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Amide Local Anesthetics Reduce Albumin Extravasation in Burn Injuries

Jean Cassuto, M.D., Ph.D., Per Nellgård, M.D.,† Lars Stage,‡ Anders Jönsson, M.D.§*

Burn injury was induced in anesthetized rats by exposing the abdominal skin to a temperature of 55° C by means of a hot aluminum rod. Temperature was registered on a Grass® polygraph. Skin exposure was interrupted when hot rod temperature had decreased to 45° C. A full-thickness burn trauma of the skin was induced as judged from histologic sections. The burned skin was dissected and extravasation of Evans blue (EB) bound plasma albumin was quantified by a spectrophotometric technique and visualized by fluorescence microscopy. In the first set of experiments, one group of rats (n = 15) was topically treated with a lidocaine–prilocaine cream 5% (25 mg of each in 1 g; EMLA®) for 1.5 h starting 15 min after inducing the burn injury. In one control group (n = 14) the thermal injury was treated with placebo cream. A second control group (n = 15) was topically treated with placebo cream without being exposed to thermal trauma. Results showed a significant inhibition of EB-albumin extravasation in the skin of burned rats treated with lidocaine–prilocaine cream compared with placebo-treated burned skin ($P < 0.001$). EB-albumin contents in the skin of burned rats treated with lidocaine–prilocaine cream did not differ significantly from unburned skin ($P > 0.05$). In the second set of experiments continuous iv lidocaine infusions at a rate of 5 (n = 10), 10 (n = 12), 20 (n = 10), or 30 (n = 10) $\mu g \cdot kg^{-1} \cdot min^{-1}$ was given. The infusions were started 15 min after the burn injury and lasted for 1.5 h. A corresponding infusion of isotonic saline was given to burned control animals (n = 10) and to animals not exposed to thermal trauma (n = 10). A maximum inhibition of albumin extravasation from the burned area was induced by iv lidocaine at an infusion rate of 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ compared with burned controls treated with isotonic saline infusions ($P < 0.01$). At infusion rates below or above this, the effect of lidocaine on albumin extravasation was significantly reduced. Results show that topical or systemic administration of local anesthetics increases vascular patency and reduces albumin extravasation at the site of experimental burn injury. Furthermore, the effect of systemic lidocaine administration on burn-induced albumin extravasation appears to be biphasic. Future trials are required to determine the clinical relevance of these results in burned patients. (Key words: Anesthetics, local: lidocaine; prilocaine. Burn injuries. Measurement techniques: spectrophotometry. Microscopy, fluorescence.)

BURN INJURIES in the skin are characterized by vascular and tissue damage leading to a pronounced inflammatory reaction and release of inflammatory mediators, such as histamine, serotonin, kinins, and prostaglandins.[1–4] As a result, blood vessels in the area of thermal injury and surrounding tissues become dilated and permeable.[5] Thermal injury will also result in activation of the complement system and the release of lipid peroxidation products at the site of burn injury, bringing about damage to remote organs.[6] In burned patients the pronounced activation of the inflammatory cascade is one of the main factors influencing the profuse plasma losses from the burned areas of the skin resulting in hypoproteinemia and hypovolemia.[5] Amide local anesthetics have been shown to inhibit the synthesis and release of various inflammatory mediators[7–11] involved in the pathophysiology of burn-induced plasma leakage.[1–5] They also reduce inflammation-induced vascular hyperpermeability.[12] The aim of the present study was to evaluate the effects of topical and systemic administration of amide local anesthetics on inflammation and plasma extravasation from skin burn injuries in rats.

Materials and Methods

Experiments were performed on Sprague Dawley rats weighing 250–300 g. The experimental protocol was approved by the Animal Care Committee. Animals were housed for at least 7 days prior to experiments in a ventilated and temperature-controlled room and had water available ad libitum. The day–night cycle was constant at 12-h light and 12-h dark. Anesthesia was induced with pentobarbital (50 mg/kg) intraperitoneally and maintained by continuous intravenous (iv) infusion of chloralose (1.5 $mg \cdot kg^{-1} \cdot min^{-1}$). A tracheostomy was performed. Blood pressure was monitored using a pressure transducer (Statham P23 AC®) connected to a cannula in a femoral artery. Heart rate was measured using a heart rate meter. Intravenous drugs were administered into a femoral vein. Body temperature was kept at 38° C with a thermoregulated heating pad.

The abdominal skin of all animals was closely shaved, taking great care not to cause any damage. Thermal injury was produced in the abdominal skin by means of an electrically heated aluminum rod (bottom surface 1 × 1 cm) connected to an adjustable transformer. A thermosensor electrode from the hot plate was connected to a Grass® polygraph for temperature measurement (fig. 1). The plate was sunk into water (5 ml) and heated until the water boiled. At this point the calibration curve leveled off and

* Associate Professor of Physiology.
† Fellow in Anesthesiology.
‡ Research Engineer.
§ Fellow in Surgery.

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Address reprint requests to Dr. Cassuto: Department of Anesthesiology, Central Hospital, S-431 80 Mölndal, Sweden.

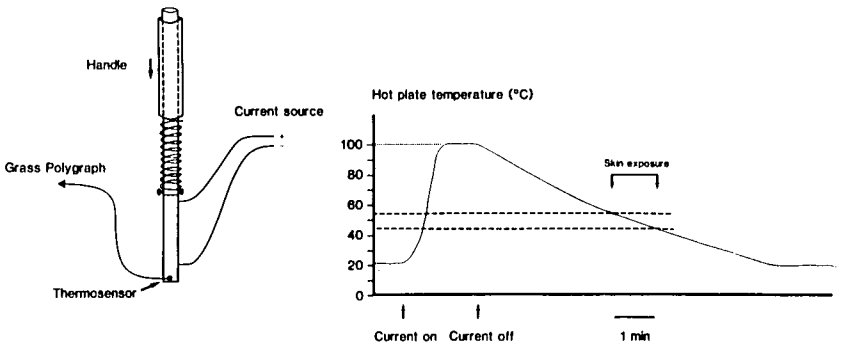

FIG. 1. The thermoprobe used for the induction of thermal skin injury (*left*). The hot plate (1 × 1 cm) is applied to the abdominal skin at constant pressure by compressing the spring under the handle. A thermosensor from the hot plate is connected to a Grass® polygraph for temperature indication. The temperature curve and temperature intervals used to induce a full-thickness skin burn injury (*right*). This method allows a continuous control and standardization of the amount of thermal energy administered to the skin independent of variations in skin temperature or blood flow.

the level for 100° C was set. Current was cut off and the hot plate was removed from the water, dried, and allowed to cool. When the temperature reached 55° C, the probe was put in contact with the abdominal skin until the temperature had reached 45° C, and then the probe was removed. This procedure allowed a constant amount of heat to be administered to each animal independent of possible variations in skin temperature or blood flow. Probe pressure was standardized by pushing down a cylindrical handle compressing in turn a spring on the probe (fig. 1). Room temperature was in the range of 21–23° C.

Two sets of experiments were performed. In the first set of experiments one group of rats (n = 15) was treated topically with 0.5 g of a lidocaine–prilocaine cream 5% (25 mg of each in 1 g; EMLA®) on the burned area 15 min after the injury. The cream was covered with an occlusive dressing and allowed to remain for 1.5 h. In one control group (n = 14) animals exposed to thermal injury received a placebo cream with identical *p*H and composition except for the local anesthetics. In a second control group (n = 15) rats not exposed to thermal trauma were topically treated with placebo cream.

In the second set of experiments a bolus injection of lidocaine (2 mg/kg) followed by a continuous iv infusion of lidocaine at 5 (n = 10), 10 (n = 12), 20 (n = 10), or 30 (n = 10) $\mu g \cdot kg^{-1} \cdot min^{-1}$ was administered 15 min after inducing the injury. A burned control group (n = 10) and a control group not exposed to thermal trauma (n = 10) received a corresponding iv bolus injection and infusion of isotonic saline. All the solutions were infused at a rate of 0.6 ± 0.03 ml/h for 1.5 h.

At the end of the period of treatment Evans blue (EB) (20 mg/kg) was dissolved in isotonic saline (1 ml) and administered intravenously in all the animals. Thirty minutes later the animals were killed by an iv injection of 1 ml saturated KCl. The experimental area of the skin (1 × 1 cm, full-thickness) and the underlying area of the rectus muscle were dissected, dried on filter paper to remove excess fluid, and immediately weighed. Each sample was placed in 4 ml formamide ($HCONH_2$) and incubated for 24 h in a water bath at 50° C as described by Gamse *et al.*[13] Colorimetric measurements were performed on the fluid in a Stasar (Gilford) spectrophotometer at the peak absorption of 612 nm. Three measurements were performed on each sample and the mean value was used for calculations. Calculations were based on external standards in formamide. All specimen analysis was performed by an assistant blinded to the experimental protocol.

All of these experiments were repeated, but rather than doing colorimetric measurements, we looked at the localization of extravasated EB-albumin in the skin and muscle by fluorescence microscopy as described previously.[14] Thirty minutes after injection of EB and dissection of the experimental area, the preparations were fixed in 4% buffered formalin for 24 h. Following this procedure tissue specimens from five animals from each group were frozen in liquid nitrogen, sectioned into 10 μm slices in a cryostat (Kryostat 1720, Leitz), mounted on glass slides, and dried by heating to 30° C for 1 min. The sections were examined in a fluorescence microscope (Microphot-FX, Nikon, lens Fluor 10 X, filter ND 4) using an epi-illumination technique (Ploek Pam System) consisting of a UV-lamp, an excitation filter (450–490 nm), a beam-splitting mirror (510 nm), and a suppression filter (515 nm). Photographs were taken using ASA 160 film (Kodak Ektachrome 135-36).

The original *p*H of the iv solutions was 5.61 for isotonic saline and 6.59–6.82 for lidocaine. Lidocaine solutions were prepared from pure crystaline lidocaine in isotonic saline. To avoid differences in *p*H among the treatment solutions, we titrated all the solutions to *p*H 7.0 ± 0.05. One hundred milliliters of each solution was continuously stirred (IKA-combimag Ret) and titrated with 0.5–1.0 mM NaOH by a micropipette (Transferpette, Brand). The *p*H of the solutions was continuously monitored by a *p*H meter (Metrohm 1632), and correction of *p*H was achieved by 0.1–0.5 mM HCl until reaching the predetermined *p*H level. The osmolarity of the solutions was controlled before and after titration by an osmometer (Advanced osmometer model 3W, Advanced Instruments), and dilution was found to be less than 2%.

All data were subject to analysis of variance followed by Duncans multiple range test for determination of significant differences at the 5% level. Contrasts were formed at a 95% confidence interval with subsequent analysis of levels of significance by Schiffe's test. Data are mean ± SEM.

Results

Histologic sections revealed that exposure of the abdominal skin to heat induced a full-thickness burn injury with extravasation of albumin within a well-defined area under the hot plate (1 × 1 cm) (fig. 2). The underlying rectus muscle was not significantly affected by heat as judged by comparison with unburned controls. In the first set of experiments topical treatment of the burned skin area with lidocaine–prilocaine cream resulted in a significant inhibition of albumin extravasation compared with placebo cream-treated thermal injury ($P < 0.001$) (figs. 2 and 3). The degree of extravasation from the burned group treated with placebo cream was significantly greater than the placebo-treated unburned group ($P < 0.001$) (fig. 3). The difference between burn injury treated with lidocaine–prilocaine cream and placebo cream-treated unburned skin was not significant ($P > 0.05$) (fig. 3). In the second set of experiments lidocaine infusion at 5, 20, and 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ did not induce any significant inhibition of EB-albumin leakage compared with saline-treated burned animals ($P > 0.05$) (fig. 4). Infusion of lidocaine at 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ induced a significant inhibition of albumin extravasation compared with burned control ($P < 0.01$) (fig. 4). EB contents in burned animals receiving lidocaine at 10 $\mu g \cdot kg^{-1} \cdot min^{-1}$ did not differ significantly from EB-albumin contents in unburned control skin ($P > 0.05$) but were significantly lower than in burned animals receiving lidocaine infusion at 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.05$) (fig. 4). No cardiovascular effects were seen during the lidocaine infusions.

Under the fluorescence microscope a bright red fluorescence was seen where EB-albumin was localized in the tissue. The red fluorescence was mainly localized to the lumen and walls of blood vessels in control skin not exposed to thermal injury. In burned control tissue of both experimental sets, rich EB fluorescence was obvious in free tissues of all layers of the skin. Topical and systemic

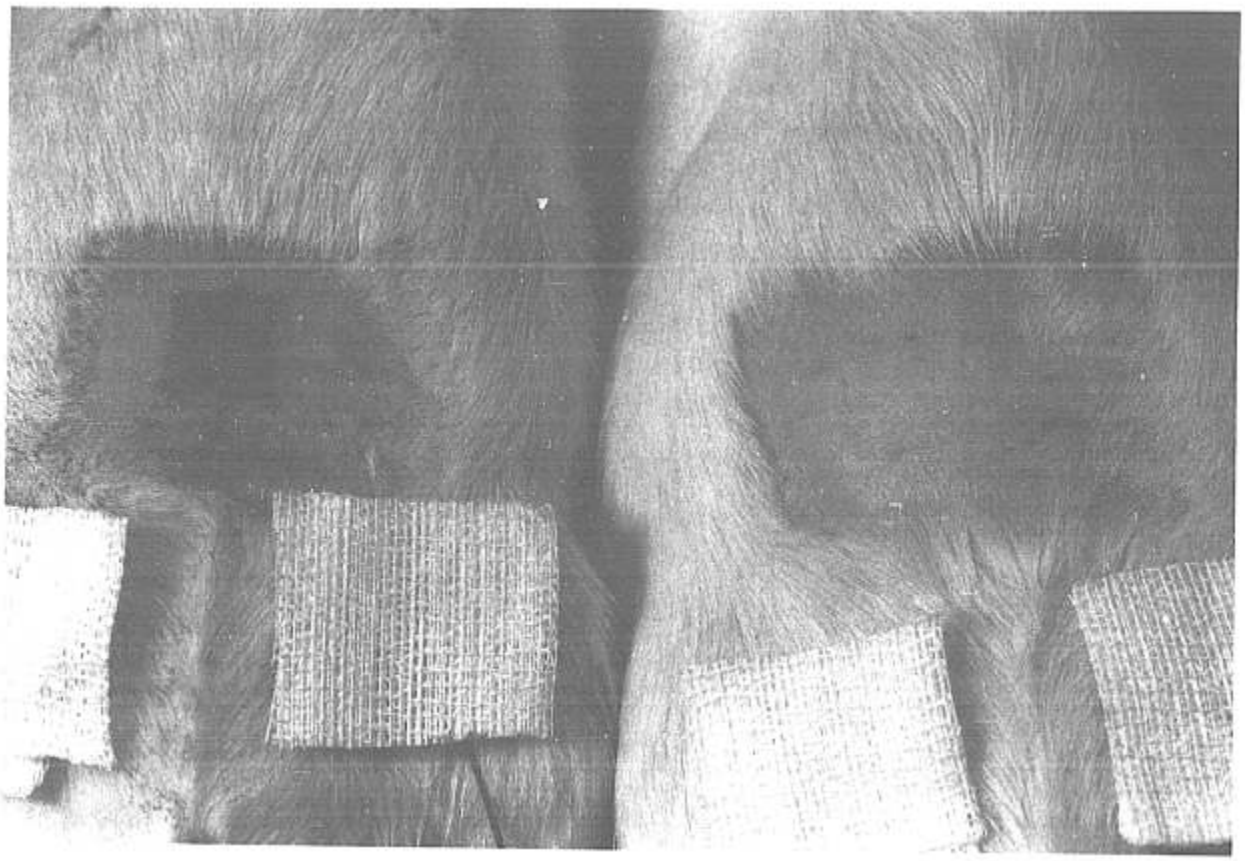

FIG. 2. The abdominal skin of both animals was exposed to thermal trauma. The animal to the left was topically treated with placebo cream, whereas the animal to the right was treated with lidocaine–prilocaine cream 5% (25 mg of each in 1 g). Treatment was started 15 min after the burn injury and lasted for 1.5 h. Both animals received iv injection of EB-albumin after termination of the treatment period. This photograph was taken 2 h after inducing the burn injury.

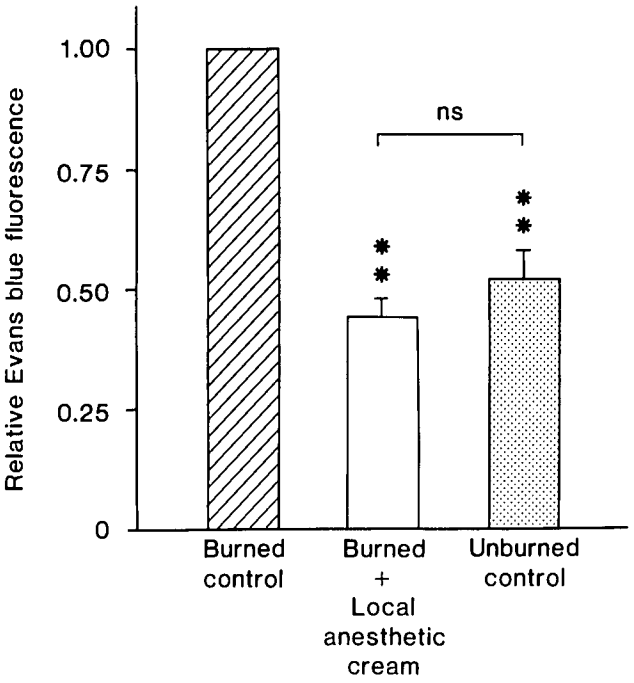

FIG. 3. Relative EB-albumin extravasation in animals exposed to skin burn injury and treated with lidocaine–prilocaine cream 5% (25 mg of each in 1 g; open bar) or placebo cream (burned control; shaded bar). Dotted bar (unburned control) indicates EB-albumin contents of skin treated with placebo cream but not exposed to thermal injury. $**P < 0.001$ *versus* placebo cream-treated animals with thermal injury (burned control); ns = not significant. Values are mean ± SEM.

administration of local anesthetics significantly reduced the amount of red fluorescence in extravascular tissues.

Discussion

Burn injury is characterized by vascular changes associated with loss of vascular patency and subsequent edema formation.[5] A variety of vasoactive substances have been proposed as mediators of plasma extravasation including substance P (SP),[15] histamine,[3] kinins,[1] and arachidonic acid metabolites (thromboxane, prostaglandins, leukotrienes).[4,16] Whether microvascular changes responsible for early burn edema are caused by direct thermal injury or local release of inflammatory mediators remains unresolved. However, data showing inhibition of burn-induced albumin extravasation by SP depletion[13] and anti-inflammatory drugs[16,17] suggest an important role for inflammatory mediators in edema formation.

Exposure of the skin to heat as described above induced a full-thickness skin burn injury without visible damage to the underlying rectus muscle. Treatment by topical or systemic administration of local anesthetics resulted in a significant inhibition of albumin extravasation. The mechanisms by which local anesthetics influence the burn-induced increase in vascular permeability can be several. By inhibition of sensory neurons, local anesthetics may inhibit the release of SP[18] shown to be of importance for early edema formation following thermal injury.[15] Another mechanism could be the interference of local anesthetics with the cellular response to thermal injury.[19] Local anesthetics have been shown to inhibit granulocyte delivery to the inflammatory site[8] and their adherence to injured vascular endothelium.[20] Moreover, local anesthetics reduce granulocyte release of superoxide anions[10,21,22] and lysosomal enzymes,[7] which play a central role in progressive postburn tissue damage and increased capillary permeability.[23,24] Several studies have shown increased production of arachidonic acid metabolites, such as prostaglandins, thromboxane, and leukotrienes from burn injuries.[4,16,23,25–28] Thromboxane and leukotrienes have both been shown to enhance granulocyte and thrombocyte aggregation, to induce changes in the tone of small vessels, and to promote microvascular permeability and tissue edema.[25–28] Such mechanisms are supported by data showing reduced burn edema and albumin extravasation by inhibition of prostaglandin and leukotriene production.[6,16] Local anesthetics have been described as antagonists of the actions of prostaglandins in the inflammatory area[29] and were shown to induce potent inhibition of leukotriene production.[30] In this respect, the actions of local anesthetics resemble those of other scavengers used experimentally in the treatment of burn injuries.[31,32] The pronounced release of thromboxane from

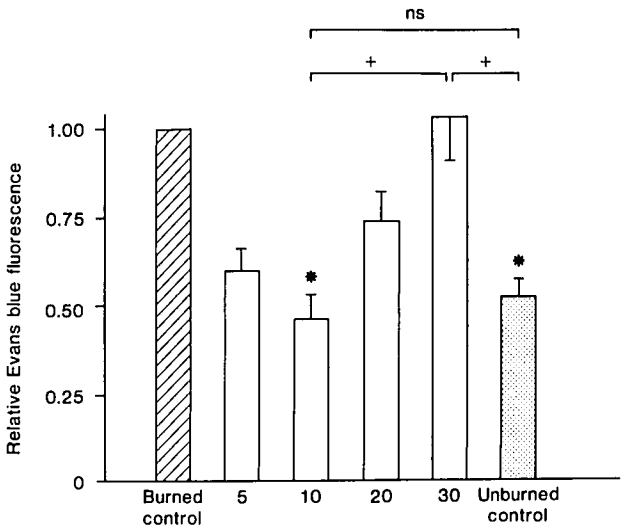

FIG. 4. Relative extravasation of EB-albumin from the skin following burn injury in animals receiving iv infusions of lidocaine (open bars). Numbers give infusion rates in $\mu g \cdot kg^{-1} \cdot min^{-1}$. Shaded bar represents albumin extravasation in animals receiving iv infusion of isotonic saline after being exposed to a skin burn injury (burned control). Dotted bar (unburned control) indicates albumin extravasation from normal skin in animals not exposed to thermal injury and treated with iv isotonic saline infusion. $*P < 0.01$ *versus* saline-treated burn injury (shaded bar); ns = not significant. $\dagger P < 0.05$. Values are mean ± SEM.