthesiology and Intensive Care, S.M. Kirov Military Medical Academy of the Ministry of Defense of the Russian Federation, https://orcid.org/0000-0001-5144-3360

INTRODUCTION

The problem of local toxicity of topical anesthetics has been studied for many years. Since local anesthetics are not highly selective blockers of voltage-gated sodium channels, they can also block calcium [1] and potassium channels [2]. In addition, local anesthetics affect various signaling pathways within the cell, activating the mitochondrial and nuclear pathways of cell apoptosis [3, 4]. All this leads to disruption of cell activity, contributing to the development of tissue damage. Since the peripheral nerve is the target structure of regional anesthesia, the most striking manifestation of local toxicity is nerve damage with the development of neurological deficit [3, 5]. Neurotoxicity has been identified for various local anesthetics [4, 6, 7]. Peripheral nerves pass in muscle tissues and intermuscular spaces, the introduction of a local anesthetic can lead to myotoxicity [7, 8]. In addition to the processes described above, damage to muscle cells is associated with calcium-dependent inhibition of contractility and modulation of ryanodine receptors of sarcoplasmic reticulum of muscle cells [9]. In clinical practice, it has been shown that damage to muscle tissue can lead to the development of myopathies [10]. However, there is currently no unequivocal opinion on the myotoxicity of local anesthetics. There are works that demonstrate insignificance in the clinical practice of myotoxicity of local anesthetics [11], but there is also clinical evidence of a serious deterioration in the quality of life of patients [12, 13].

Despite the interest of researchers in the problem of local toxicity of local anesthetics, the mechanisms of damage development are not yet fully understood. There is no answer to the question of the restoration of cells damaged by local anesthetics.

Objective: to study the damaging effect of bupivacaine on the sciatic nerve and biceps of the thigh of rats.

MATERIAL AND RESEARCH METHODS

The experimental study was approved by an independent Ethics Committee on the basis of S.M. Kirov Military Medical Academy (protocol No. 203 dated 03/20/2018). The work was carried out in compliance with the rules for the use and keeping of laboratory animals in accordance with Order No. 755 M3 of the USSR dated 07/12/1977 and recommendations of the Helsinki Declaration. A series of experiments was carried out on 25 outbred adult albino female rats weighing 160-200 g.

RANDOMIZATION AND BLINDING

Simple tabular randomization of laboratory animals into nine groups by generating random numbers was performed using the *Research Randomizer (https://www.randomizer.org)*. Rats were blinded in the study by an external specialist who prepared and encoded syringes with anesthetics for administration to laboratory animals following the rules of aseptic and antiseptic according to the random number table. After preparation of drugs, the distribution table was sealed in an envelope. The researcher introduced an encoded drug unknown to him, removed animals from the experiment, and collected material. The encoded material was transferred to a laboratory where a pathomorphologist performed a histological examination. The pathomorphologist did not have information on drugs administered to laboratory animals. After receiving all the results, the envelope with the table of distribution of laboratory animals into groups was opened and the information obtained was disclosed.

A 0.9% sodium chloride solution was used as a placebo. The distribution of the groups depending on the drug administered is presented in Table 1.

T a b l e 1

The distribution of experimental animals in groups

Group number	n	Concentration	Drug
1 group (placebo)	10	0.9%	Sodium chloride
2 group	10	0.2%	Bupivacaine
3 group	10	0.5%	Bupivacaine
4 group	10	0.75%	Bupivacaine
5 group	10	one%	Bupivacaine

Sciatic nerve blockade was performed on all laboratory animals — 0.2 ml of the drug was injected paranevally. The accuracy of the approach was monitored using ultrasonic navigation using the *Sono Site Edge* linear sensor *L* 25 *X* with a frequency of 6–13 MHz. After paraneural administration, 0.2 ml of the drug were injected into the biceps of the thigh under the ultrasound guidance.

To assess the effect of bupivacaine on skeletal muscle and peripheral nerve, 5 animals of each group were withdrawn from the experiment 1 hour after drug administration, and to assess tissue repair after 14 days. An overdose of sodium thiopental was used for excretion. After excretion, a section of the sciatic nerve and biceps of the thigh was taken, which was fixed in 10% formalin solution for 48 hours. Next, the nerve and muscle sections were dehydrated and poured into paraffin blocks. Tissue sections 5–6 μm thick were placed on glass slides. Histological sections were stained with hematoxylin and eosin, and then examined under a light microscope. Assessment of tissue damage was performed separately for the sciatic nerve and muscle tissue in accordance with the modified criteria of *P. Benoit et al.* (1980) from 0 to 3 points [14] by a pathomorphologist who had no information on drugs and study groups. Inflammatory changes were ranked as follows: 0 points — no signs of inflammation, 1 point — single inflammatory cells, 2 points — few inflammatory cells, located mainly around the vessels, 3 points — inflammatory cells fill the entire interstitial space, impregnate muscle tissue, surround vessels and nerves. Cell damage was ranked as follows: 0 points — no damage, 1 point — single cells or fibers with signs of necrosis or apoptosis, 2 points — multiple cells with signs of cell damage by the type of necrosis or apoptosis, 3 — the drug shows the destruction of large volumes of fibers with involvement shells, fascia and other structures.

STATISTICAL ANALYSIS

Statistical data processing was carried out using the computer program *IBM SPSS Statistics* 25.0. Damage data are presented as the median (Me) (quartile 1 (*Q*1); quartile 3 (*Q*3)). Pairwise data comparisons are presented using nonparametric methods for unrelated samples *(Mann - Whitney)*. Differences were statistically taken into account as significant at $p < 0.05$.

RESEARCH RESULTS

Injection of saline into laboratory animals of the control group caused an expansion of the intercellular spaces and connective tissue septa. No signs of cell necrosis or apoptosis were found. In two laboratory animals in this group, single macrophage-type cells and neutrophils were found in the injection area of a 0.9% sodium chloride solution.

The introduction of local anesthetics caused inflammatory infiltration and damage to muscle and neural tissue 1 hour later (Table 2). Low concentrations of bupivacaine (0.2, 0.5%), acting on the biceps muscle and peripheral nerve, caused a slight inflammatory infiltration and weak signs of damage. High concentrations (0.75, 1.0%) are more pronounced signs of inflammation and damage.

T a b l e 2

Changes in muscle tissue and sciatic nerve 1 hour after the administration of bupivacaine and sodium chloride

Groups	Biceps femoris		Sciatic nerve	
	Inflammation	Damage	Inflammation	Damage
Sodium Chloride 0.9% (n = 5)	0 (0; 1)	0 (0; 0)	0 (0; 0.5)	0 (0; 0)
Bupivacaine 0.2% (n=5)	1 (1; 2)	1 (1; 2)	1 (1; 1.5)	1 (1; 1.5)
Bupivacaine 0.5% (n=5)	2 (1; 2)	2 (1; 2)	1 (1; 2)	2 (1; 2)
Bupivacaine 0.75% (n=5)	2 (2; 2.5)	2 (2; 2)	2 (1,5; 2)	2 (1,5; 2)
Bupivacaine 1.0% (n=5)	2 (2; 3)	2 (2; 2.5)	2 (2; 2)	2 (2; 2)

Damage in the form of dystrophic changes in muscle fibers in the form of polychromasia, uneven perimuscular edema, the disappearance of transverse striation of muscles, apoptosis and the formation of contractile contractures were revealed in muscle tissue (Fig. 1).

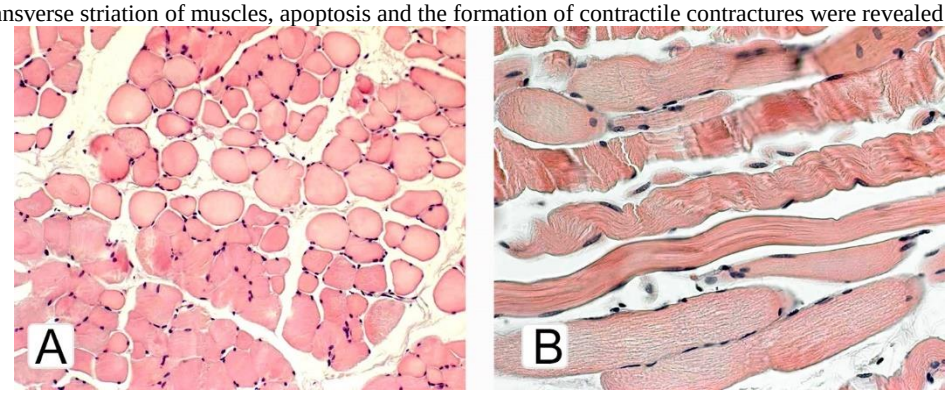

Fig. 1. Signs of damage to muscle fibers 1 hour after administration. A — perimuscular edema, groups of muscle fibers with dystrophic changes, polychromasia, apoptosis of cells. B — perimuscular edema, muscular fibers with contractile contractures, polychromasia, and less-pronounced cross-striation (stained with hematoxylin and eosin, magnification x400)

In the tissue of the peripheral nerve, changes were less significant. After paraneural administration of bupivacaine, uneven edema of nerve structures, dystrophic changes in nerve fibers and cells with signs of damage like necrosis and/or apoptosis were revealed in the sciatic nerve (Fig. 2).

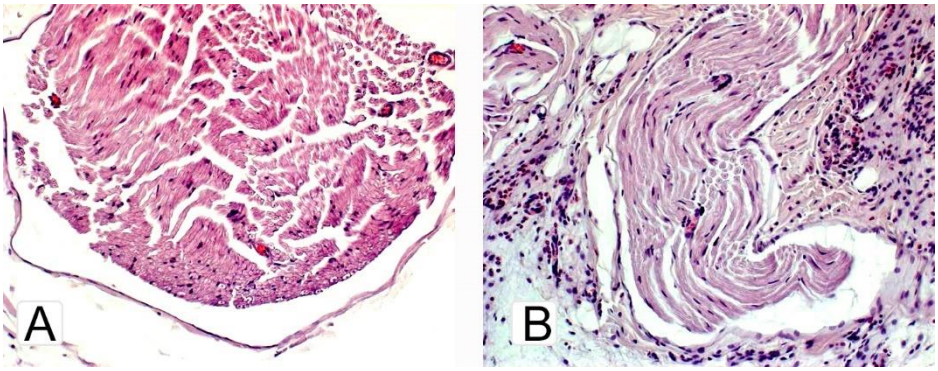

Fig. 2. Signs of damage to the nerve 1 hour after administration. A — edema of neural structures and pronounced dystrophic changes in nerve fibers with occasional cells with signs of apoptosis. B — irregular edema of neural structures, dystrophic changes in nerve fibers with occasional cells with signs of apoptosis and necrosis (stained with hematoxylin and eosin, magnification x400)

In addition to signs of damage, tissue infiltration by inflammatory cells was detected (Fig. 3). If the infiltration was weak (1 point), then single inflammatory cells were within the interstitium around the vessels, individual cells were found around the nerves and in muscle tissue. Among inflammatory cells neutrophils prevailed, there were single macrophages and lymphocytes. With moderate inflammatory infiltration (2 points), clusters of cells were located mainly in the interstitium around the vessels, individual cells were detected around the nerves and in muscle tissue. Among inflammatory cells neutrophils and macrophages prevailed, a few lymphocytes were found. With severe infiltration (3 points), inflammatory cells filled the entire interstitial space, impregnated muscle tissue, surrounded vessels and nerves, and were detected inside the walls of blood vessels and nerves. Among inflammatory cells neutrophils and macrophages prevailed, there was a moderate number of lymphocytes.

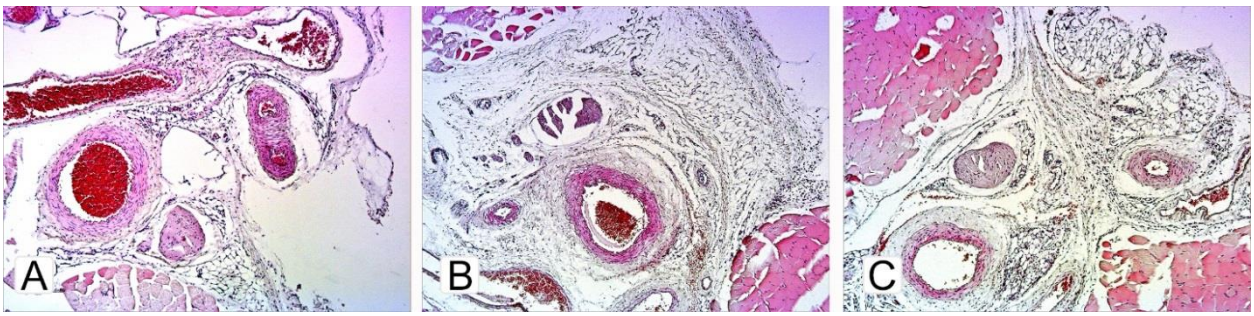

Fig. 3. Tissues infiltrated with inflammatory cells 1 hour after administration. A — occasional cells within the interstitium around the vessels, around the nerve, in the muscles; neutrophils prevail, macrophages and occasional lymphocytes are present. B — moderate quantity of inflammatory cells in the interstitium around the vessels, in the muscles, in the nerves; basically, cells of macrophage type and a small amount of neutrophils and white blood cells are found. C — inflammatory cells fill the entire interstitial space, impregnate muscle tissue, surround vessels and nerves; prevailing cells: neutrophils, macrophages, occasional lymphocytes (stained with hematoxylin and eosin, magnification x200)

Damage rates in the biceps femoris and sciatic nerve in the groups with bupivacaine were statistically significantly different from those with placebo (Table 3). In addition to markers of apoptosis and necrosis of muscle cells and peripheral nerve trunks at the injection sites of local anesthetics, statistical differences were also found in the severity of inflammatory infiltration compared with the picture after administration of a 0.9% sodium chloride solution (see Table 3). Since statistical processing of categorical data was carried out, pairwise comparisons of groups are presented using nonparametric methods for unrelated samples (Mann-Whitney).

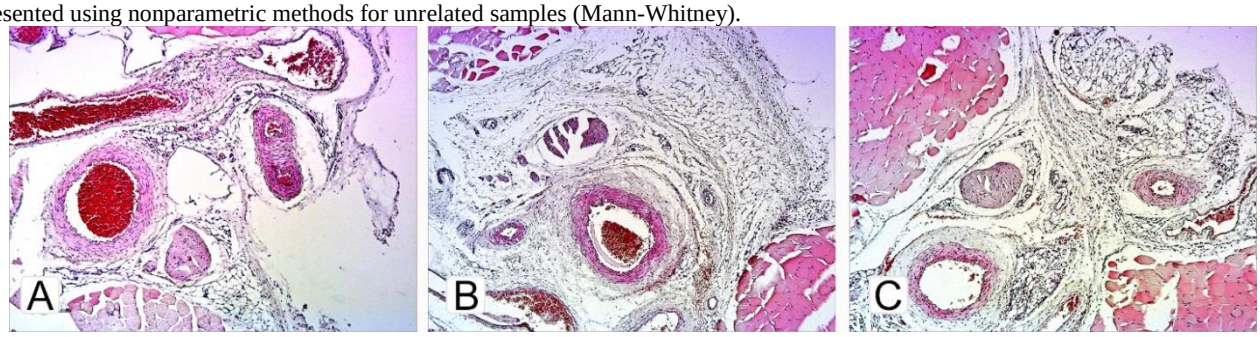

Fig. 3. Tissues infiltrated with inflammatory cells 1 hour after administration. A — occasional cells within the interstitium around the vessels, around the nerve, in the muscles; neutrophils prevail, macrophages and occasional lymphocytes are present. B — moderate quantity of inflammatory cells in the interstitium around the vessels, in the muscles, in the nerves; basically, cells of macrophage type and a small amount of neutrophils and white blood cells are found. C — inflammatory cells fill the entire interstitial space, impregnate muscle tissue, surround vessels and nerves; prevailing cells: neutrophils, macrophages, occasional lymphocytes (stained with hematoxylin and eosin, magnification x200)

Two weeks later, signs of damage and inflammatory infiltration decreased, but continued to persist (Table 4). At the injection sites of bupivacaine, uneven perimuscular edema was detected in muscle tissue, muscle fiber groups with dystrophic changes in the form of polychromasia, and uneven edema of nerve structures and dystrophic changes continued to remain in the sciatic nerve (Fig. 4).

Table 4

Changes in muscle tissue and sciatic nerve 14 days after the administration of bupivacaine and sodium chloride

Groups	Biceps femoris		Sciatic nerve	
	Inflammation	Damage	Inflammation	Damage
Sodium Chloride 0.9% (n=5)	0 (0; 0.5)	0 (0; 0)	0 (0; 0.5)	0 (0; 0)
Bupivacaine 0.2% (n=5)	1 (1; 1.5)	1 (0.5; 1)	1 (0; 1)	1 (1; 1.5)
Bupivacaine 0.5% (n=5)	1 (1; 1.5)	1 (1; 1)	1 (0.5; 1)	1 (1; 2)
Bupivacaine 0.75% (n=5)	1 (2; 2)	2 (1; 2)	1 (1; 1.5)	1 (1; 2)
Bupivacaine 1.0% (n=5)	1 (1; 2)	2 (1; 2)	1 (1; 1.5)	1 (1; 2)

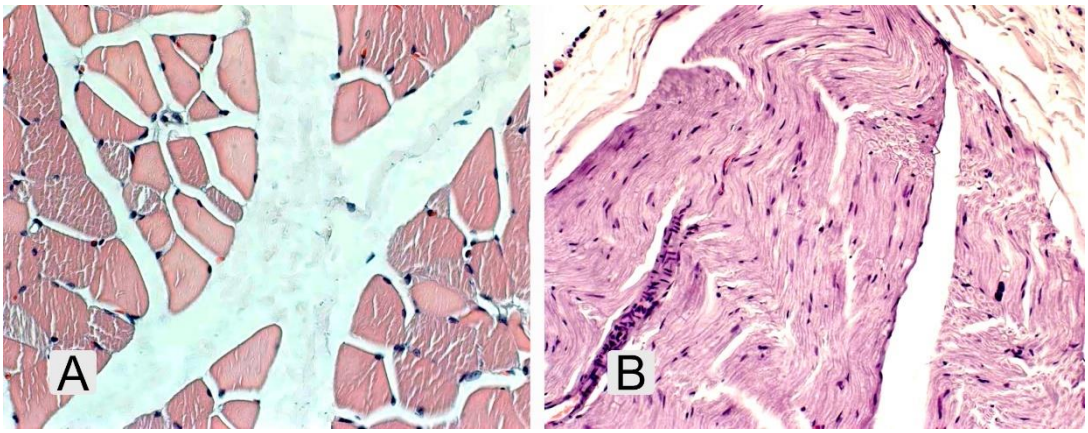

Fig. 4. Changes in biceps femoris and sciatic nerve 14 days after administration. A — irregular perimuscular edema, muscle fiber groups with dystrophic changes in the form of polychromasia. B — irregular edema of neural structures and mild dystrophic changes

Despite the fact that the intra-group study after administration of the drug was carried out at different stages, the material was taken from different experimental animals, therefore, the statistical processing of data was carried out using non-parametric methods for unrelated samples *(Mann-Whitney)*. A pairwise comparison of changes in muscle tissue after 1 h and 14 days after injection showed statistically significant differences in the signs of inflammation in the muscle with the introduction of 0.75 and 1% solutions of bupivacaine, and in terms of damage to the muscle tissue - only in the ratio of 0.5% bupivacaine solution (Table 5). Changes in the sciatic nerve on the basis of inflammation were statistically different when 0.5 and 1% bupivacaine solutions were administered, and on the basis of damage, only when a 1% bupivacaine solution was administered. The absence of statistically significant differences indicates that there was no significant reduction in inflammatory infiltration or damage. Those concentrations at which a significant decrease in inflammation and damage was detected did not lead to the complete disappearance of these signs. Inflammation and damage persisted after administration of all test concentrations of bupivacaine.

Table 5

Statistical indices of pairwise comparison of signs of bupivacaine and placebo inflammation and damage 1 hour after administration, and 14 days after the administration (Mann-Whitney test)

	Biceps femoris		Sciatic nerve		Indicators
Comparison groups	Inflammation	Damage	Inflammation	Damage	
Bupivacaine 0.2% (n=10)	7.000	6.000	6.000	12.500	U
	-1.247	-1.678	-1.678	0.000	Z
	0.212	0.093	0.093	1.000	p
Bupivacaine 0.5% (n=10)	7.500	5.000	4.000	10.000	U
	-1.225	-1.964	-2.032	-0.600	Z
	0.221	**0.050**	**0.042**	0.549	p
Bupivacaine 0.75% (n=10)	4.000	7.500	5.000	7.500	U
	-2.032	-1.500	-1.800	-1.225	Z
	0.042	0.134	0.072	0.221	p
Bupivacaine 1.0% (n=10)	3.000	6.000	2.500	5.000	U
	-2.154	-1.678	-2.449	-1.964	Z
	0.031	0.093	**0.014**	**0.050**	p

DISCUSSION

The study compares the myotoxic and neurotoxic effects of bupivacaine with 0.9% sodium chloride solution. The toxic effect of local anesthetics on muscle and neural tissue was revealed in various studies in the study of cultured [4, 15], isolated tissues [16] and in the tissues of laboratory animals [17]. However, the severity of the effects of bupivacaine on muscle and nerve fibers is estimated ambiguously.

In our study, the presence of a myotoxic effect of all concentrations of bupivacaine was revealed in comparison with the result of the administration of a 0.9% sodium chloride solution. Similar data on the myotoxicity of bupivacaine were obtained by *Yildiz et al*. (2011) [18], *Oz Gergin et al.* (2019) *W. W. Zink et al*. (2003), investigating the effect of its 0.5% solution [17]. As a rule, only 0.5% bupivacaine solution was studied. In our study, in a comparative aspect, a damaging effect on the muscle tissue of four concentrations of bupivacaine was revealed. In most studies, inflammatory cells or markers are determined together with signs of necrosis and apoptosis, combining them with a single concept of damage [15, 17, 18]. In our study, signs of an inflammatory reaction at the sites of anesthetic administration were evaluated separately from signs of damage. Statistical differences in the infiltration of inflammatory muscle cells by the administration of all concentrations of bupivacaine were also revealed compared with data regarding the administration of a 0.9% sodium chloride solution.

In addition to the myotoxic effect, the presence of a neurotoxic effect of all bupivacaine concentrations was revealed compared with the action of a 0.9% sodium chloride solution. As in most studies, we revealed signs of sciatic nerve edema, apoptosis, and necrosis [3, 7]. Considering the damaging effect on neurons, it is believed that the neurotoxicity of local anesthetics can cause postoperative neurological complications [3, 19]. In the literature, the neurotoxicity of bupivacaine has been studied quite extensively [6, 3, 16]. In our study, signs of axonal degeneration of individual fibers that appeared after the administration of bupivacaine were detected. Neurotoxicity of bupivacaine according to *CMS Cereda et al*. (2012) also manifested itself in the form of destruction of Schwann cells and damage to neurons themselves [7].

In our study, complete restoration of muscle tissue and the peripheral nerve did not occur by day 14, although *K. Yildiz et al.* (2011) determined the complete recovery of muscle tissue by the 3rd week from the administration of bupivacaine.

The advantage of the study was the study of myotoxicity and neurotoxicity of various concentrations of bupivacaine and the assessment of tissue recovery 14 days after administration. The limitation of the study was a categorical translation of histological changes in tissues with their ranking from 0 to 3 points, depending on the subjective assessment of a pathomorphologist.

CONCLUSION

The study showed the presence of neurotoxicity and myotoxicity of all four concentrations of bupivacaine compared with a 0.9% sodium chloride solution. In the sciatic nerve, dystrophic changes in nerve fibers, neutrophilic infiltration were detected . In the biceps femoris muscle perimuscular edema, apoptosis, necrosis and polychromasia with the disappearance of the transverse striation of the muscles, the appearance of clusters of inflammatory cells were determined. Preservation of signs of damage and inflammatory infiltration was revealed 14 days after the administration of bupivacaine.

FINDINGS

1. According to the studied parameters, damage and inflammatory infiltration in the biceps femoris muscle 1 hour after the administration of 0.2, 0.5, 0.75 and 1% solutions of bupivacaine are more pronounced compared with the results of the introduction of 0.9% sodium chloride solution.

2. Damage and inflammatory infiltration in the sciatic nerve 1 hour after the administration of 0.2, 0.5, 0.75 and 1% solutions of bupivacaine are more pronounced compared with the results of the introduction of 0.9% sodium chloride solution.

3. On the 14th day after the administration of bupivacaine, complete restoration of muscle tissue and peripheral nerve did not occur.

REFERENCES

1. Putrenko I, Ghavanini AA, Meyer Schöniger KS, Schwarz SK. Central Nervous System-Toxic Lidocaine Concentrations Unmask L-Type Ca^{2+} Current-Mediated Action Potentials in Rat Thalamocortical Neurons: An In Vitro Mechanism of Action Study. *Anesth Analg*. 2016;122(5):1360–1369. PMID: 26771269 https://doi.org/10.1213/ANE.0000000000001158
2. Nakahira K, Oshita K, Itoh M, Takano M, Sakaguchi Y, Ishihara K. Clinical Concentrations of Local Anesthetics Bupivacaine and Lidocaine Differentially Inhibit Human Kir2.x Inward Rectifier K+ Channels. *Anesth Analg*. 2016;122(4):1038–1047. PMID: 26756912 https://doi.org/10.1213/ANE.0000000000001137
3. Verlinde M, Hollmann MW, Stevens MF, Hermanns H, Werdehausen R, Lirk P. Local Anesthetic-Induced Neurotoxicity. *Int J Mol Sci*. 2016;17(3):339. PMID: 26959012 https://doi.org/10.3390/ijms17030339
4. Lirk P, Haller I, Colvin HP, Frauscher S, Kirchmair L, Gerner P, et al. In vitro, lidocaine-induced axonal injury is prevented by peripheral inhibition of the p38 mitogen-activated protein kinase, but not by inhibiting caspase activity. *Anesth Analg*. 2007;105(6):1657–1664. PMID: 18042864 https://doi.org/ 10.1213/01.ane.0000286171.78182.e2
5. Neal JM, Barrington MJ, Brull R, Hadzic A, Hebl JR, Horlocker TT, et al. The Second ASRA Practice Advisory on Neurologic Complications Associated With Regional Anesthesia and Pain Medicine: Executive Summary 2015. *Reg Anesth Pain Med.* 2015;40(5):401–430. PMID: 26288034 https://doi.org/10.1097/AAP.0000000000000286
6. Lirk P, Haller I, Colvin HP, Lang L, Tomaselli B, Klimaschewski L, et al. In Vitro, Inhibition of Mitogen-Activated Protein Kinase Pathways Protects Against Bupivacaine- and Ropivacaine-Induced Neurotoxicity. *Anesth Analg*. 2008;106(5):1456–1464. PMID: 18420860 https://doi.org/10.1213/ane.0b013e318168514b
7. Cereda CMS, Tofoli GR, Maturana LG, Pierucci A, Nunes LA, Franz-Montan M, et al. Local Neurotoxicity and Myotoxicity Evaluation of Cyclodextrin Complexes of Bupivacaine and Ropivacaine. *Anesth Analg*. 2012;115(5):1234–1241. PMID: 22822189 https://doi.org/10.1213/ANE.0b013e318266f3d9
8. Hussain N, McCartney CJL, Neal JM, Chippor J, Banfield L, Abdallah FW. Local anaesthetic-induced myotoxicity in regional anaesthesia: a systematic review and empirical analysis. *Br J Anaesth.* 2018;121(4):822–841. PMID: 30236244 https://doi.org/10.1016/j.bja.2018.05.076
9. El-Boghdadly K, Pawa A, Chin KJ. Local anesthetic systemic toxicity: current perspectives. *Local Reg Anesth*. 2018;11:35–44. PMID: 30122981 https://doi.org/ 10.2147/LRA.S154512
10. Neal JM, Salinas FV, Choi DS. Local Anesthetic-Induced Myotoxicity After Continuous Adductor Canal Block. *Reg Anesth Pain Med*. 2016;41(6):723–727. PMID: 27662067 https://doi.org/10.1097/AAP.0000000000000466
11. Rhee SM, Chung NY, Jeong HJ, Oh JH. Subacromial Local Anesthetics Do Not Interfere With Rotator Cuff Healing After Arthroscopic Repair. *Am J Sports Med*. 2018;46(5):1097–1105. PMID: 29470095 https://doi.org/10.1177/0363546517753827
12. Smolka W, Knoesel T, Mueller-Lisse U. Local anesthetic-induced myotoxicity as a cause of severe trismus after inferior alveolar nerve block. *Quintessence Int*. 2018;49(5):391–396. PMID: 29532814 https://doi.org/10.3290/j.qi.a40051
13. Blum RA, Lim LT, Weir CR. Diplopia following sub-tenon's anaesthesia: an unusual complication. *Int Ophthalmol*. 2012;32(2):191–193. PMID: 22350117 https://doi.org/10.1007/s10792-012-9535-3
14. Benoit PW, Yagiela A, Fort NF. Pharmacologic correlation between local anesthetic-induced myotoxicity and disturbances of intracellular calcium distribution. *Toxicol Appl Pharmacol*. 1980;52(2):187–198. PMID: 7361318 https://doi.org/10.1016/0041-008x(80)90105-2
15. Kim HJ, Sung SR, Seo KS, Lim SW, Yoon TG. Bupivacaine-induced Apoptosis in the Primary Cultured Cardiomyocytes via p38 MAPKs. *Korean J Anesthesiol*. 2006;50(6):S48-S56. https://doi.org/10.4097/kjae.2006.50.6.S48
16. Barsa J, Batra M, Fink BR, Sumi SM. A comparative in vivo study of local neurotoxicity of lidocaine, bupivacaine, 2-chloroprocaine, and a mixture of 2-chloroprocaine and bupivacaine. *Anesth Analg*. 1982;61(12):961–967. PMID: 7149299
17. Oz Gergin O, Bayram A, Gergin IS, Aksu R, Yay A, Balcıoglu E, et al. Comparison of myotoxic effects of levobupivacaine, bupivacaine and ropivacaine: apoptotic activity and acute effect on pro-inflammatory cytokines. *Biotech Histochem*. 2019;94(4):252–260. PMID: 30712387 https://doi.org/10.1080/10520295.2018.1548711
18. Yildiz K, Efesoy SN, Ozdamar S, Yay A, Bicer C, Aksu R, et al. Myotoxic effects of levobupivacaine, bupivacaine and ropivacaine in a rat model. *Clin Investig Med*. 2011;34(5):E273 PMID: 21968269
19. Sondekoppam RV, Tsui BC. Factors Associated With Risk of Neurologic Complications After Peripheral Nerve Blocks: A Systematic Review. *Anesth Analg*. 2017;124(2):645–660. PMID: 280067709 https://doi.org/10.1213/ANE.0000000000001804

Received on 03.05.2019

Accepted on 07.06.2019