The Effect of Local Anesthetics on Wound Healing in Rats

RATLARDA LOKAL ANESTEZİKLERİN YARA İYİLEŞMESİNE ETKİSİ

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Summary

Purpose: This study has been performed to evaluate the effects of local anesthetics on wound healing. The study was accomplished at the Experimental and Surgical Research Center of Erciyes University.

Material and Methods: A total of 4 groups were formed, each including 10 rats. 5 cm midline skin incision was made on the abdominal wall of the rats. In the control group physiologic saline was injected into wound edges. 2.Group : Prilocaine, 3.Group: Lidocaine were used as local anesthetics. 4.Group received vasoconstrictor agent (adrenaline) in addition to lidocaine. Incision was closed with 4/0 atraumatic silk suture. In the postoperative 7th day the skin including the incision was excised 5 mm away from the wound and reserved for histopathological and biochemical analyses. Sutures were examined and photographed after removal.

Results: When hydroxyproline levels were compared, hydroxyproline level in the 4. group was lower than the other groups and the difference was statistically significant. There was no difference between the control group and the local anesthetic groups. In the histopathological examination, only local anesthetic+vasoconstrictor group had necrosis foci without enough granulation tissue.

Conclusion: This effect has been related to local tissue hypoxia caused by local anesthetics + vasoconstrictor. We think the phenomenon will be revealed more clearly with the measurements of hypoxia.

Key Words: Wound healing, Local anesthetics

T Klin J Med Res 2001, 19:13-19

Özet

Amaç: Bu deneysel çalışma günümüzde yaygın olarak kullanılan lokal anesteziklerin yara iyileşmesini nasıl etkilediğini göstermek amacıyla yapıldı. Çalışmamız Erciyes Üniversitesi Deneysel ve Cerrahi Araştırma Merkezi'nde yapıldı.

Materyal ve Metod: Herbiri on rattan oluşan dört grupta toplam kırk rat üzerinde çalışma yapıldı. Kontrol grubunda 5 cm.'lik deri insizyonu yapılan ratlara yara dudaklarına serum fizyolojik injekte edildi. 2.Grupta prilokain, 3.Grupta lidokain 4.Grupta lidokain+adrenalin injekte edildi. 4/0 atravmatik ipekle cilt kapatıldı. Postoperatif yedinci gün bu insizyonu içine alan 0,5 cm.'lik bir uzaklıktan cilt tamamen eksize edilerek histopatolojik ve biyokimyasal çalışma için saklandı. Sürürler alındıktan sonra sürürler fotoğraflanarak fizik muayeneyle değerlendirildi.

Sonuçlar: Hidroksiprolin seviyeleri karşılaştırıldığında lokal anestezik verilen 2. ve 3. gruplarla kontrol grubu arasında bir fark yokken, lokal anestezik ilaca vazokonstrüksör ajan ilavesinin yapıldığı 4.grupta hidroksiprolin değerleri diğer gruplara göre istatistiksel olarak anlamlı düşmüştür. Yaraların histopatolojik değerlendirilmesinde sadece lokal anestezik + vazokonstrüktör ilaç verilen grupta yeterli bir granülasyon teşekkülü olmamış, yer yer nekroz sahaları gözlenmiştir. Sonuç olarak lokal anesteziklerin yara iyileşmesini kısmen olumsuz etkilediği vazokonstrüktör ajan ilavesiyle yara iyileşmesinin iyice bozulduğu görüldü.

Tartışma: Lokal anesteziklerin ve vazokonstrüktör ilaçların bu etkiyi dokuda oluşan lokal hipoksiye bağlı olarak ortaya çıkardığı kanaatine varıldı. İleride yapılacak çalışmalarda dokudaki parsiyel oksijen basınç değerlendirilmesinin yapılmasıyla konunun daha da aydınlanacağını düşünmekteyiz.

Anahtar Kelimeler: Yara İyileşmesi, Lokal anestezikler

T Klin Araştırma 2001, 19:13-19

Received: May 09, 2000

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Wound is the distortion of normal anatomical and functional continuity of a living tissue. Wound healing is the well-regulated sequential process of various cellular and biochemical events to form a new tissue after tissue trauma.

Wound healing is an important subject for all surgical branches with ongoing intensive studies. There are series of studies still continuing about the effects of every drug or technique on wound healing. These studies have revealed a lot of factors affecting wound healing positively or negatively.

Distortion of wound healing in surgical patients increases the mortality and morbidity rates. Wound healing problems such as anastomotic leaks and prolonged healing of wounds are still problems of surgical clinics all around the world. Problems in wound healing increase not only the mortality and morbidity rates but also the hospital stay and the treatment costs. Therefore, wound healing should be taken into account before deciding the procedures and the drugs to be used in the treatment.

In this study, we investigated the local procedures which are frequently used in surgery clinics and all the other clinics. As known, local anesthetics are widely used in many surgical procedures. We wanted to investigate how local anesthetics affect wound healing. Recently, infiltration of the wound edges with local anesthetics after a procedure under general anesthesia is the subject of studies. With this technique, which is gaining popularity, local pain in the incision area and early postoperative pains are reduced, and respiratory problems are decreased after large abdominal and thoracal incisions.

In the present study, the effects of local anesthetics infiltrated in the wound edges after simple surgical procedures or after operations under general anesthesia are investigated with an experimental model and the results are discussed in the light of previous reports.

Materials and Method

This study was performed in the Experimental and Clinical Research Center of Erciyes University. Male Wistar-Albino type rats which were reproduced in the laboratory and fed with standard diets, weighing between 250 and 300gr, were used in the experiments. One control and three study groups, each containing 10 rats, were formed.

Group 1: Control group; received physiological saline.

Group 2: Prilocaine infiltrated group; (Citanest, %2 Prilocaine HC1, 20ml vial, Eczacibasi.)

Group 3: Lidocaine without vasoconstrictor was infiltrated. (Aritmal %2, 5ml, lOOmg Lidocaine HC1.)

Group 4: Lidocain plus vasoconstrictor. (Jetocaine, 2ml, 40mg Lidocaine HC1 + 0.025mg Adrenaline, Adeka.)

Surgical procedure: The rats underwent general anesthesia with intraperitoneal injection of 25-30mg/kg ketamin HC1. A11 the hairs in the abdominal skin was shaved and povidon iodine was used to clean the site of the incision. Abdominal skin was incised 5cm long till the fascia. After hemostasis, subcutaneous tissue of the wound edges were infiltrated with 2ml of local anesthetic agent in the study groups or physiological saline in the control group via an insulin injector without traumatising the tissue. Then, the wound edges were sutured with 4/0 atraumatic silk sutures using microsurgical instruments. The operation was completed after cleaning the wound with povidon iodine.

The rats were fed with standard diet in the postoperative period. In the postoperative 7th day, the rats were anesthetized again with ketamin HC1 and the wound area was cleaned with povidon iodine and the sutures were removed. After physical examination and the photography of the wounds and the rats, the wounds were scored as good (properly healed), moderate (partially healed) or bad (without healing). Then, the incision areas were excised together with 0.5cm adjacent tissue, one centimeter of the excised scar tissues was fixed with 10% formalin for histopathological examination. The specimens of 6 micron thick slides were examined in Hematoxylene-Eosin stain. The remaining specimen was rinsed with physiological saline and processed with Vistis freezediyer and freeze-dried procedure to form dry specimens before keeping it under -72°C temperature. The dry weights of the tissues were measured and hydrolized in a deiodinized glass tube with 6 N HC1 for 8 hours at 100°C temperature and, then processed with 5 N NaOH

Table 1. The distribution of wound healing status among groups after evaluation with physical examination

Group	good	moderate	bad
Control Group 1	6	3	1
Group 2 (Prilocaine)	4	3	3
Group 3 (Lidocaine)	4	4	2
Group 4 (Lidocaine+ Epinephrine)	2	5	5

till pH reached 6.6. Afterwards, spectrophotometry technique as described by Bergman et al. was used to measure the hydroxyproline levels (1). The HP levels in all of the groups were compared statistically with the help of Anowa-Scheffer test. The scores of the wound healing states were analysed according to X^2-test.

Results

After the physical examination and photography of the wounds the scoring was made and the results were represented in Table 1. There was no significant difference between all of the groups with the help of X^2-test.

The hydroxyproline levels for each gram dry weight of the tissues were shown in Table 2.

The histopathological examination revealed somewhat different features in each group.

1. Control group: A proper formation of granulation tissue was observed by the presence of connective tissue and increased neovascularisation. There were congested vessels in some fields and the findings showed a normal primary wound healing process. There was no necrosis or inflammatory cell infiltration.

2. Prilocaine group: There was granulation tissue formation with connective tissue and neovascularisation. Additionally, a few number of chronic inflammatory cells were observed. Generally, wound healing was good without any necrosis in all sections.

3. Lidocaine group: There was an increase of connective tissue formation and neovascularization in an irregular fashion. A regular granulation tissue was not detected. Some fields revealed intensive chronic inflammatory cell infiltration.

4. Lidocaine plus adrenaline group: There was no evidence of granulation tissue formation in this group. There was fibrin and polymorphonuclear leukocytes with cellular debris. Wound healing was not observed and some fields showed fibrin and necrosis findings.

Table 2. The distribution of hydroxyproline levels among groups (microgram/gram)

Rat No	Group I	Group II	Group III	Group IV
1	109. 48	97. 63	92. 44	59. 83
2	92. 62	86. 61	72. 79	74. 08
3	97. 39	115. 99	119.23	52. 92
4	103. 22	76. 03	95. 04	55. 08
5	97. 39	94. 17	84. 24	61. 12
6	98. 00	93. 74	92.44	59. 18
7	107. 04	91. 58	95.47	60. 04
8	98. 60	95. 04	89. 42	60. 03
9	103.25	93. 74	92. 23	60. 04
10	101. 27	93. 75	92. 44	60. 04

Groups	n	Mean hydroxyproline levels ± SE
1	10	108±5.0
2	10	93.8±9.9
3	10	92. 6±11.5
4	10	58. 2±8.4

f=44.55

As a result, the wound healing was not good in the fourth group. In the other groups, there were signs of wound healing; however, local anesthetic received groups showed some degrees of chronic inflammatory cell infiltration. The healing in the control group was satisfactory to a large extend.

The Anowa-Scheffer test for the significance of differences in the hydroxyproline (HP) levels in all of the groups was shown The different HP levels in the control group , the second group and the third group were statistically insignificant ($p>0.05$). The values in the fourth group were significantly lower than the control group (pO.OOT). There was a significant difference between the fourth group and the second group and the third group (pO.OOT).

Discussion

Various studies have been and are still being performed on wound healing by different surgical departments. The measurement of tissue HP levels is the most widely used method to assess wound healing. Besides, baric measurements in anastomotic wounds or tension force measurements are other widely used evaluation criteria. However, histopathological examination is the hallmark way of evaluating wound healing.

Wound healing is one of the basic subjects of surgery. Any disturbance in wound healing can sometimes easily lead to drastic outcomes. A lot of factors have been studied for their effects on wound healing.

In gastrointestinal surgery, wound healing have been widely studied, especially in colon anastomosis cases. In a study, the sutures placed in close vicinity to each other have been found to affect wound healing negatively by the observation of decreased dermal seperation pressure (2).

Yesilkaya et al.(3) have compared, in a similar study involving distal colon anastomoses, the single layered and double layered anastomosis. They have found the wound healing being better in the single layer sutured group together with a significantly higher level of HP in the wound tissue. In the double layer sutured anastomosis, the blood flow was found to be decreased and the resulting ischemia distorted the wound healing together with a reduced HP level (3).

Local anesthetic agents have a variety of usage. In the simple surgical procedures local anesthesia is the most preferred mode of anesthesia. There are some results related to the surgical procedures performed in this manner. Bonadio studied 103 children with minor trauma and performed a total of 670 sutures after topical application of tetracaine + epinephrine + cocaine solution. He obtained a sufficient level of anesthesia with a good wound healing except for one patient and observed no side effects (4). The same author studied another 75 children with face and lip trauma, and applied the same solution topically with no side effects or any distortion of wound healing (5).

Sinclair et al. used local anesthetics to decrease postoperative incisional pain. Ten patients undergoing inguinal herniorapby received lidocaine aerosol on wound edges just before skin closure, 10 patients received placebo aerosol and another 10 patients received no topical agent. In the postoperative first day, the study group had significant decreases in pain scores and they had significantly less need to have analgesics. The plasma lidocaine levels were very low and there was no side effect or negative effect on wound healing in the study group (6).

Wakamatsu et al.(8) studied the effects of lidocaine and lidocaine + epinephrine solutions at 1/20000, 1/80000 and 1/200000 concentrations on wound healing with comparison to a control group. The second molar tooth was extracted with instillation of local anesthetics on general anesthesia. Control group received no instillation. The wounds were examined histopathologically in the postoperative 1st and 14th days. A11 the wounds healed without complication in all of the groups. There are some studies related to the effects of lidocaine to delay wound healing (7). Besides that, lidocaine has been found to decrease the number of leukocytes, migration and the metabolic effects, therefore causing distortion in wound healing (8).

In another study to show the effects of two xylocaine preparations on the dermal wound healing of albino rats, xylocaine was found to decrease the epitelization and tension force in the wound in both gel and solution forms. However, the basic compounds of xylocaine gel and solution forms increased reepitelization without affecting tension

force. The evidence suggested that the topical agents should be carefully and selectively used for their effects on wound healing (9).

Benzofracaine, etaden and xanthinol nicotinamide were studied on mice for their effects on wound healing. BF at doses of 15, 30, 60 and 120 mg/kg, etaden (20 mg/kg) and xanthinol were intramuscularly given in the 1st and 14th days of postmechanic injury. Wound healing and microcirculation around the wound normalized quickly. While xanthinol and etaden proved to be beneficial, benzofracaine had the optimal dose of 60mg/kg in the wound healing process.Wound healing with benzofracaine and etaden at the 14th day was observed to be 57% and 28.6%, respectively (10).

Oxygen has an important role in wound healing. Tissue oxygenation is vital for collagen synthesis, angiogenesis, epithelization and resistance to infection in the wound. Oxygen is needed for the hydroxylation of lysine and proline. Epithelial replication and simultaneous angiogenesis need oxygen, as well. There is an increased tendency to infection in the ischemic tissue. Oxygen is also needed for destruction of bacteria by leukocytes and the stimulation of phagocytosis. Destruction of tissue perfusion disturbs wound healing after operation (11-14).

Arterial blood pressure , heart rate and urinary output in the operated patient are indirect measures of tissue perfusion. The direct measure can be the evaluation of tissue oxygenation. In a study by Hartman et al. (15) 15 patients in the control group and 14 patients in the treatment group were evaluated for the subcutaneous partial oxygen pressure $Psc0_2$ with a preplaced subcutaneous silicon catheter. The values were taken in the 2-4th hours and the 1st and 2nd days of postoperative period. The patients were oxygenated with 60-70% oxygen via a mask. The control group received standard fluid replacement while the study group received an additional 500-2300 ml of 2.5% glucose and physiological saline solutions. The statistical analysis showed a significantly higher level of HP in the treatment group in the postoperative 7th day. As a result, the patients with increased levels of tissue perfusion had higher levels of HP in the wound tissue with increased granulation tissue and resulting good wound healing.

In a similar study, 44 patients undergoing major abdominal operations were investigated with their tissue oxygen pressures. One group received the fluid replacement according to the heart rate, blood pressure and urinary output and the other group of patients received fluids with the monitorization of tissue partial oxygen pressure. In the postoperative 7th day, tissue HP levels were measured and found to be higher in the second group, which led to the idea that the tissue oxygen pressure was a good index of tissue perfusion (16).

Direct measurement of tissue oxygen is important for the follow-up of postoperative patient. After major abdominal, cardiac and vascular operations, postoperative perfusion can be disturbed with resulting wound hypoxia and that can distort wound healing. Therefore, after these major operations, the patients' tissue perfusion should be well regulated and the tissue oxygen pressures should be well-monitored (17).

In some operations, it is possible to monitor $p0_2$ with a preplaced special catheter. In a study, a preplaced silicon catheter is used to monitor $p0_2$ of colon anastomosis in operated rats. In this study, continuous sutures were compared with the interrupted ones and $p0_2$ was found to be lower in the first one and HP levels were higher in the second group. As a result, hypoxia in the wound edge was found to destroy wound healing (18).

The evaluation of HP levels in the studies of wound healing is the most important objective criterion for the evaluation of wound healing. The levels increase in the wound especially in the fifth and seventh postoperative days. The HP levels detected in these postoperative days will give much information about the state of wound healing. The tension force measurement of the wound is another method used in the studies. The baric measurements of anastomotic bursts in anastomosis cases and the evaluation of dermal seperation pressures of incision are other used methods. However, the high levels of HP with a good progression of collagen synthesis in the wound will correlate with the seperation pressures of the incision. Therefore, in the present study, HP levels are regarded to assess the state of wound healing.

Evaluation of wound healing with physical examination is possible. The observation of a thor-

ough healing of the incision can be regarded as a normal wound healing. However, some healing defects in the incision line can relate to a defective wound healing process. In this study, physical examination was also used to assess wound healing together with HP levels. The photography of the wound after the removal of sutures in the seventh day were examined to score the state of wound healing as good, moderate or bad. Histopathological examination of granulation tissue correlated well with these other techniques of evaluation.

The histopathological examination of the control group showed well-developed granulation tissue. The same group had higher levels of HP and a good output in the physical examination with a good wound healing in 6 of 10 rats.

The 2nd and 3rd groups, which received local anesthetics, showed the formation of granulation tissue histopathologically. Even though the HP levels were lower than that of the control group there was no statistically significant difference. The examinations of the incisions showed a good healing in 4 of 10 rats in the local anesthetic groups, somewhat in lower rate than the control group but without statistical significance. The granulation tissue formation in the histopathological examination in the control group and local anesthetic groups were not different. Therefore, the results can not suggest the possibility of wound healing defects with the use of local anesthetics. In fact, the formation of granulation tissue is the evidence for normal wound healing. However, local anesthetics could be partially accused to cause delay in wound healing. We partially agree with the idea that local anesthetics distort wound healing by increasing the wound infection rate. However, the addition of vasoconstrictors to local anesthetics to prolong the anesthesia time can affect the results significantly. The vasoconstriction can decrease tissue oxygenation by decreasing the regional blood flow. If the important place of oxygen in the process of wound healing is thought, the resulting hypoxia can be said to delay or distortion in wound healing. Especially the tissues with limited oxygen supply such as finger tips, ear lobule or nose tips will be much affected from this situation. The results of the present study prove the fact with the lower level of HP in the wound and the histopathological findings with insufficient formation of granulation tissue and necrotic foci in the wound tissue. The physical examination in this fourth group revealed a good healing in only two rats. Therefore, the addition of vasoconstrictors to local anesthetics will disturb wound healing but the long term results could not be discussed out of this study. Since the wound healing is a continuous process, the long term results can not be learnt from this study. Therefore, it is necessary to carry out studies with long term results.

As a result, local anesthetics can be accused to disturb wound healing; however, the worst effect will be with the addition of vasoconstrictors to local anesthetics. Therefore, the dose and the injection site of local anesthetics should be carefully decided. Especially in the anesthesia of tip parts, vasoconstrictors should be avoided to prevent defective wound healing and development of tissue loss after the process of necrosis.

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