Original Article

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The Effects of Different Concentrations of Epinephrine Adjuvant to Levobupivacaine on Wound Healing

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Abstract

Objective: Local anesthetics (LAs) and epinephrine are often combined for infiltration of surgical sites for their wide-range effects. The aim of our study is to investigate the effects of different concentrations of epinephrine added to levobupivacaine on wound healing.

Methods: Forty female albino Wistar rats were divided into four groups. 3 ml of 3,75mg/ml levobupivacaine was applicated for all groups. In the control group (C) (n=10), 1ml isotonic saline was added, and in group A10 (10 µg/ml) (n=10), 1ml 1/100,000 adrenaline; in group A5 (5 µg/ml) (n=10), 1ml 1/200,000 adrenaline; and in group A2,5 (2,5 µg/ml) (n=10), 1ml 1/400,000 adrenaline. Two minutes after the infiltration of the drug combination into subcutaneous tissue, 3cm longitudinal cutaneous-subcutaneous incisions were performed on the mid-dorsal line under sterile conditions. Incisions were sutured with 4/0 sharp prolen with six sutures. Postoperative 8th-day rats were sacrificed to evaluate wound healing. Tissue burst pressures (TBP) and tissue hydroxyproline levels (THP) were measured, and histopathological evaluation for a fibrotic index was performed. A One-Way ANOVA and Chi-square test were used for statistical analyses.

Results: There was no statistically significant difference between groups according to TBP and THP levels (p=0.4, p=0.201 respectively). Fibrotic index values were significantly higher in epinephrine groups (p=0,001), and were highest in A2,5.

Conclusions: Epinephrine added to levobupivacaine in low concentrations accelerates wound healing in the early phase by stimulating fibrosis, and has no adverse effects on surgical sites. Long-term studies are needed for late effects of epinephrine adjuvant levobupivacaine.

Key words: *Wound Healing, levobupivacaine, adrenaline, tissue burst pressure, hydroxyproline, fibrosis*

Introduction

Infiltration of an incision site with LAs is an easy and less complicated way of supplying postoperative analgesia [1-3]. Preemptive infiltration or the infiltration of the incision at the end of the surgery with the LAs decreases the need for analgesic drugs peri- and/or post-operatively [4]. Uncomplicated wound healing is one of the major goals of every surgical procedure.

Among many factors affecting wound healing, medications like LAs used locally during the perioperative period have an important role in this process. Besides their analgesic effects, LAs interfere with the healing process at the inflammation phase [5]. Lidocaine and bupivacaine are used for infiltration anesthesia, and bupivacaine is preferred for its longer duration of action. Levobupivacaine has been used for less neurotoxic and cardiotoxic effects than bupivacaine, with their long duration of action [6,7]. It was shown that bupivacaine and lidocaine decrease the collagen synthesis by blocking cell membrane stability, sodium conduction, and intracellular calcium entrance in rat fibroblast tissue culture acquired from the surgical wound [7]. Dere et al. reported that levobupivacaine in clinical doses at infiltration anesthesia has a positive effect on wound healing and an increasing effect with the increasing doses of levobupivacaine [4].

Adding vasopressor agents to LAs as an adjuvant provides well-known effects like slow absorption of LAs and reduced bleeding. Studies report that systemic and locally secreted epinephrine impair wound healing [8]. It was reported that β-2 adrenergic receptor activation delays wound healing, thereby affecting keratinocyte migration speed [8,9]. The aim of our study is to investigate and compare the effects of different concentrations of adrenaline adjuvant to levobupivacaine on wound healing with well-known and widely used wound-healing evaluation methods, including tissue burst pressures (TBP), hydroxyproline levels (THP), and histopathological evaluation of the fibrotic index [4].

Material and Methods

Animals

This study was performed at the Experimental Animal Laboratory in the Medical Faculty of Marmara University with the approval of the Animal Ethics Committee. All protocols were in accordance with the regulations concerning the care and use of laboratory animals, as in the Declaration of Helsinki.

Forty out-bred female albino Wistar rats (mean weight 200 ± 30 g, mean age 7 months) were divided into 4 groups, and kept by applying a 12-hour light/dark cycle with a stable temperature between 19 and 22 oC in standard rat cages, all of which contained a maximum of five rats in each. They were fed with standard rat pellets and tap water.

Operation and Designing Model

After 12 hours of starvation, 75 mg/kg of ketamine hydrochloride was used for anesthesia intraperitoneally (IP). After the loss of cornea reflex and extremity withdrawal response, the surgical area was shaved. The incision area was scrubbed with povidon iodine solution and was swapped with sterile gauze after two minutes.

Before the incision, the rats in the groups were infiltrated with 3 ml of 3,75mg/ml Levobupivacaine [4], adding the following:

- Saline in control group C (n=10) (1ml saline solution 0,9% NaCl)
- 10 μg/ml of adrenaline in group A10 (n=10) (1ml 1/100,000 adrenaline)
- 5 μg/ml of adrenaline in group A5 (n=10) (1ml 1/200,000 adrenaline)
- 2,5 μg/ml of adrenaline in group A2,5 (n=10) (1ml 1/400,000 adrenaline)

Two minutes after the infiltration of the preparations, 3cm longitudinal cutaneous-subcutaneous incisions were performed on the mid-dorsal line under sterile conditions. Incisions were sutured with 4/0 sharp prolen with six sutures. 100 mg/kg of paracetamol was injected IP for analgesia. The rats were allowed to eat normally at 6 hrs. postoperatively.

All rats were sacrificed on the 8th postoperative day by giving a high dose (100-150 mg/kg) of sodium thiopental. Sutures were cautiously taken from the skin with a sharp scalpel so as not to affect the results. 6x2cm tissue samples were resected and divided into three equal parts for mechanical tissue burst pressures (TBP), biochemical tissue hydroxyproline (THP) levels, and histopathological fibrotic index evaluation.

Tissue Burst Pressure Assessment

Tissue samples were collected in normal saline. A 10-N uni-actional tensiometer was used for measurement. Tissues were attached to the tensiometer from both sides of the incision line. A force of 1 N/min was applied. The pressures at the time of burst were recorded.

Tissue Hydroxyproline Assessment

This procedure is based on detecting free hydroxyproline levels in tissue homogenate after the alkaline hydrolysis of the homogenate.

Histopathological Assessment

Tissue samples were placed in 10% formalin for 24 hours for stabilization. After stabilization paraffin blocks were prepared to obtain 6μm cross sections. Mason trichrome was used to stain the samples. Samples were evaluated by an experienced pathologist for their fibrosis levels. The pathologist in the study was blinded during the interpretation of the results. Fibrosis in the incision line was classified as follows: no fibrosis (0), mild fibrosis (+1), moderate fibrosis (+2), and high-level fibrosis (+3) [4].

Statistical Analysis

All statistical analyses were performed by a statistical software package (SPSS 17.0). We used a One-Way ANOVA test for comparison of defining statistical methods and quantitative data. A chi-square test was used for ordinal data. Results were evaluated in a 95% confidence interval, and a p-value <0.05 was accepted as significance.

Results

There were no postoperative complications such as infection and wound separation. No mortality was observed in all groups during the study.

Tissue Burst Pressure Assessment

The means of tissue burst pressure in groups C, A10, A5, and A2,5 were 108.75 ± 16.06, 105.0 ± 14.67, 113.75 ± 18.11, and 103.75 ± 17.72, respectively (Mean ± SD). There was no significant difference between groups according to tissue burst pressures (p=0.4) (Table I) (Figure 1).

Tissue Hydroxyproline Assessment

The means of tissue hydroxyproline levels in groups C, A10, A5, and A2,5 were 12.99 ± 9.70, 17.31 ± 9.81, 22.08 ± 13.78, and 24.00 ± 14.90, respectively (Mean ± SD). There was no significant difference between groups according to tissue hydroxyproline levels (p=0.201) (Table I) (Figure 2).

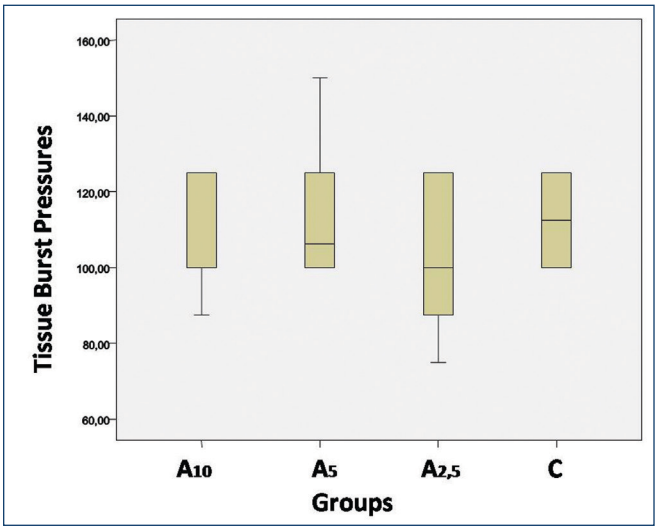

Figure 1. Tissue burst pressure of groups (newton).

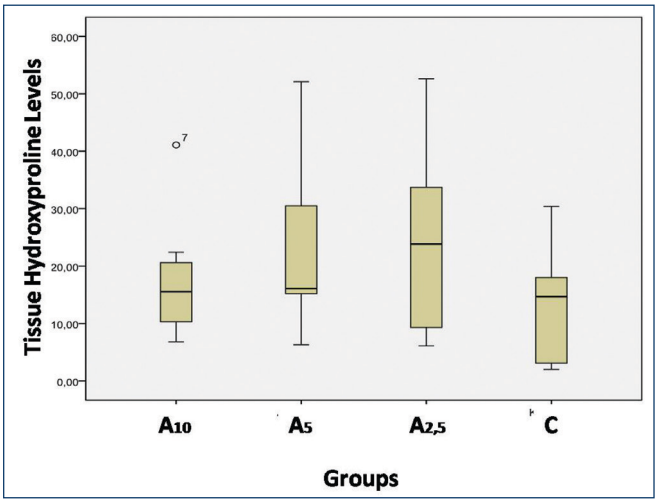

Figure 2. Tissue hydroxyproline levels of groups (gr/gr.protein).

Histopathological Assessment

The means of the fibrotic index in groups C, A10, A5, and A2,5 were 2.3 ± 0.483, 0.5 ± 0.527, 0.9 ± 0.737, and 2.1 ± 0.567, respectively (Mean ± SD). Fibrosis was significantly higher in groups C and A2,5 (p=0.001). The means of group A10 were statistically lower than groups C and A2,5. There is no difference

Table 1. Tissue burst pressures, tissue hydroxyproline levels, and tissue fibrotic index results of groups (mean ± SD, p).

Groups	Tissue Burst Pressures (Newton)		Tissue Hydroxyproline Levels (gr/gr.protein)		Tissue Fibrotic Index (0-3)	
	Mean ± SD	p	Mean ± SD	p	Mean ± SD	p
C	108,75 ± 16,06	0.4	12,99 ± 9,70	0.201	2,3 ± 0,483	0.001
A10	105,0 ± 14,67		17,31 ± 9,81		0,5 ± 0,527	
A5	113,75 ± 18,11		22,08 ± 13,78		0,9 ± 0,737	
A2,5	103,75 ± 17,72		24,00 ± 14,90		2,1 ± 0,567	

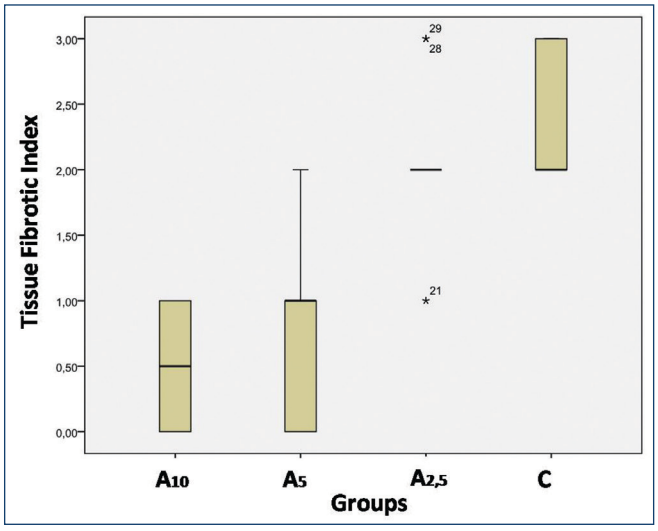

Figure 3. Fibrotic indexes of groups (0-3).

between groups A10 and A5. There is no difference between groups C and A2,5 (Table I) (Figure 3).

Discussion

Infiltration anesthesia is used for decreasing postoperative pain and prolonging the analgesia [4]. Adrenaline is used for reducing blood flow and slowing the rate of absorption of the local anesthetic agent, thereby reducing the plasma concentration and prolonging the duration of action [10]. Knowledge about the effects of adrenaline adjuvant LAs on wound healing is limited. In our study, we investigated the effects of different epinephrine concentrations added to levobupivacaine on wound healing.

Cutaneous wound healing is a complex and well-organized process requiring the coordinated migration and proliferation of both keratinocytes and fibroblasts, as well as other cell types [8], and includes inflammation, granulation-proliferation and remodeling phases [5]. Waite et al. reported that despite lidocaine and bupivacaine influencing local inflammatory and proteolytic factors, they didn't impair the rate of healing in mimicking normal human wounds and impaired age-related healing in mice [9]. Brower et al. reported that local infiltration of LAs in concentrations inhibit the activation, migration, and metabolic activity of inflammatory cells [5]. Antibacterial and myotoxic properties of LAs effect wound healing, besides other factors [4]. It was reported that the levobupivacaine and sufentanil combination had antibacterial effects and was safe for use in local anesthesia [11], and levobupivacaine has no risk of myotoxicity [12]. In our study, no infection or myotoxicity was found.

The effect of LAs such as lidocaine, procaine, bupivacaine, proparacaine, benzocaine, tetracaine, dubicaine, mepivacaine, prilocaine, ropivacaine, and cocaine on wound healing is evaluated in humans, animals and in vitro studies [5]. Dere et al. studied the effect of levobupivacaine in different doses on wound healing. They found higher breaking strength, higher hydroxyproline levels and a higher fibrotic index in levobupivacaine groups than in the control (saline-applicated) group. Because it was reported that 3,75 mg/ml of levobupivacaine had a significantly higher positive effect on wound healing than other groups [4], we used a 3,75mg/ml concentration of levobupivacaine in our study.

It was shown that locally or systemically elevated epinephrine levels as a response to injury impairs wound healing by slowing re-epithelization via the stimulation of beta-2-adrenergic receptors of keratinocytes [8,13]. Wakamatsu et al. compared the effects of lidocaine with combinations of 1/20,000, 1/80,000 and 1/200,000 epinephrine solutions on wound healing with the control group, concluding that lidocaine alone has no effects on wound healing, but when combined with epinephrine, it facilitates healing [14]. Morris et al. reported that procaine has negative effects on experimental wound healing, and adding epinephrine to procaine elevates these effects [15]. Banli et al. reported that there was no difference between control, prilocaine and lidocaine groups, and the HP levels were significantly lower in the prilocaine-epinephrine group in the experimental wound-healing study [16]. In our study, epinephrine concentrations higher than 1/400,000 caused significantly lower fibrotic indexes. In epinephrine groups, HP levels were higher compared to the control group, with the highest level being in A2,5 (Table I).

In our study, tissue burst pressure results and tissue hydroxyproline levels were similar to the results of the study with 3,75mg/ml levobupivacaine, which was reported by Dere et al. This showed that adding saline or epinephrine solutions does not affect the breaking strength and tissue hydroxyproline levels. Fibrotic index results of control and A2,5 groups were similar, but A10 and A5 groups had a lower fibrotic index. We con-

sidered that adding 1/100,000 and 1/200,000 concentrations of adrenaline to 3,75mg/ml levobupivacaine would decrease wound healing in comparison with 3,75mg/ml levobupivacaine alone.

Because of their short duration, in vitro studies generally evaluated the first phase (inflammation) of wound healing [5]. In our study, we sacrificed the animals on the 8th postoperative day. The second-phase (3-4 weeks) and third-phase (6-12 months) studies are needed to evaluate late effects of epinephrine adjuvant to levobupivacaine. High concentration of epinephrine (1/100,000 and 1/200,000) may be effective in phases two and three.

In conclusion, in the early stage of wound healing, although the results of the TBP measurements, and partly the THP measurements, do not support our opinion, we concluded that epinephrine added to levobupivacaine in low concentrations accelerates wound healing by stimulating fibrosis, as well as having no adverse effects on surgical sites, and long-length studies are needed for late effects of other concentrations of epinephrine.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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