SCIENTIFIC ARTICLE

Effects of local anesthetics on wound healing

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Abstract
Introduction: Local anesthetic infiltration is used widely for post-operative analgesia in many situations. However the effects of local anesthetics on wound healing are not demonstrated clearly. This study planned to evaluate the effects of lidocaine, prilocaine, bupivacaine and levobupivacaine on wound healing, primarily on wound tensile strength and on collagen ultra-structure.
Methods: This study was conducted on male Sprague Dawley rats. On days 0, 8th, 15th, and 21st, all animals were weighed and received a preincisional subcutaneous infiltration of 3 mL of a solution according the group. Control saline (C), lidocaine (L) 7 mg.kg^{-1}, prilocaine (P) 2 mg.kg^{-1}, bupivacaine (B) 2 mg.kg^{-1} and levobupivacaine (LVB) 2.5 mg.kg^{-1}. The infiltrations were done at the back region 1.5 cm where incision would be performed at the upper, middle and lower part along the midline, under general anesthesia. Wound tensile strengths were measured after 0.7 cm × 2 cm of cutaneous and subcutaneous tissue samples were obtained vertical to incision from infiltrated regions. Tissue samples were also obtained for electron microscopic examination. Evaluations were on the 8th, 15th and 21st days after infiltration.
Results: There was no difference between groups in the weights of the rats at the 0th, 8th, 15th and 21st days. The collagen maturation was no statistically different between groups at the 8th and 15th days. The maturation scores of the B and L groups at the 21st day was significantly lower than the Group C (1.40, 1.64 and 3.56; respectively). The wound tensile strength was no statistically different between groups at the 8th and 15th days but at the 21st day the Groups B and LVB had significantly lower value than Group C (5.42, 5.54 and 6.75; respectively).

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Conclusion: Lidocaine and prilocaine do not affect wound healing and, bupivacaine and lev-obupivacaine affect negatively especially at the late period.
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Efeitos dos anestésicos locais na cicatrização de feridas

Resumo
Introdução: A infiltração de anestésico local é amplamente utilizada para analgesia pós-operatória em muitas situações. No entanto, os efeitos dos anestésicos locais na cicatrização de feridas não foram demonstrados claramente. Neste estudo planejamos avaliar os efeitos de lido-caína, prilocaína, bupivacaína e levobupivacaína sobre a cicatrização de feridas, principalmente sobre a força tênsil da ferida e a ultraestrutura do colágeno.
Métodos: Este estudo foi realizado em ratos machos da linhagem Sprague Dawley. Nos dias 0, 8, 15 e 21, todos os animais foram pesados e receberam uma infiltração subcutânea pré-incisional de 3 mL de uma solução, de acordo com a designação dos grupos: Grupo C recebeu salina (controle); Grupo L recebeu lidocaína (7 mg.kg^{-1}); Grupo P recebeu prilocaína (2 mg.kg^{-1}); Grupo B recebeu bupivacaína (2 mg.kg^{-1}); Grupo LVB recebeu levobupivacaína (2,5 mg.kg^{-1}). As infiltrações foram feitas na região posterior a 1,5 cm de onde a incisão seria realizada na parte superior, média e inferior ao longo da linha média, sob anestesia geral. A força tênsil da ferida foi medida após amostras de 0,7 × 2 cm de tecido cutâneo e subcutâneo serem obtidas das regiões infiltradas, verticalmente à incisão. Amostras de tecido também foram obtidas para exame microscópico eletrônico. As avaliações foram realizadas nos dias 8, 15 e 21 após a infiltração.
Resultados: Não houve diferença entre os grupos em relação ao peso dos ratos nos dias 0, 8, 15 e 21. A maturação do colágeno não foi estatisticamente diferente entre os grupos nos dias 8 e 15. Os escores de maturação dos grupos B e L no dia 21 foram significativamente inferiores aos do Grupo C (1,40, 1,64 e 3,56, respectivamente). A força tênsil da ferida não foi estatisticamente diferente entre os grupos nos dias 8 e 15, mas no dia 21, os grupos B e LVB apresentaram valores significativamente menores que o Grupo C (5,42, 5,54 e 6,75, respectivamente).
Conclusão: Lidocaína e prilocaína não afetam a cicatrização de feridas, enquanto bupivacaína e levobupivacaína afetam negativamente, especialmente no período tardio.
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Introduction

Normal wound healing consists of 4 phases: hemostasis, inflammation, proliferative, and remodeling. Collagen production begin on the 3rd day and continue for 3 weeks. Collagens released from fibroblasts and their cross-linkage enhances wound tension strength. Local anesthetic infiltration is used widely both for post-operative analgesia and in performing many surgical operations.[1] However the effects of local anestheticss on wound healing are not revealed clearly yet, despite the studies in literature.[1–6] Because, different results are reported for the effects of local anesthetic agent on wound healing in the literature.[1–6] In the studies about the effects of local anesthetics on wound healing, generally wound tensile strength, tissue hydroxyproline level, amount of collagen fiber and fibrotic index are used.[1,4,6] In our review of English literature we did not find any study showing collagen fiber ultrastructure. In this study, we planned to present the effects of local anestheticss which

are used widely in clinics such as lidocaine, prilocaine, bupivacaine and levobupivacaine on wound healing, primarily on wound tensile strength and of collagen ultrastructure.

Methods

This study was conducted at the Karadeniz Technical University Surgical Research Center, using rats from Karadeniz Technical University Surgical Research Center after approval by the Karadeniz Technical University Animal Care and Local Ethics Committee (Approval Number: 2013/47). This study was conducted on 50 male Sprague Dawley rats with a mean weight of 330 ± 20 g (10–12 weeks). During the study all rats were kept in metal cages – 1 rat per cage – 12 h in illuminated and 12 h in dark environment at normal room temperature (21 ± 2 °C) and humidity (40–60%) and were fed with standard rat feed and tap water. Rat care in cage was performed regularly with daily controls. All rats in the study were treated humanely in accordance with ''Guide for

the Care and Use of Laboratory Animals''. All the surgical interventions on the rats were performed under anesthesia. Ketamine hydrochloride (Ketalar® vial, 50 mg.mL^{-1}, Eczacıbasi. Istanbul, Turkey), 50 mg.kg^{-1} intraperitoneal (ip) and xylazine hydrochloride (Rompun® vial, 23.32 mg.mL^{-1}, Bayer. Istanbul, Turkey) 5 mg.kg^{-1} ip were used for anesthesia induction. Weight of all rats was measured on initially, 8th, 15th, and 21st days. The back region of the rats was shaved under anesthesia and surgery region disinfection was performed under operating room conditions with povidone iodine. Pre-incisional total 3 mL saline was infiltrated to subcutaneously control group and different local anesthetic agent (lidocaine, prilocaine, bupivacaine and levobupivacaine) with the same volume was infiltrated to both groups at the region of 1.5 cm where incision would be performed at the upper, middle and lower part along the midline (1 mL upper, 1 mL middle, 1 mL lower part of the back region). 1 cm of healthy tissue was kept between the incision at the lower and upper region. Two minutes later cutaneous and subcutaneous incision was made and incision regions were closed primarily with 3/0 sharp silk suture. Wound dressing was applied daily to rats. For analgesia 20 mg.kg^{-1} dose paracetamol was added to daily drinking waters of the rats in all groups. Rats were separated into 5 equal groups with 10 rats in each group.

Group control (Group C): 3 mL pre-incisional saline.
Group lidocaine (Group L): 7 mg.kg^{-1}/3 mL pre-incisional lidocaine.
Group prilocaine (Group P): 2 mg.kg^{-1}/3 mL pre-incisional prilocaine.
Group bupivacaine (Group B): 2 mg.kg^{-1}/3 mL pre-incisional bupivacaine.
Group levobupivacaine (Group LVB): 2.5 mg.kg^{-1}/3 mL pre-incisional levobupivacaine.

Rats in all groups numbered respectively, 1–10. Wound tensile strengths were measured after 0.7 cm × 2 cm of cutaneous and subcutaneous tissue samples were obtained vertical to incision from the upper, middle and lower incision regions respectively at the 8th, 15th and 21st days. Tissue samples were also obtained for electron microscopic examination. The samples for electron microscopic examination were stored in 2% glutaraldehyde solution at +4 °C. Upper and middle incision regions were separately closed with 3/0 sharp silk suture after samples were obtained at the 8th and 15th day. Sutures were removed from the lower and middle incision region at the 10th day of the study. All the rats were sacrificed with intracardiac puncture under anesthesia after tissue samples were obtained at the 21st day.

Measurement of wound tensile strength

The tests were performed using a uniaxial tensile testing system (Instron 3382 test frame). This machine has two crossheads; one is adjusted for the length of the specimen and the other is driven to apply tension/compression to the test specimen. The load is applied by electro-mechanical system in that test machine. The test system has also a video extensometer for measuring strain (sensitive displacement of cross-head of test machine). During testing in that system, the samples are subjected to a controlled tension or compression until failure. The test machine has a digital time, force and elongation measurement systems consisting of electronic sensors connected to a data collection device (computer) and software to manipulate and output the data. The tests of tissue samples were done using the described test system at a strain rate of 10^{-3} s^{-1}. The force was determined at tearing or fracturing points of the tissue samples as tensile strength of the tested tissues. For each case, two to three experiments were conducted on companion specimens to check the repeatability of the results. These results were reported at Newton (N).

Ultrastructural method

The tissues obtained from all the rats in the study groups for examination in transmission electron microscope were fixed in 3% glutaraldehyde (Sigma, G5882 USA) fixative liquid in the millonings phosphate buffer of pH 7.3 in the refrigerator at +4 °C for 2–4 h. The stiffened tissues were cut into smaller pieces of 1 mm^3. The samples were washed with millonings phosphate buffer of pH 7.3 and then were fixed with 1% osmium tetroxide (OsO$_4$) in the same buffer for 1 h. Thereafter tissues were washed in the same buffer three times each for 15 min. Pieces were treated for dehydration in 10%, 30%, 50%, 70%, 80%, 96% alcohol for 10 min, in 100% alcohol two times for 20 min each. After this they were kept in propylene oxide two times for 15 min each, in 1/1 propylene + araldite for 45 min, 1/3 propyleneoxide + araldite for 45 min and at last in pure aralidite (G4901, Sigma Chemical Co., St. Louis, MO, USA) for one night. Next day the pieces were embedded in plastic containers filled with pure araldite. They were polarized by keeping in 600 °C drying oven for 48 h. 0.5 microns of semi thin slices were cut from the electron microscope blocks obtained with Reichart UM 2 and UM 3 ultramicrotomes. This semi thin slice were stained with toluidine blue and the desired region was determined and then thin slices of 60–70 nm were transferred on to (200 mesh) copper grids. These slices first stained with saturated uranyl acetate prepared with 70% ethanol and then with lead citrate of Reynold. The slices were examined with Jeol JEM 1011 EM and the photographs were taken with Veleta brand camera using Olympos soft imaging system iTEM program. An experienced histologist examined 150 collagen fibers for each tissue sample without knowing to which group it belongs and scoring was performed according to the objective evaluation of the histologist and sequence and maturation of the collagen fibers and evaluation was performed on the basis of total value. In accordance with this, '0', '1' and '2' was given respectively to the ones with irregular sequence, partly regular sequence and regular sequence. '0', '1' and '2' was given respectively, to unmatured, partly matured and matured according to collagen maturation. The scores obtained according to the sequencing and maturation of collagen fibers was added and the results were evaluated as total maturation score.

Statistical analysis

When 10 rats per group was taken to the study for 5 groups, the study power (1β) was determined as 55% at significance

Table 1 Average weight of the rats in the groups.

W	Group C Mean (Sd)	Group P Mean (Sd)	Group L Mean (Sd)	Group B Mean (Sd)	Group LVB Mean (Sd)	F	p
0th day	321.4 (20.1)	330.6 (22.4)	338.7 (29.1)	339.7 (23.2)	338.3 (26.6)	0.94	0.450
8th day	325.8 (10.1)	330.8 (10.7)	327.6 (12.9)	328.8 (11.0)	326.5 (31.1)	0.12	0.974
15th day	340.9 (17.0)	345.9 (16.6)	341.5 (23.0)	344.1 (18.3)	339.5 (15.0)	0.20	0.938
21st day	347.4 (16.7)	350.7 (18.8)	350.6 (22.9)	349.7 (18.3)	346.0 (18.1)	0.11	0.977

Sd, standard deviation; W, weight; C, control; P, prilocaine; L, lidocaine; B, bupivacaine; LVB, levobupivacaine; F, frequencies; p, probability.

Table 2 Average maturation scores of the rats in the groups.

M	Group C Mean (Sd)	Group P Mean (Sd)	Group L Mean (Sd)	Group B Mean (Sd)	Group LVB Mean (Sd)	F	p
8th day	0.44 (0.73)	0.20 (0.42)	1.10 (0.74)	0.30 (0.68)	0.55 (0.82)	2.57	0.051
15th day	1.11 (0.78)	1.00 (0.67)	1.10 (0.57)	0.90 (0.57)	0.91 (0.54)	0.26	0.904
21st day	3.56 (0.53)	2.50 (1.27)	2.60 (0.97)	1.40 (0.97)	1.64 (0.81)	7.98	0.000

Sd, Standard deviation; M, maturation; C, control; P, prilocaine; L, lidocaine; B, bupivacaine; LVB, levobupivacaine; F, frequencies; p, probability.

level of 5% and effect size 0.40. When findings obtained in the study are evaluated, SPSS 21.0 Statistics packet program was used for statistical analysis. When data of the study are evaluated descriptive statistics methods (frequency, percent, average, standard deviation) as well as Kolmogorov–Smirnov distribution test was used to examine the normal distribution. If there are more than two groups in the comparison of quantitative data, one-way Anova test was used in the comparison of parameters between groups and Bonferroni test was used in the determination of group causing the difference. In the intra-group comparisons repeated measures ANOVA was used. Pearson correlation analysis was used to analyze the relation between maturation values and wound tensile strength. The results were evaluated at 95% confidence interval and $p < 0.05$ significance level.

Results

B3 (rat number: 3, in Group B), LVB1 (rat number: 1, in Group LVB) numbered rats and P8 (rat number: 8, in Group P) numbered rat became exitus caused by anesthesia induction respectively after tissue sample of the post-operative 8th day and 15th day was obtained. Therefore, wound tension strength, and ultrastructural tissue samples could not be examined (There was no tissue samples B3 and LVB1 rats at 15th and 21st days. There was no tissue sample P8 rat at 21st day).

There was no statistically significant difference between groups the average weights of the rats at the 0th, 8th, 15th and 21st days ($p > 0.05$). Average weights of the rats of the groups are presented in Table 1.

While, there was no statistically significant difference between groups at the 8th and 15th days with regards to collagen maturation ($p > 0.05$), average maturation scores of the bupivacaine and levobupivacaine groups at the 21st day was significantly lower than the control group ($p = 0.000$). Average maturation scores of the rats of the groups are presented in Table 2.

While, there was no statistically significant difference between groups at the 8th and 15th days with regards to wound tensile strength ($p > 0.05$), average wound tensile strength of bupivacaine and levobupivacaine groups at the 21st day was significantly lower than control group ($p = 0.000$). Average Wound Tensile Strengths (WTS) of the rats in the groups were presented in Table 3.

Electron microscopic images of all the groups at the 8th day, 15th day and 21st day are shown respectively in Figs. 1–3.

Table 3 Average wound tensile strengths (WTS) of the rats in the groups.

WTS	Group C Mean (Sd)	Group P Mean (Sd)	Group L Mean (Sd)	Group B Mean (Sd)	Group LVB Mean (Sd)	F	p
8th day	4.63 (0.48)	4.54 (0.34)	5.11 (0.51)	4.82 (0.65)	4.62 (0.35)	2.36	0.067
15th day	5.13 (0.37)	5.05 (0.34)	5.21 (0.55)	5.29 (0.66)	5.10 (0.47)	0.34	0.852
21st day	6.75 (0.71)	6.26 (0.70)	6.20 (0.61)	5.42 (0.75)	5.54 (0.63)	6.06	0.001

Sd, Standard deviation; WTS, wound tensile strengths; C: control; P, prilocaine; L, lidocaine; B, bupivacaine; LVB, levobupivacaine; F, frequencies; p, probability.
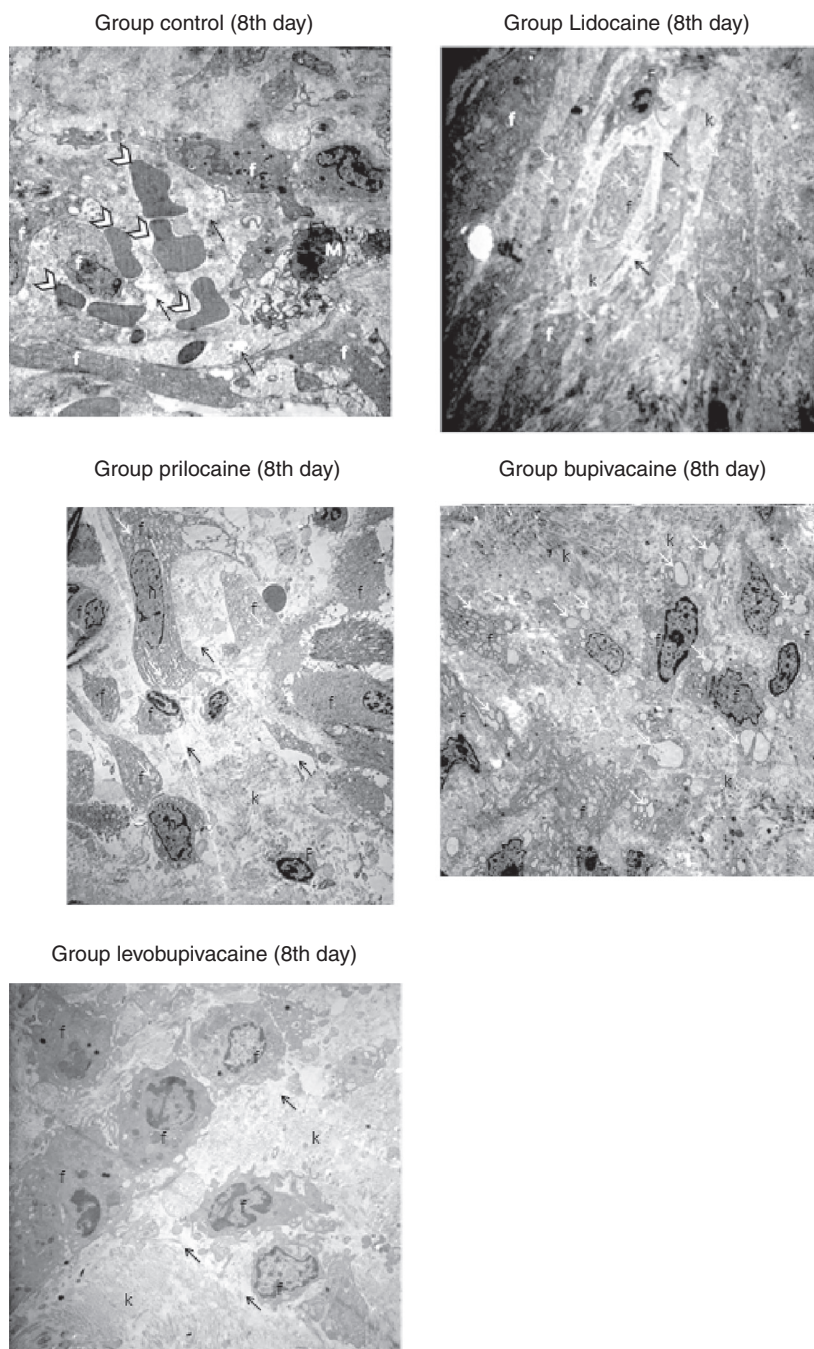
Group control (8th day)   Group Lidocaine (8th day)

Group prilocaine (8th day)   Group bupivacaine (8th day)

Group levobupivacaine (8th day)

Figure 1   Electron microscopic images of all the groups at the 8th day.

Discussion

During a normal wound healing period, increase of fibroblasts at the proliferative phase is determined (increase in fibrotic index). Collagen released from fibroblasts and cross linkage of this collagen increases wound tensile strength.[7] Wound tensile strength increases based on sequence of collagen fibers rather than collagen amount.[1] Sequence of collagen fibers is irregular in the early periods of wound healing. This may explain the weakness of wound strength despite high collagen amount in the early period of wound healing.[8] Data shows that there may not be a posi-

tive correlation between ''OH-pyroline level'' and ''wound tensile strength''. Nagler et al.[9] reported that halofuginone decreased collagen α 1 gen expression but did not significantly decrease wound tensile strength. All this information shows that principal factor designating wound tensile strength is based on sequence of collagen fibers rather than on collagen amount.

The discussion regarding the effects of local anesthetics used widely for post-operative analgesia in clinical practice and for many local surgical procedures on wound healing stays up to date. Because when the studies in literature are reviewed, fairly contradictory results are detected in
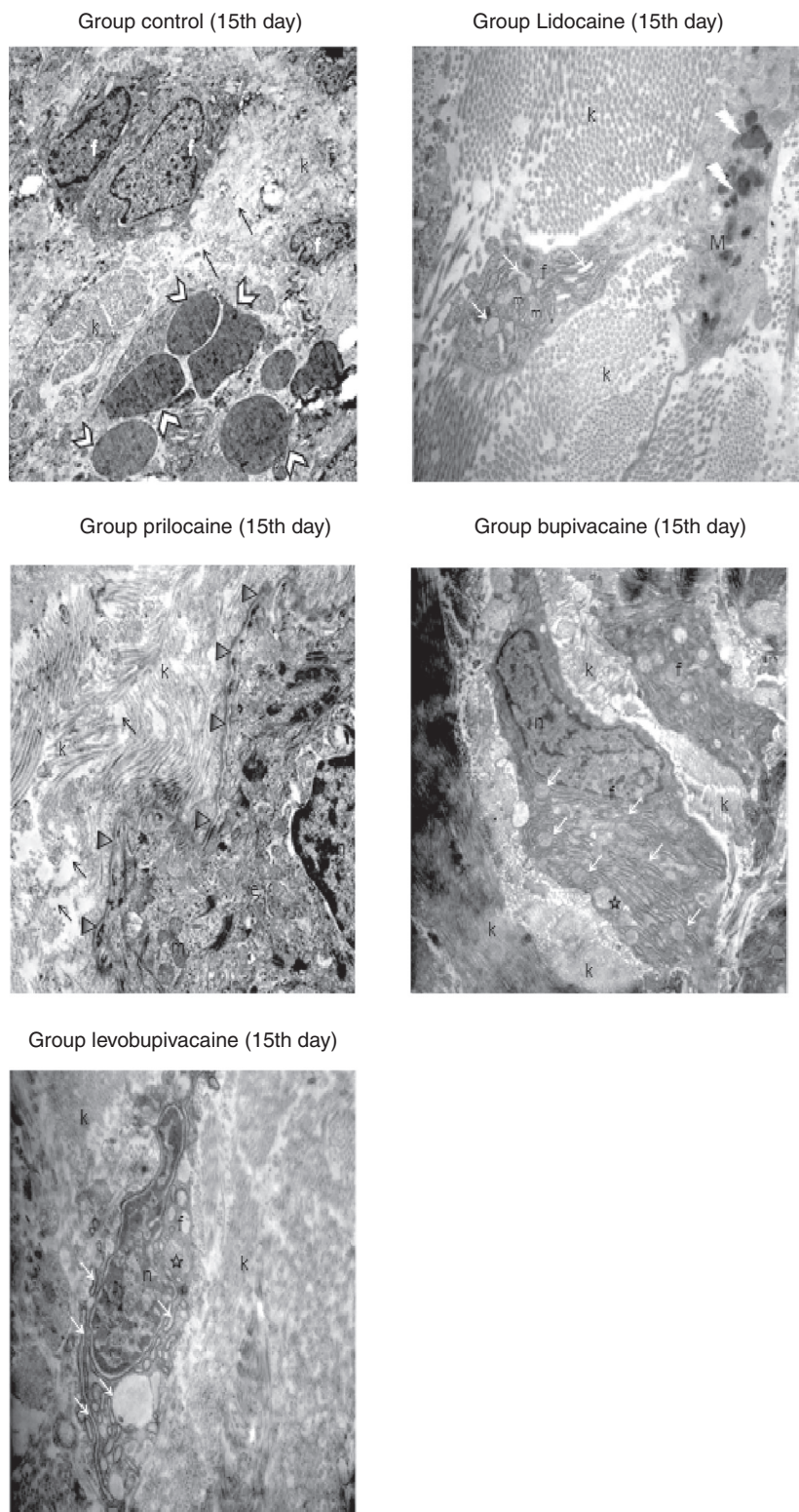
Group control (15th day)   Group Lidocaine (15th day)

Group prilocaine (15th day)   Group bupivacaine (15th day)

Group levobupivacaine (15th day)

Figure 2   Electron microscopic images of all the groups at the 15th day.

different study results on the same local anesthetics. These results increases the uncertainty about which local anesthetics is more reliable in terms of wound healing. Since sequence of collagen fibers are more determinative than collagen amount, in this study it is aimed to reveal the most reliable local anesthetics in terms of wound healing by presenting the effects of local anesthetics on sequence and maturation of collagen fibers. The results obtained from the study show that local anesthetics does not cause a significant change with regard to wound tensile strength and

Group control (21st day)

Group Lidocaine (21st day)

Group prilocaine (21st day)

Group bupivacaine (21st day)

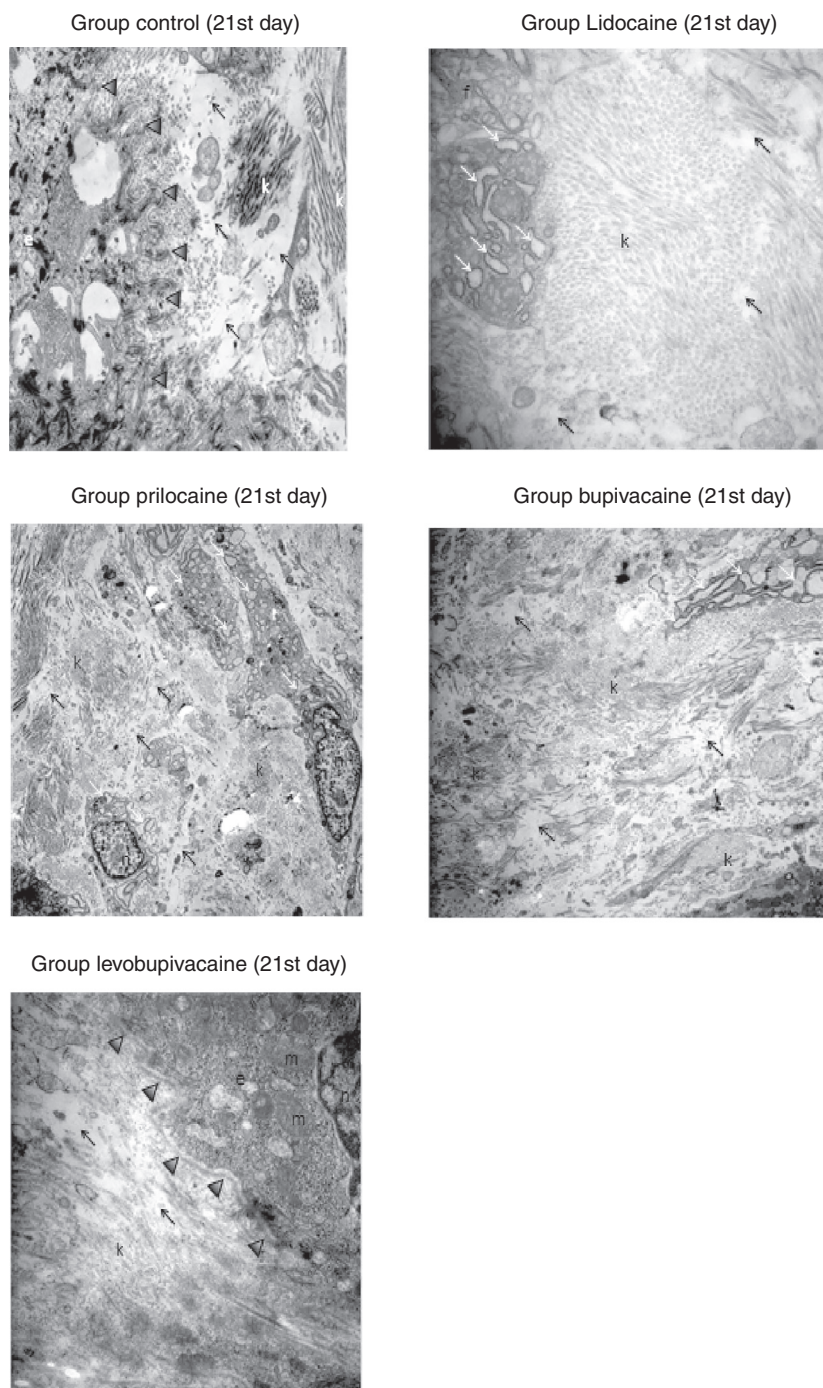Group levobupivacaine (21st day)

Figure 3 Electron microscopic images of all the groups at the 21st day.

collagen maturation at the 8th and 15th days however bupivacaine and levobupivacaine significantly decreased at the 21st day. This situation presents that bupivacaine and levobupivacaine affects wound healing negatively.

In the studies conducted by Hanci et al.,[6] it was reported that bupivacaine and lidocaine significantly decreased wound tensile strength at the 8th day however on the contrary in our study it was determined that although lidocaine increased collagen maturation and wound tensile strength at the 8th day it did not cause a statistically significant change but although bupivacaine decreased collagen maturation

and wound tensile strength, it did not cause a statistically significant change.

In the study conducted by Waite et al.[2] it was reported that bupivacaine and lidocaine did not cause any negative or positive change in wound healing at the 3rd day. In our study because the data is measured at the earliest 8th day, it was determined in line with the results of this study that both bupivacaine and lidocaine did not cause a statistically significant change in wound healing at the 8th day.

In the studies stated in literature it was reported that estrogen deprivation is responsible from poor wound

healing.[10,11] Therefore, it is possible that wound healing may be different with regards to age and gender differences. In a study we performed previously, it was determined that while levobupivacaine affected wound tensile strength negatively at the 8th day, it increased wound tensile strength at the 21st day.[1] But in this study, it was determined that while no significant change was detected in wound tensile strength at the 8th and 15th day, a significant decrease in wound tensile strength is determined at the 21st day. The difference in our two studies, like the differences in the other studies increases the uncertainty and discussion regarding the effects of local anesthetics. But when it is considered that this study was conducted on male rats, it is predicted that this difference may be due to the estrogen effect of the female rats used in the previous study, based on the positive effect of estrogen on wound healing. Because there is no study or data regarding the differences in the effects caused by levobupivacaine based on gender differences. These conclusions of the study reveal the need to perform studies about effects of levobupivacaine on different genders. In parallel with our researches we did not find a comparative study where effects of lidocaine, prilocaine, bupivacaine and levobupivacaine on ultrastructure of collagen is presented.

Conclusion

Both the limited numbers of study in literature and differences between the present results of the studies show that many studies are required to reveal the effects of local anesthetics on wound healing. Since wound tensile strength is primarily based on sequence of collagen fibers, we consider that ultrastructure of collagen must be presented in the new studies to be conducted. The uncertainties about the of local anesthetics on wound healing may be eliminated with the new studies to be conducted. When the results obtained from this study is based on ultrastructure of collagen, we consider that lidocaine and prilocaine do not affect wound healing and, bupivacaine and levobupivacaine affect negatively especially at the late period.

Conflicts of interest

The authors declare no conflicts of interest.

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