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Influence of Lidocaine on Leukocyte Function in the Surgical Wound

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The inflammatory response of the wound is mediated to a large extent by leukocytes, which play an important role in the wound healing process. Local anesthetics, which are routinely administered before minor skin surgery and for postoperative pain relief, have been shown to have diverse effects on wound healing. Local anesthetics have also been reported to induce potent inhibition of leukocytes *in vitro*, although their effects on leukocyte activity in the surgical wound have not been elucidated. The present study investigated the *in vivo* effects of lidocaine on leukocyte function in the surgical wound of rats by sampling leukocytes from hollow titanium implants. The surgical wound was treated with lidocaine or placebo after implantation of the titanium chamber and before skin closure. Leukocyte metabolic activity was measured by chemiluminescense. Cell count was analyzed in a Bürker chamber. Results showed progressive increase in leukocyte counts in the wounds of control animals and significantly lower cell counts in the wounds of lidocaine-treated animals 48 h ($P < 0.05$) and 72 h ($P < 0.05$) after surgery. A pronounced inhibition of the metabolic response to serum-opsonized zymosan was seen after 8 h in the lidocaine-treated animals *versus* controls ($P < 0.05$). After 24 h, leukocyte metabolic activity decreased dramatically in the control group and remained at a low level until 72 h after surgery. In the lidocaine-treated group, the leukocyte response to zymosan remained constantly low throughout the study. The effects of lidocaine were not a result of impaired leukocyte viability. Our results showed that administration of lidocaine in the surgical wound reduced leukocyte migration and metabolic activation in the wound area. These *in vivo* results suggest, in accordance with previous *in vitro* studies, a reduced release of tissue-toxic substances such as oxygen free radicals and lysozymes known to impair wound healing. Previous reports on improved wound healing with lidocaine may be explained by the present results. (Key words: Anesthetics, local: lidocaine. Anesthetic techniques: topical. Biocompatible materials. Blood: leukocytes. Inflammation. Measurement techniques: luminescence. Titanium. Wound healing.)

DAMAGE TO THE SKIN will invariably induce an inflammatory reaction. A major component of this reaction is the migration of leukocytes into the wound, where these cells play an important role in the defense against foreign agents, removal of tissue debris, and wound healing.[1] In a sterile wound, however, excessive production of tissue-toxic substances by the leukocytes will primarily cause tissue damage leading to impaired wound healing.[2,3]

Local anesthetics are routinely used to prevent pain in connection with minor surgery and for postoperative pain relief. The effects of local anesthetics on wound healing have therefore attracted the interest of several investigators, but with conflicting results. Some investigators have reported delayed wound healing with local anesthetics,[4] whereas others have reported no negative effects[5] or even improved wound healing.[6,¶] These conflicting reports on wound healing and the importance of leukocytes in this process led us to investigate the actions of local anesthetics on leukocyte activity in the area of surgery.

Previous studies have shown that amide local anesthetics interfere with various steps of the inflammatory response of leukocytes.[7–15] However, little information is available regarding the *in vivo* effects of local anesthetics on leukocyte activity in the area of surgery. In the present *in vivo* study, leukocytes were sampled from the wound by means of a titanium implant,[16] and the effects of lidocaine administered in the surgical wound on leukocyte accumulation and metabolic activation were investigated.

Materials and Methods

Experiments were performed on male Sprague-Dawley rats weighing 250–300 g. The experimental protocol was approved by the Animal Use Committee. Animals were housed for at least 7 days prior to experiments in a ventilated and temperature-controlled room and had food

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¶ Missavage AE, Gunther RA: Healing of porcine wounds with topical lidocaine cream (abstract). Proceedings of the American Burn Association 22:172, 1990.

and water available *ad libitum*. The day–night cycle was constant, with 12 h light and 12 h dark. Anesthesia was induced with a mixture of diazepam (5 mg/ml), pentobarbital (Nembutal) (50 mg/ml), and isotonic saline in a 2:1:1 volume ratio (0.15 ml/100 g body weight) administered intraperitoneally.

In all experiments, a midline incision (1–1.5 cm) was made through the abdominal skin, which had been closely shaved and sterilized by an iodine solution (0.1% Jodopax® in ethanol). After blunt dissection of the right rectus muscle, a titanium chamber was placed in direct contact with the peritoneum[16] (fig. 1). The rectus sheath and fascia were closed over the chamber by resorbable sutures (Vicryl® 5-0, Ethicon). The skin was sutured with two single silk sutures (3-0, Ethicon).

The fenestrated hollow chambers were made of commercially pure titanium and ultrasonically cleaned for 15 min in ethanol (70%), trichloroethylene, and ethanol (70%), in that order, and then kept in absolute ethanol until implantation, before which they were rinsed in sterile isotonic saline. In one group of rats (n = 24), the surgical wound was pretreated with 10 mg lidocaine (0.1 ml, lidocaine aerosol, 100 mg/ml) prior to implantation of the chamber. In the second group of rats (n = 24), the wound was treated with a corresponding volume of a placebo aerosol with identical contents except for lidocaine. We chose topical administration of the aerosol solutions rather than infiltration of the local anesthetic because of the adhesive properties of the aerosol to biologic tissues, since the loose skin of the rat does not allow an infiltration to remain in the area of administration. Before skin closure, 30 μl phenol-red-free Hank's balanced salt solution (HBSS) was injected into the titanium chamber in all of the animals (experimental and control).

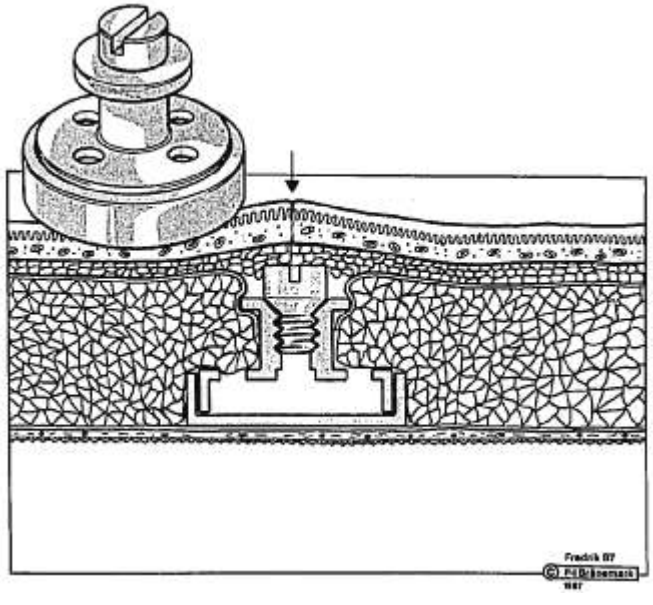

FIG. 1. Cross section of the titanium chamber illustrating the procedure by which exudate is sampled. After reincision of the skin, the cover screw is removed; a syringe is inserted into the body of the chamber; and its content is aspirated for further analysis. (Reproduced from Eriksson *et al.*,[16] with permission from Butterworth-Heinemann Ltd.)

After 8 h (n = 6 lidocaine, n = 6 control), 24 h (n = 6 lidocaine, n = 6 control), 48 h (n = 6 lidocaine, n = 6 control), and 72 h (n = 6 lidocaine, n = 6 control), the rats were killed by administration of 2.5 mg diazepam and 25 mg pentobarbital intraperitoneally followed by cutting of the heart. Subsequently, the surgical wound was reopened, and the chamber contents were collected by repeated washing with HBSS followed by aspiration, yielding a total sample of 150 μl. A volume of 5 μl of the collected exudate was used for counting the total number of leukocytes in a Bürker chamber. Leukocyte viability was measured by trypan blue dye exclusion in order to exclude the possibility that the results of the study were a consequence of cell death. Differential cell counts were performed on slides after Ehrlich's hematoxylin-eosin staining.

The remaining exudate was immediately diluted with HBSS and adjusted to 0.5×10^6 leukocytes/ml and kept on ice until analysis of leukocyte metabolic activation by chemiluminescense.[17] Zymosan particles (Sigma), used to stimulate phagocytic activity, were washed in HBSS and incubated with rat serum for 30 min at 37° C. The particles were subsequently washed and suspended in HBSS to a concentration of 12.5 mg/ml. Luminol (5-amino-2,3-dihydro-1,4-phtalazinedione) (Sigma) was dissolved in dimethyl sulfoxide (Merck, Germany) to give 5.5 mg/ml, stored at −20° C, and diluted 1:20 in HBSS before use. Before analysis by chemiluminescense, the cell suspension (100 μl) was preincubated in a plastic vial for 10 min at 37° C. The chemiluminescense reaction was started by addition of luminol (100 μl) and rat serum-opsonized zymosan particles (100 μl). Chemiluminescense recordings were performed at 37° C in a luminometer (LKB 1251, Wallac, Finland) and recorded by computer at 40-s intervals for 30 min. The peak chemiluminescense (millivolts) was determined and used for statistical analysis. All of the analyses were performed by an assistant blinded to the study protocol.

All data were subject to analysis of variance followed by Duncan's multiple-range test for determination of significant differences at the 5% level. Contrasts were formed at a 99% confidence interval. Data are mean ± SEM.

Results

In control animals, the number of leukocytes in the surgical wound was progressively increased and reached a level significantly greater than that in lidocaine-treated animals at 48 h ($P < 0.05$) and 72 h ($P < 0.05$) after surgery (fig. 2).

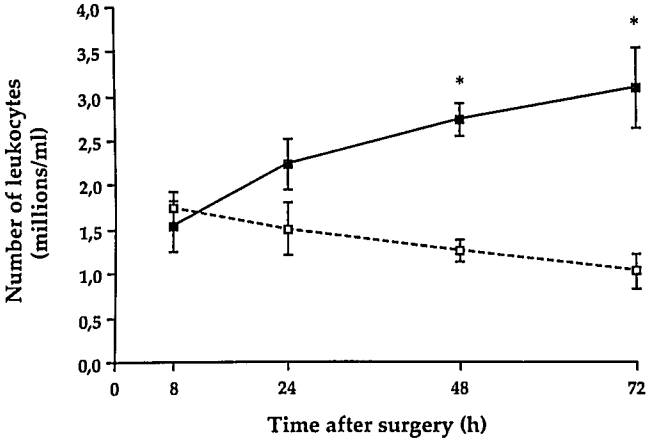

FIG. 2. Granulocyte count in exudate sampled from a titanium implant in the surgical wound of animals topically treated with lidocaine (dashed line) and in control animals (solid line). Leukocyte count was performed in a Bürker chamber. *$P < 0.05$ *versus* control group. Other differences between the groups were not significant. Data are mean ± SEM.

Leukocyte metabolic responsiveness measured by chemiluminescense was significantly greater in the control group 8 h after surgery as compared to lidocaine-treated animals ($P < 0.05$) (fig. 3). Chemiluminescense was markedly reduced 24 h after surgery in the control group and remained low. In the lidocaine group, leukocyte metabolic responsiveness was constantly low throughout the study (fig. 3).

Leukocyte viability (percent) after surgery did not differ significantly between the groups at 8 h (lidocaine 99.3 ± 0.38, control 99.8 ± 0.15), or 24 h (lidocaine 89.5 ± 1.71, control 90.8 ± 0.83). Differences between the groups were significant at 48 h (lidocaine 87.0 ± 1.20, control 78.5 ± 2.34) ($P < 0.05$) and 72 h (lidocaine 91.3 ± 1.99, control 81.5 ± 3.19) ($P < 0.05$) after surgery.

Discussion

In the present study we used a titanium chamber to harvest wound exudate for further *in vitro* analysis. This could be questioned on the grounds that the chamber itself may add to the inflammatory reaction induced by the surgical trauma. However, pure titanium has been shown to have good biocompatible properties, as was demonstrated in soft tissues where titanium induced an inflammatory reaction significantly less than that observed with commonly used polymer surgical implants.[16–18] This biocompatibility of titanium has also been observed in patients in whom screw-shaped titanium implants have been used to anchor artificial teeth.[19] In clinical practice, where interactions between cells and implants may play a crucial role for the acceptance of biomaterials,[20] the use of agents with antiinflammatory properties could have potential influence on the healing of surgical implants. Although the present study shows that the inflammatory response is favorably modulated by lidocaine, its possible effects on the unwanted fibrous capsule formation invariably seen around soft tissue implants remains to be shown.

The attraction of leukocytes to an inflammatory site involves several vascular and extravascular events in which leukocytes interact with chemotactic factors, other cells, and the three-dimensional matrix until eventually reaching the inflammatory focus.[21] The present observation of a reduced number of leukocytes in lidocaine-treated animals (fig. 2) could therefore be attributed to an interference with several of these events. Lidocaine has been shown to inhibit leukocyte adherence *in vitro*[7,8] and cause a dose-dependent reduction of leukocyte adherence to the vessel wall[9] and inflammatory peritoneum *in vivo*.[10] Since leukocyte emigration from blood vessels involves adhesion to endothelial cells,[1,9] a decreased leukocyte adhesiveness may explain part of the drastic reduction of accumulated cells in the chamber.

Another possible explanation for the reduced number of leukocytes in the wound is the inhibition by lidocaine of the migratory properties of leukocytes in the three-dimensional extracellular matrix between the blood vessels and the chamber. *In vitro* studies have shown that motility and migration on a two-dimensional substratum is reduced by lidocaine,[8,10,12] but we lack knowledge with regard to the effects of lidocaine on leukocyte migration in a three-dimensional collagenous matrix, which does

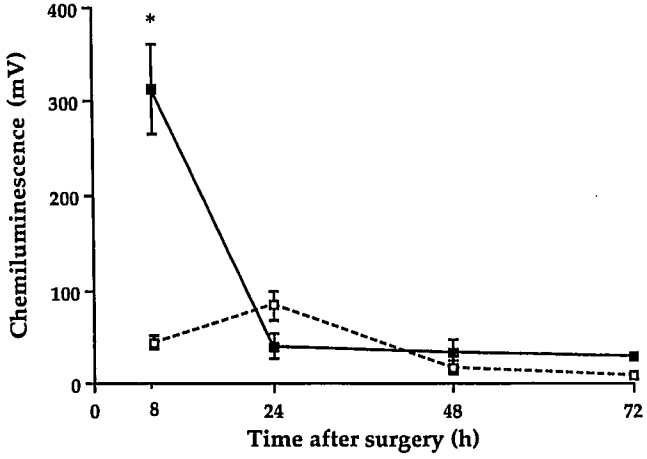

FIG. 3. Chemiluminescence (peak levels) of leukocytes from a surgical wound by the use of a titanium chamber after stimulation *in vitro* with serum opsonized zymosan. The wound was treated in connection with the surgical procedure with lidocaine (dashed line) or placebo (solid line). Base line indicates chemiluminescence in leukocytes prior to stimulation *in vitro*. *$P < 0.05$ *versus* control group. Other differences between the groups were not significant. Data are mean ± SEM.

not appear to be adhesion-dependent.[22–24] An additional explanation for the reduced number of accumulated cells in lidocaine-treated animals could be that, by reducing the metabolism of cells that already have reached the inflammatory site, the release of chemoattractants from these activated cells is inhibited, and, as a consequence, the continued accumulation of leukocytes also is inhibited. This is supported by recent *in vitro* data showing that the leukocyte-derived release of the potent chemoattractant substances leukotriene B_4[25–28] and interleukin-1[28,29] is strongly inhibited by local anesthetics.[30]

Considering that infiltration of lidocaine normally blocks nerve conduction for about 1–3 h,[31] the inhibition of leukocyte migration by lidocaine was surprisingly long-lasting. Several possible explanations can be offered. Reduced venous outflow, capillary and venous stasis, and slowed capillary blood flow in the incisional area due to shunting or thrombosis of injured vessels[32] could have reduced washout of the drug from the wound area and prolonged its effect. Alternatively, the local anesthetic formulation used in the present study could have acted as a slow-release unit because of its adhesive properties, as described for similar anesthetic formulations.[33] This possibility is supported by clinical data showing measurable plasma levels of lidocaine 48 h after a single aerosol administration in the surgical wounds of patients undergoing hysterectomy[34] and 72 h after surgery in postcholecystectomy patients (unpublished results).

In accordance with earlier *in vitro* studies showing inhibition of leukocyte metabolism by local anesthetics,[11–13] we found in the present *in vivo* study a strongly inhibited chemiluminescense in lidocaine-treated animals in response to zymosan particles (fig. 3). This metabolic inhibition by lidocaine was not the result of cell damage, since cell viability was similar in both groups.

It is generally accepted that one of the main purposes of wound inflammation is to protect the body against foreign agents such as microorganisms. The inhibition by lidocaine of leukocyte metabolic activation to zymosan (fig. 3) may suggest an impaired host response to microorganisms.[35] However, several studies have shown local anesthetics to possess potent antimicrobial properties *in vitro*[36,37] and *in vivo*.[38] It is also a general impression that the oxidative damage that occurs immediately follows tissue injury, and that the need for that oxidative activity for killing infection probably occurs much later.[39] Moreover, in a clean surgical wound there is little need for antimicrobial activity by leukocytes in the early postoperative period.

The present results showing reduced inflammatory response in the surgical wound by the local anesthetic may imply impaired wound healing by the agent. The high metabolic activity of granulocytes seen in the control group of this study (fig. 3) is equivalent to increased release of superoxide anions and lysosomal enzymes,[14,15] which play a central role in progressive tissue damage.[40] In addition, increased release of other inflammatory mediators, such as thromboxanes, leukotrienes, and interleukins, have been shown to promote microvascular permeability and tissue edema[41] and to induce platelet aggregation and vascular damage,[42] which will interfere with normal wound healing. Lidocaine has been shown to inhibit the release of both leukotrienes and interleukins[30] and to dissolve thrombus formation,[43] which, along with the present results, may in part explain the improved healing induced in partial-thickness wounds by lidocaine¶; this suggests that local anesthetics reduce harmful wound activity normally resulting in impaired healing and excessive scarring.

In conclusion, the current results show that administration of a local anesthetic in the area of surgery reduces granulocyte accumulation and metabolic activation, suggesting reduced release of tissue-toxic substances in the traumatized tissue by lidocaine, which may explain the positive effects reported for the agent on wound healing.

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