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EXPERIMENTAL STUDY

Intraperitoneal EMLA (lidocaine/prilocaine) to prevent abdominal adhesion formation in a rat peritonitis model

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Abstract: *Objective:* The accelerative effect of EMLA (eutectic mixture of lidocaine 2.5 % and prilocaine 2.5 %) in the wound healing process is known. We hypothesised that post-operative peritoneal adhesions may be reduced with intra-peritoneal EMLA administration in a model of bacterial peritonitis.
Study design: Bacterial peritonitis was induced in 24 rats by cecal ligation and puncture. The rats were randomly assigned to one of four groups. Group 1 (n=6)) received EMLA intraperitoneally, group 2 (n=6) received 2 % lidocaine hcl solution intraperitoneally, the third group received one dose (100 mg/kg) of ceftriaxone sodium (Rocephin^R, Roche, 1 g) intraperitoneally one day after cecal ligation and puncture procedure, and in control group (group 4, n=6), no fluid or medicine was introduced into the abdomens of the rats. All animals were killed 14 days later in order to assess the adhesion score. Tissue antioxidant levels were measured in 1 g tissue samples taken from the abdominal wall.
Results: The adhesion score was significantly lower in the EMLA group than in the lidocaine and control groups. The catalase levels were higher in the lidocaine and control groups than in EMLA group.
Conclusions: Intraperitoneal EMLA inhibited the formation of postoperative intra-abdominal adhesions without compromising the wound healing in this bacterial peritonitis rat model. EMLA also decreased the oxidative stress during peritonitis *(Tab. 1, Fig. 7, Ref. 27)*. Full Text (Free, PDF) *www.bmj.sk.*
Key words: intraperitoneal EMLA, lidocaine/prilocaine, prevent abdominal adhesion, peritonitis model.

The inflammatory response has been recognized as a common cause in all pathways for adhesion formation (1). Adhesions are the results of the inflammatory response to tissue injury in the peritoneal space.

Many animal studies have shown that local anesthetics have anti-inflammatory effects. These effects were related to the inhibition of neutrophils with these agents (2–4). The anti-inflammatory effects may impair the host's defence, but may also improve the condition of destructive inflammation.

There are no reports on the inhibition of adhesion formation of EMLA cream (eutectic mixture of lidocaine 2.5 % and prilocaine 2.5 %). We hypothesised that post-operative peritoneal adhesions may be reduced with intra-peritoneal EMLA administration in a model (cecal ligation and puncture) of bacterial peritonitis.

Materials and methods

Twenty-four female Wistar rats weighing 200–220 g were divided into four random groups. Animals were housed at 21 °C and given standard rat chow diet and water *ad libitum*. The study protocol was approved by the Animal Ethics Review Committee of the Faculty of Medicine, University of Kahramanmaras.

Surgical procedures

After overnight fasting, all animals were anesthetized with a mixture of 40 mg/kg ketamine and 5 mg/kg xylazine hydrochloride. In all rats, bacterial peritonitis was induced by performing a cecal ligation and puncture (CLP) procedure using the methods of Wichterman et al (5). The abdomen was shaven and swabbed with povidone iodine solution preoperatively. The same researcher performed all surgical procedures. A 3-cm midline incision was made and the abdomen was opened under clean surgical condi-

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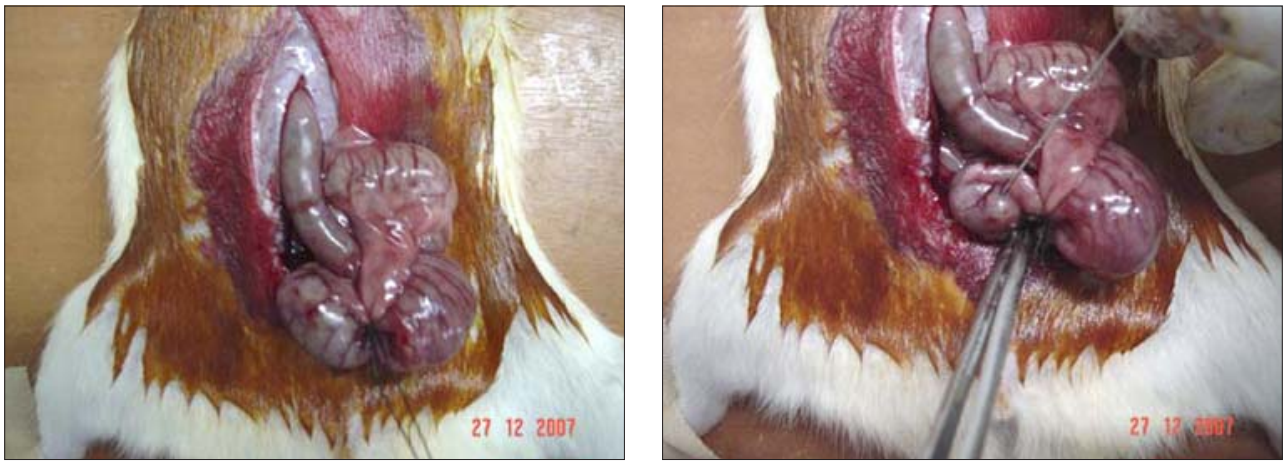


Fig. 1. The cecal was ligated just proximal to the ileocecal valve, with a 3-0 silk suture, and at the anti-mesenterial site the cecal was punctured twice with a 22-gauge needle.

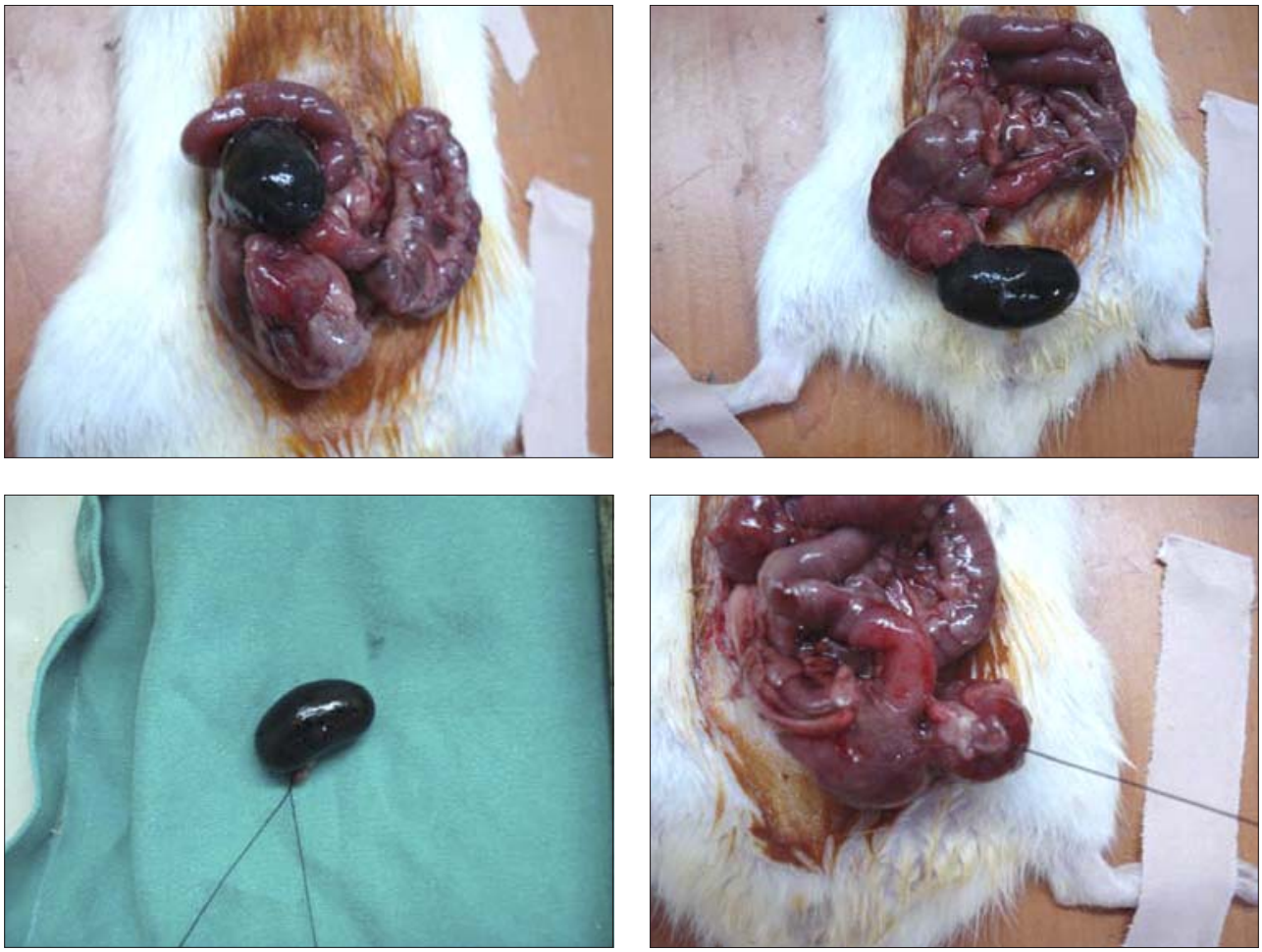


Fig. 2. Necrotic stump was isolated and resected.

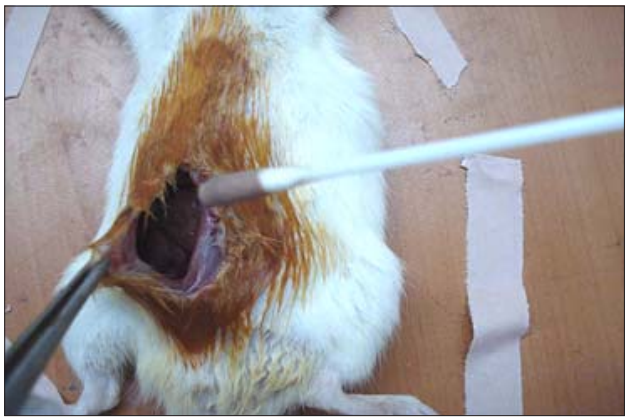


Fig. 3. Microbiological examination.

Fig. 4. Administration of 5 % EMLA cream (eutectic mixture of lidocaine 2.5 % and prilocaine 2.5 %, Astra Zeneca, Sweden).

tions. The caecum was dissected without damaging the vascularization and refilled backwards with faeces. Thereafter, the caecum was ligated just proximal to the ileocecal valve, with a 3/0-silk suture, and at the anti-mesenterial site it was punctured twice with a 22-gauge needle, squeezed gently to force out a small amount of faeces, and then returned to the abdominal cavity (Fig. 1). Thereafter the midline incision was closed in one layer with a 3/0-silk suture. Immediately after the operation, all animals were resuscitated with 5 ml of isotonic sodium chloride solution administered subcutaneously. On day 1, all animals were reoperated on under anaesthesia, and peritoneal fluid samples were taken for microbiological examination to examine the state of peritonitis (Fig. 2). The ligated cecal stump was isolated and resected (Fig. 3). Before closing the abdomen, animals were randomly assigned to receive either 1 g of 5 g EMLA cream (eutectic mixture of lidocaine 2.5 % and prilocaine 2.5 %, Astra Zeneca, Sweden) intraperitoneally (Fig. 4) (group 1, n=6), or 1 mg/kg of 2 % lidocain hcl solution (Jetokain Simplex 20 mg/2 mL, Adeka, Turkey) intraperitoneally (group 2, n=6). The third group received one dose (100 mg/kg) of ceftriaxone sodium (Rocephinr, Roche, 1gr) intraperitoneally one day after CLP procedure while in control group (group 4, n=6), no fluid or medicine was introduced

Tab. 1. Cumulative adhesion scoring scale (7).

Points	
0	No adhesions
+1	One adhesion band from the omentum to the target organ
+1	One adhesion band from the omentum to the abdominal scar
+1	One adhesion band from the omentum to another place
+1	One adhesion band from the adnexa/epididymal fat bodies to the target organ
+1	One adhesion band from the adnexa/epididymal fat bodies to the abdominal scar
+1	One adhesion band from the adnexa/epididymal fat bodies to another place
+1	Any adhesive band other than described above
+1	Target organ adherent to the abdominal wall
+1	Target organ adherent to the abdominal scar
+1	Target organ adherent to the bowel
+1	Target organ adherent to the liver or the spleen
+1	Any other organ adherent

* Target organ was scraping serosa of cecum.

into the abdomens of the rats. On the first postoperative day, all animals were given only water; standard rat chow and water were provided on the second postoperative day.

Microbiological examination

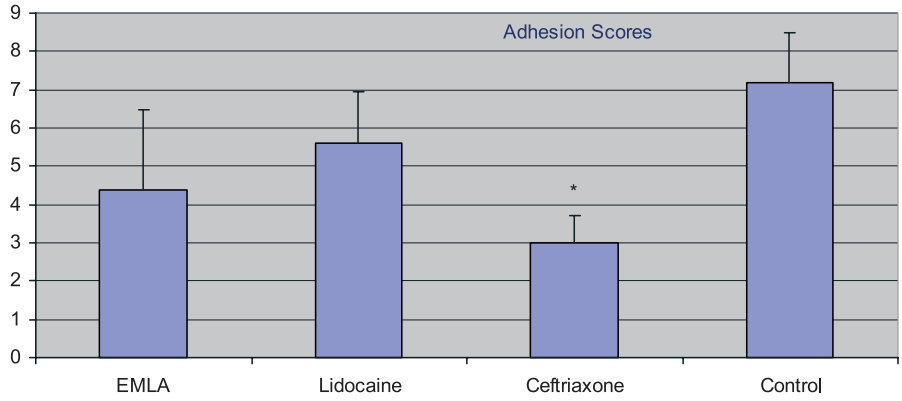
Samples of peritoneal fluid were cultured in aerobic and anaerobic conditions. For aerobic cultivation, the samples were inoculated onto 5 % sheep blood agar, chocolate agar and Mac Conkey's agar, and incubated for 24–48 h at 35 °C. For anaerobic cultivation, samples were inoculated onto chocolate agar, Mac Conkey's agar, 5 % anaerobic sheep blood agar containing canamycin and vancomycin, and incubated for 48–72 h at 35 °C in a GasPak anaerobic system. In addition, the samples obtained by swabbing for the anaerobes were taken into the enriched thioglycolate broth, and incubated for 4–7 days at 35 °C in a GasPak anaerobic jar (AnaeroGen, OXOID, Basingstoke, England). Aerobic microorganisms were identified by standard laboratory methods and API ID32E. Anaerobic microorganisms were identified by using OXOID Anident discs.

Evaluation of adhesions

All rats were killed on the 14th day by being anaesthetized with an overdose of ethyl ether before relaparotomy. The abdomen was opened with an inverted U incision. One point was given for each adhesion and a cumulative adhesion score was calculated. Adhesions were scored in a blinded manner according to the method of Bothin et al (Tab. 1) (6).

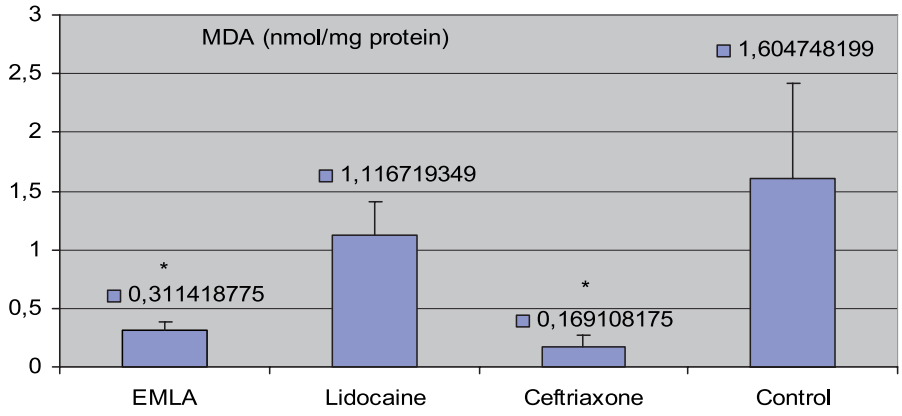
Antioxidant study

In order to determine tissue antioxidant levels, 1x1 cm^2. Tissue samples were taken from the lateral of the incision line on the abdominal wall. The samples were preserved in a deep freezer until examination. The tissues were homogenized with three



The differences of adhesion scores among all the groups were significant **(P<0.05, Kruskal-Wallis test)**
* The adhesion score of ceftriaxiaxone group were significantly lower than the control groups. **(P<0.05, Mann-Withney-*U*)**

Fig. 5. Adhesion scores.



MDA levels in control and lidocaine groups were higher than in the EMLA and ceftriaxone groups.
The differences of MDA levels among all the groups were significant **(P<0.05, Kruskal-Wallis test).**
* The MDA levels of EMLA and ceftriaxone group were significantly lower than the lidocaine and control groups. **(P<0.05, Mann-Withney-*U*)**

Fig. 6. MDA levels (nmol/mg protein).

volumes of ice-cold 1.15 % KCI. The activities of antioxidant enzymes and the levels of lipid peroxidation were measured in the supernatant obtained from centrifugation at 14 000 r.p.m. Superoxide dismutase (SOD) activity was measured according to the method described by Fridovich (7). Catalase (CAT) activities were determined by measuring the decrease in hydrogen peroxide concentration at 230 nm by the method of Beutler (8). Lipid peroxidation level in tissue samples was expressed in malondialdehyde and measured according to the procedure of Ohkawa et al (9). Protein concentration was determined according to the method of Lowry (10).

Morbidity and mortality

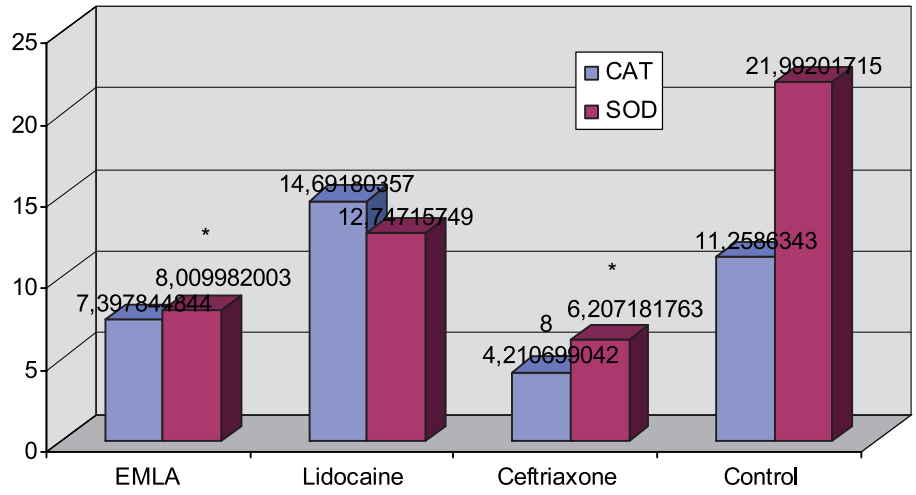
Dead rats were autopsied and the causes of deaths were recorded. The presence of intra-abdominal abscesses was noted.

Statistical analysis

All variables were expressed as mean and range. Differences between groups were evaluated by Kruskal-Wallis variance analysis followed by a Mann–Whitney U-test. p-values <0.05 were considered statistically significant. All data were entered into and processed by SPSS 9.05 for Windows statistical package.

Results

One point was given for each adhesion and a cumulative adhesion score was calculated. Results of the adhesion scores in all rats are presented in Figure 5. The mean scores of the animals killed at the end of week 2 were summarized as: group 1, 4.4; group 2, 5.8; group 3, 3 and group 4, 7.2. The differences of adhesion scores among all the groups were significant (p<0.05,



* The SOD levels of EMLA and ceftriaxiaxone group were significantly lower than the control and lidocaine groups. **(P<0.05, Mann-Withney-*U*)**
* The CAT levels of ceftriaxiaxone group were significantly lower than the control group. **(P<0.05, Mann-Withney-*U*)**
The differences of CAT and SOD levels among all the groups were significant **(P<0.05, Kruskal-Wallis test)**

Fig. 7. SOD and CAT levels.

Kruskal–Wallis test). In comparison with the lidocaine and control groups, the adhesion score in the antibiotic treated groups were significantly lower ($p<0.05$, Mann–Whitney test). Adhesion scores of EMLA treated groups were significantly lower according to the control group ($p>0.05$), but it did not reach statistical significance.

When antioxidant levels of the lateral wall of the abdomen were evaluated on day 14, the MDA values of EMLA and the antibiotic-treated groups were significantly lower than in the control group ($p<0.05$), and levels in the lidocaine group were higher than in these treated groups (Fig. 6). CAT levels were high in control and lidocaine groups, however only the differences to the antibiotic-treated group were statistically significant. When the SOD levels were taken into account, the control group was higher than the EMLA and antibiotic-treated groups (statistically siginificant). The results of the lidocaine group for SOD were proved to be higher compared to EMLA and antibiotic-treated groups. Nevertheless they did not reach statistical significance (Fig. 7).

The culture results of the samples taken on the first postoperative day revealed polymicrobial intra-abdominal infection. The most frequently isolated microorganisms were Escherichia coli, Enterobacter aerogenes, Proteus mirabilis, Proteus vulgaris, group D Streptococcus, Enterococcus, Staphylococcus aureus. Clostridium difficile and Bacteroides fragilis were the most frequently isolated anaerobic agents. Within 1 day after CLP, 1 rat in each group died due to sepsis.

Discussion

EMLA cream is widely being used in various superficial surgical procedures such as needle insertion, circumcision, skin biopsies and skin grafting (11, 12). In recent years, some researchers have shown that local anesthetics impair normal wound healing (13, 14) whereas Karacal et al have shown that local anesthetics have a benefical effect on skin flap survival due to vasomotor activities (15) while Goodman has shown that wound healing appeared to be faster (16). It is known that wound healing and adhesion formation have similar pathways, following the sequence of tissue inflammation, fibrin deposition, fibrin organization, collagen formation, and maturation (17, 18).

EMLA is effective in inhibiting the movement of leucocytes and phagocytosis (19) and it is shown that EMLA has antibacterial properties *in vitro*. The results of Kerenyi et al study show that EMLA killed E. coli and P. aeruginosa within 1 hr, Micrococcus spp. after 1 hr, and S. aureus after 3 hrs of exposure at room temperature (20). These results suggest that EMLA has antibacterial properties in vitro. We have established our present study on the assumption that EMLA may be beneficial in infection control as well as in reducing the intraabdominal adhesions. In our present study, bacterial peritonitis was induced by performing the CLP procedure. A significantly lower score of adhesions in the abdominal cavity was found 14 days after the instillation of EMLA when compared with lidocaine group and no fluid-no medicine instillation group (control group) but not lower than in antibiotic-treated group. This findings may support the thesis of beneficial effect of EMLA on bacterial peritonitis.

The essential importance of the findings presented in this study resides in how EMLA shows this effect. Several *in vitro* studies have demonstrated a reduced production of free oxygen radicals from neutrophils after treatment with local anesthetics (21, 22). Other *in vivo* models documented reduced adherence and migration of leucocytes (2, 3, 23). Local anesthetics inhibit

the movement of leucocytes and phagocytosis *in vitro* (4, 22). This is important because phagocytes are essential for controlling infections and are the mediators of inflammation (24, 25). The anti-inflammatory effects may impair the host defence, however they may also improve the conditions of destructive inflammation. In Azuma Y et al study, local anesthetics caused not only the modulation of neutrophil functions in host defense, but also the inhibition of bacterial growth. This is the effect that we mainly require in reducing the intraperitoneal adhesion formation. In our present study, lower adhesion score and MDA levels support this thesis. It is an interesting fact of our study that the results of antibiotic-treated group was better than EMLA and other groups. This result has showed that EMLA is effective in reducing the adhesion formation but its antibacterial effect is not as good as that achieved by antibiotic therapy.

Lidocaine at 10 mg/ml reduced the bacterial growth, while lidocaine at 2 mg/ml did not inhibit the bacterial growth (26). It is likely that the use of local anesthetics at 1 mg/ml causes an increase in the infection rate or a delay in recovery by inhibiting the neutrophil functions. Similar results were obtained in the present study. It indicates that the treatment with EMLA led to lower SOD and lower MDA levels when compared to those in the group treated with lidocaine. Moreover, the treatment with antibiotics resulted in lower SOD and lower MDA levels when compared to the control, EMLA and lidocaine groups. These results are consistent with the idea that an oxidant/antioxidant imbalance is involved in animal peritonitis. The use of EMLA in peritonitis was effective in decreasing the oxidative stress of tissue during peritonitis and it is more conceivable that EMLA is more effective than lidocaine for potentiating superoxide anion production. This effect depends on the usage of lidocaine (jetocaine simplex 2 %, 20 mg/2 mL) low dose (1 mg/kg) and 1 g of 5 g EMLA 5 % cream (lidocaine 25 mg, prilocaine 25 mg). Similiar dose-related effects have been shown by Azuma et al. In their study, lidocaine at a dose of 1 mg/ml caused an almost 50 % inhibition of phagocytosis of E. coli in neutrophils. In contrast, these at doses of 0.01 and 0.1 mg/ml failed to affect phagocytosis (27).

We concluded that intraperitoneal EMLA decreases the incidence of postoperative intra-abdominal adhesion formation without impairing the healing of wound in rats bacterial peritonitis model. Further experiments are necessary to elucidate the mechanisms of action of EMLA in a bacterial peritonitis environment. Some degree of oxidative stress occurs in most, if not in al, human diseases and the major question to be answered is whether it makes a significant contribution to the disease pathology.

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