Lidocaine Inhibits Granulocyte Adherence and Prevents Granulocyte Delivery to Inflammatory Sites

By Rob Roy MacGregor, Richard E. Thorner, and David M. Wright

The effectiveness of the cationic local anesthetics in blocking the generation and transmission of nerve impulses has been known since Sigmund Freud's study of the physiologic effects of cocaine in 1884. More recent work has shown that these agents affect the membranes of most cells, influencing the flux of sodium and calcium, causing expansion of the membrane, and promoting membrane stability.

When leukocytes are exposed to local anesthetics, their cell morphology changes dramatically: pseudopod formation ceases, ruffling of the membrane is reduced, and the cells take on a rounded, smooth configuration. These changes are associated with inhibition of the cells' capacity to flatten out (spread) and adhere to surfaces.

Adherence-inhibiting property of the cationic anesthetics has been used to elute polymorphonuclear leukocytes (PMN) from the nylon columns used in filtration leukapheresis. Transillumination studies of microvasculature have shown that the adherence-inhibiting action of the local anesthetics can be demonstrated in vivo as well: direct application of these agents to traumatized vessels prevents or reverses the adherence of PMNs to their surfaces.

Our laboratory has described a direct correlation between the intensity of granulocyte adherence (GA), measured in vitro with a nylon fiber column assay, and the extent of PMN delivery to sites of inflammation. In all cases, agents that inhibited GA were found to suppress the inflammatory exudation of PMNs. Therefore, the action of the local anesthetics on cell shape and adherence suggested that this group of compounds might have antiinflammatory properties. We report here our investigation of lidocaine's effect on GA and on delivery of PMNs to sites of inflammation.

MATERIALS AND METHODS

Assay System

The assay for granulocyte adherence has been reported in detail previously. Spun nylon fiber is carefully weighed and packed to a column length of exactly 15 mm in Pasteur pipettes. One-milliliter specimens of heparinized whole blood are added to triplicate columns and allowed to flow through by gravity. Comparison of pre and post column granulocyte counts allows calculation of the percent of granulocytes adhering in the column (%GA), and the average of triplicate column results represents the adherence value for each specimen.

In Vitro Studies

Lidocaine was prepared by our hospital pharmacy as a 10^-1 molar solution in normal saline and kept at 4°C until used. To determine the effect of in vitro incubation of PMNs with lidocaine, heparinized whole blood was obtained from normal human donors and incubated at 37°C for 30 min with lidocaine before GA was determined. In some experiments a preparation of pure PMNs was made from normal whole blood by Ficoll-Hypaque density gradient sedimentation after the method of Boyum. Following separation, the PMNs were washed 3 times with modified Hanks' balanced salt solution (HBSS) at a concentration of 5-10 x 10^6 PMN/ml. Smears showed no contamination with platelets or RBCs and >98% PMNs. Lidocaine was added to this suspension to reach the desired concentrations, and the specimens incubated for 30 min at 37°C before determination of GA. Controls

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were cells prepared similarly, but without addition of lidocaine. The reversibility of the lidocaine effect was determined by incubation of heparinized whole blood in 10^{-2} molar lidocaine for 30 min at 37°C, followed by washing of the whole cell button 3 times with modified Hanks' solution and resuspension of the cells in normal plasma. Adherence of these cells was compared to that of cells washed in a similar manner but not incubated with lidocaine, and with cells incubated with lidocaine but not washed. All experiments were repeated at least 3 times, and results expressed represent means ± standard errors of the means for each experiment. Differences were tested for significance by Student's t test unless otherwise noted. Viability of the PMNs following the incubation and wash steps was greater than 95% by trypan blue dye exclusion.

In Vivo Studies

The effect of lidocaine in vivo on granulocyte adherence was determined by intravenous administration of the drug to 2–3 kg New Zealand white male rabbits. In all animal experiments, blood was obtained by cardiac puncture. First, a single bolus dose of lidocaine, 2.5 mg/kg, was given to the animals and specimens of heparinized whole blood tested for GA before dose, and 5, 15, 30, and 60 min thereafter. A subsequent group of animals received a bolus dose of 1.5 mg/kg lidocaine followed by a constant infusion of 0.3 mg/kg/min lidocaine solution in HBSS. This scheme resulted in a mean lidocaine blood level of 2.7 µg/ml after 4 hr. (The therapeutic range in humans being treated for arrhythmias is 1.2-6.0 µg/ml.) Blood was taken from the animals for measure of GA preinfusion, and at 5 min, 1, 2, 3, and 4 hr after beginning the infusion. To determine whether similar adherence changes occur in humans, 9 patients who received bolus dose of 100 mg/kg lidocaine and at the 0.01 level for the 10^{-2} M concentration (Student's t test). When a pure preparation of PMNs was suspended in HBSS, GA was 73.4% ± 3.8%, compared to 49.1% ± 6.2% when incubated with 10^{-2} M lidocaine in HBSS. Thus, the action of lidocaine on PMN adherence also occurs in the absence of other formed elements and components of normal plasma. Inhibition of GA by lidocaine was completely reversible: PMNs incubated in 10^{-2} or control animals not given methylprednisolone. The weekly dose of 6 mg/kg was selected as analogous to 60 mg methylprednisolone/day for a 70 kg man. In other experiments, pairs of animals were inoculated intraperitoneally with 3 × 10^9 Staphylococcus aureus from an overnight culture. One animal was infused with HBSS while the other received lidocaine in HBSS for the first 8 hr following inoculation, and the survival of both animals was monitored. Blood cultures were obtained 8 hr after infection, and at 1 wk in survivors.

Pathology of Infection

The microscopic appearance of the inflammatory reaction in lidocaine-infused and normal animals was determined by injecting pairs of 2–3 kg rabbits intracutaneously with 0.5 ml of a suspension of an overnight culture of S. aureus containing 10^9 organisms/ml. One animal was infused for 12 hr with lidocaine, 0.3 mg/kg/min, following a bolus dose of 1.5 mg/kg. The other received only HBSS as a control. Twelve hours following inoculation, the site of injection was excised down to the muscle layer, fixed, and stained for microscopical examination.

RESULTS

Incubation of normal human whole blood with lidocaine caused a dose-dependent inhibition of granulocyte adherence (Fig. 1); the reduction from normal adherence values was significant at the 0.01 level for 10^{-2} M lidocaine, and at the 0.05 level for the 10^{-4} M concentration (Student's t test). When a pure preparation of PMNs was suspended in HBSS, GA was 73.4% ± 3.8%, compared to 49.1% ± 6.2% when incubated with 10^{-2} M lidocaine in HBSS. The microscopic appearance of the inflammatory reaction in lidocaine-infused and normal animals was determined by injecting pairs of 2–3 kg rabbits intracutaneously with 0.5 ml of a suspension of an overnight culture of S. aureus containing 10^9 organisms/ml. One animal was infused for 12 hr with lidocaine, 0.3 mg/kg/min, following a bolus dose of 1.5 mg/kg. The other received only HBSS as a control. Twelve hours following inoculation, the site of injection was excised down to the muscle layer, fixed, and stained for microscopical examination.

Peritonitis

To assess the potential antiinflammatory effect of lidocaine infusion, sterile peritonitis was induced in 2–3 kg rabbits by the intraperitoneal instillation of 120 ml of normal saline over 1–2 min through a multiply fenestrated polyethylene catheter as described previously. Pairs of animals were studied, both of which were given peritonitis. One received a bolus dose of 1.5 mg/kg lidocaine followed by infusion by ear vein with 0.3 mg/kg/min for the duration of the experiment. The lidocaine was infused in HBSS, at a rate of 10 ml/hr; controls received HBSS infusion alone. Blood for adherence determination was obtained from both animals by cardiac puncture before, and 5 min, 2, 4, and 6 hr after beginning peritonitis, and 2–3 ml of peritoneal fluid were drawn at 2-hr intervals for PMN count. To compare the antiinflammatory effect of lidocaine with that of methylprednisolone, 2–3 kg rabbits were given 15 mg doses of the depot form of methylprednisolone subcutaneously twice at 7-day intervals, and 1–3 days after the second dose, sterile peritonitis was induced as above. Peritoneal exudate was measured 3 and 6 hr following induction and compared to counts in
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10^{-4} M lidocaine returned to normal adherence following washing 3 times with modified Hanks' solution and resuspension in normal plasma.

Intravenous administration of lidocaine, 2.5 mg/kg, to rabbits as a bolus dose produced a transient fall in adherence (Fig. 2). Mean GA before lidocaine was 68.8% ± 4.0%, and fell to 30.7% ± 5.9% 5 min after administration (p < 0.001); at 15 min the inhibition was not statistically significant (p < 0.1, > 0.05). When animals were infused with lidocaine, 0.3 mg/kg/min, following a bolus dose of 1.5 mg/kg i.v., adherence remained significantly inhibited at the same level for the duration of the infusion (p < 0.01 at all times). No consistent change in circulating PMN count was noted. Fluid administration without lidocaine had no effect on GA. To determine whether the continued inhibition of GA during infusion was due to lidocaine or the accumulation of its metabolites, the infusion was discontinued abruptly after 3 hr, and adherence was monitored for 2 hr thereafter (Fig. 3). The rate of return to normal was indistinguishable from that observed following the single bolus dose; this suggests either that accumulated metabolites of lidocaine were not responsible for the continued suppression of GA during infusion or that any such metabolites were excreted or inactivated at the same rate as the parent compound.

Adherence in 9 humans receiving an i.v. bolus of lidocaine for acute management of arrhythmias (mean dose 100 mg) fell from a mean of 51.1% ± 6.4% predose to 36.9% ± 5.3% 5 min after dose (p < 0.001, paired sample t test), demonstrating that the drug has the same effect on adherence in humans as in rabbits.

Because of the acute clinical setting of the patients, the time course of the adherence changes was not determined nor were serum lidocaine levels measured.

Dialysis of postlidocaine plasma from rabbits showed that the factor responsible for the inhibition of GA was completely dialyzable. Suspension of normal cells in normal plasma resulted in adherence of 62.6% ± 3.1% with undialyzed normal plasma and 55.8% ± 4.7% with dialyzed normal plasma (p < 0.2). When plasma drawn 5 min following a lidocaine dose was used to suspend normal cells, a mean adherence of 30.9% ± 3.2% resulted, and dialysis of this lidocaine plasma resulted in an adherence of normal cells equivalent to that seen in normal plasma (64.1% ± 3.0%).

Lidocaine infusion markedly inhibited the delivery of granulocytes into the exudate of rabbits with sterile peritonitis (Fig. 4). The left-hand panel demonstrates that adherence increased significantly in normal rabbits given peritonitis (p < 0.01 versus preperitonitis values) but that lidocaine infusion overcomes the influence of peritonitis, reducing adherence to approx-

Fig. 2. Effect of an i.v. bolus of lidocaine on granulocyte adherence. Points represent means from 3 rabbits, brackets ± standard errors of the means. Adherence was significantly inhibited only at 5 min (p < 0.001, Student's t test); at 15 min the difference was p < 0.1, > 0.05.

Fig. 3. Granulocyte adherence during and after lidocaine infusion. Three rabbits were infused with lidocaine, 0.3 mg/kg/min, for 3 hr, at which time the infusion was stopped and adherence measured sequentially.

Fig. 4. Effect of lidocaine infusion on granulocyte adherence and exudate formation in animals with sterile peritonitis.
technically to accurately measure the total exudate at each interval, delivery was expressed as volume approximately 50% ered a mean of 1,2770 ± 19,280 ± 2,836 both significantly inhibit and methylprednisolone after 4 hr of peritonitis and for ethanol-treated animals, 40.4% for those control effects from the drug. Table 1 compares the inhibition of peritoneal exudate formation by lidocaine, ethanol, and methylprednisolone acetate as a depot injection 15 mg subcutaneously 7–10 days before and again 1–3 days before initiating peritonitis.

Table 1. Peritoneal Exudate PMN Counts (cells/cu mm) in Rabbits Given Sterile Peritonitis and Concomitant Lidocaine, Ethanol, or Methylprednisolone

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PMN Counts by Hours of Peritonitis</th>
<th>Percent of Control</th>
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<tbody>
<tr>
<td></td>
<td>2 hr</td>
<td>3 hr</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>62 ± 12</td>
<td>170 ± 19</td>
</tr>
<tr>
<td>Control</td>
<td>1,053 ± 521</td>
<td>12,770 ± 1,764</td>
</tr>
<tr>
<td>Ethanol</td>
<td>609 ± 97</td>
<td>3,404 ± 389</td>
</tr>
<tr>
<td>Control</td>
<td>798 ± 171</td>
<td>10,874 ± 1,573</td>
</tr>
<tr>
<td>Methylprednisolone</td>
<td>1,787 ± 218</td>
<td>7,124 ± 861</td>
</tr>
<tr>
<td>Control</td>
<td>4,337 ± 1,217</td>
<td>17,633 ± 3,417</td>
</tr>
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Lidocaine was given by intravenous infusion, 0.3 mg/kg/min for 6 hr; ethanol as a 25% solution in normal saline, 35 ml at time 0, 20 ml 4 hr later; methylprednisolone acetate as a depot injection 15 mg subcutaneously 7–10 days before and again 1–3 days before initiating peritonitis.

Lidocaine infusion adversely affected how animals responded to an intraperitoneal inoculation of *S. aureus*. Six pairs of animals were given 3 × 10⁸ colony-forming units of an overnight growth of *S. aureus* intraperitoneally in 100 ml of normal saline; both members of each pair were restrained for 8 hr while receiving infusions of HBSS. One infusion contained lidocaine (0.3 mg/kg/min). All animals were bacteremic at 8 hr. Five of the 6 lidocaine-infused animals died within 48 hr of inoculation, whereas only 1 of the 6 animals that were infected but not lidocaine-infused died. The other 5 had sterile blood cultures and normal peritoneal cavities when sacrificed 1 wk after inoculation.

The histologic appearance of skin was examined 12 hr following inoculation of 5 × 10⁸ *S. aureus*. The epidermal and dermal layers of skin in the control rabbit were edematous and intensely infiltrated with PMNs; in contrast, the same area in the lidocaine-infused animal had a great deal of edema but virtually no PMNs were present. In the lidocaine-treated animal, clumps of darkly staining staphylococci could be seen, with no surrounding inflammatory reaction. Figures 5 and 6 demonstrate a deeper level in the skin: the normal animal (Fig. 5) had extensive edema and PMN infiltration; the lidocaine-infused animal (Fig. 6) had virtually no PMNs in the tissue, although marked edema was present. Note the vessel clogged with PMNs between 2 lymphatics dilated with edema fluid.

**DISCUSSION**

The cationic local anesthetics have been shown to inhibit a number of leukocyte functions in vitro. In 1974, Cullen and Haschke showed that 10⁻²–10⁻³ M concentrations of lidocaine significantly inhibited PMN nitroblue tetrazolium dye reduction, phagocytosis of latex particles, and the postphagocytic burst of oxygen consumption. Then Rabinovitch and De Stefano showed that cultivated macrophages incubated with lidocaine or tetracaine underwent rounding, characterized by cell contraction and withdrawal of cell processes. They found this change in shape to be completely reversible on withdrawal of the anesthetic agent. Nicolson et al., studying BALB/3T3 cells showed that the rounding of the formerly flat adherent cells by exposure to tertiary amine local anesthetics was associated with a loss of plasma membrane-associated microtubules and microfilaments. They also reported that these effects were reversible with washing. Further evidence that lidocaine’s rounding effect might be secondary to its effects on the cytoskeleton was provided by the report that lidocaine reversibly inhibits the polymerization of rabbit brain tubulin. Goldstein et al. showed that local anesthetics reduced extracellular release of lysosomal enzymes after PMN stimulation by zymosan and reduced superoxide anion production. They showed these effects...
Fig. 5. Subcutaneous tissue of normal rabbit injected 12 hr before with $5 \times 10^6$ S. aureus.

Fig. 6. Subcutaneous tissue of rabbit given same injection of S. aureus but also perfused with lidocaine, 0.3 mg/kg/min, for 12 hr following injection.
to be associated with a marked rounding of the cells but did not find a loss of microtubules and microfilaments. They suggested that the inhibitory effects of the anesthetics were not secondary to effects on these cytoskeletal elements because they were not mimicked by the combined actions of cytochalesin-B and colchicine.4 Regardless of the precise mechanism, it is established that the cationic local anesthetics inhibit phagocytosis, lysosomal enzyme release, postphagocytic oxygen consumption, and superoxide anion production in various types of leukocytes.4,5,15 In each case, inhibition is associated with a marked rounding of cells and withdrawal of cell processes.3-6,16

When adhering to surfaces, PMNs change from the round shape, characteristic of circulating cells, and adopt a flat configuration, spreading out upon the surface to which they are adhering.18 The change in cell configuration induced by the cationic local anesthetics might be anticipated to interfere with the PMN's ability to adhere. Van Oss et al. showed that anticoagulants that chelate divalent cations caused PMNs to adopt a rounded cell shape without pseudopodia, which markedly inhibited their adherence.19 Low temperatures,20 colchicine,21 and cyclic AMP22 also prevent PMNs from flattening out and adhering to other surfaces. Recently Schiffer et al., taking note of the prevention by local anesthetics of cell spreading and flattening, used tetracaine and lidocaine to elute PMNs from nylon columns used in filtration leukapheresis.7 They found that the eluted cells rounded and had a reduced number of filopodial cytoplasmic projections when suspended in the lidocaine-eluting fluid, but regained normal function and appearance when washed free of lidocaine.

We became interested in lidocaine's adherence-inhibiting effect because of our interest in granulocyte adherence as a critical step in the exudation of PMNs at sites of inflammation. In prior studies, we have shown that all antiinflammatory agents share the common property of adherence-inhibition4 and have found a direct correlation between inhibited adherence and poor exudate formation.11,12 Further, we have shown that pharmacologic correction of inhibited GA by propranolol or ascorbic acid improves poor delivery of PMNs to sites of inflammation.11,23 Because of this past work demonstrating that inhibition of GA is associated with inhibition of PMN delivery, we predicted that lidocaine infusion would inhibit the movement of PMNs out of the intravascular compartment into inflamed tissues. We were surprised to find that the inhibition of inflammation was more than tenfold greater with lidocaine (2% of control exudate PMN counts) than with methylprednisolone (40.4% of control) or with ethanol (43.5% of control). This comparative effectiveness suggests that lidocaine might perform well in comparison with glucocorticoids in the suppression of some episodes of acute inflammation. In vivo transillumination of vessel walls in inflamed tissues has shown that local application of the cationic anesthetics caused adhering PMNs to detach from injured endothelium and to return to circulation.8 This finding supports inhibition of adherence as the mechanism responsible for poor exudation.

The profound antiinflammatory effect of lidocaine that we have shown raises several questions. First, are patients who are receiving lidocaine infusions for arrhythmias at increased risk of developing and being unable to control bacterial infections? A brief retrospective review of such patients in our medical intensive care unit did not demonstrate an unanticipated frequency of infection, but most patients were infused for only 12–36 hr, perhaps insufficient time for the development of a spontaneous infection. Second, in addition to the known antiarrhythmic effects of lidocaine, could the drug's inhibition of inflammation around areas of myocardial infarction contribute to its effectiveness in controlling arrhythmias in this disease? Finally, could the antiinflammatory properties of lidocaine infusion be used in some of the inflammatory reactions that cause destruction rather than protection in the host's tissues, such as vasculitis, arthritis, and other autoimmune reactions? A therapeutic trial of lidocaine infusion in these conditions when other antiinflammatory therapy is failing would seem rational on the basis of our findings.

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