Comparison of the Antibacterial Activity of Lidocaine 1% Versus Alkalinized Lidocaine In Vitro

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ABSTRACT

Background: Infections after epidural and spinal blocks are rare. The topical anesthetic lidocaine used in these procedures has been found to have antibacterial effects on various microorganisms.

Objective: The aim of this study was to assess the antibacterial effects of alkalinized lidocaine on *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Methods: Lidocaine 2%, alkalinized lidocaine, and physiologic saline (as a control solution) were added to standard bacterial preparations. The final concentration of the lidocaine was 10 mg/mL (1%). At baseline and 3 and 6 hours after incubation at 37°C, 3-mL aliquots were vortexed and pipetted into sterile polystyrene spectrophotometer cuvettes. *Baseline* referred to the end of the period of preparation of the solution (≤20 minutes). Growth was measured as the optical density at a wavelength of 540 nm.

Results: Compared with the control, lidocaine significantly inhibited the growth of *S aureus*, *E coli*, and *P aeruginosa* at baseline and 3 and 6 hours after incubation (all, $P < 0.05$). Alkalinized lidocaine significantly inhibited the growth of *S aureus* at baseline and 3 and 6 hours (all, $P < 0.05$), while it significantly inhibited the growth of *E coli* and *P aeruginosa* only at 6 hours (both, $P < 0.05$). The growth of *E coli* was significantly less in lidocaine than in alkalinized lidocaine at 0 and 3 hours (both, $P < 0.05$).

Conclusion: The antibacterial effect of lidocaine 1% on *S aureus* was not changed after alkalinization. The effect of alkalinized lidocaine on *E coli* and *P aeruginosa* was significant only at 6 hours. Lidocaine significantly inhibited the growth of these 3 microorganisms at all study periods. (*Curr Ther Res Clin Exp.* 2007;68:242–248) Copyright © 2007 Excerpta Medica, Inc.

Key words: lidocaine, alkalinization, sodium bicarbonate, microbiologic phenomena.

Accepted for publication March 23, 2007.
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doi:10.1016/j.curtheres.2007.08.007
0011-393X/$32.00

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INTRODUCTION

Various studies have found that bupivacaine, ropivacaine, lidocaine, and levobupivacaine inhibit the growth of bacteria in vitro.[1–3] Lidocaine is thought to inhibit bacterial growth through its effects on either the cell wall or cytoplasmic membrane.[4] It has been shown to inhibit the incorporation of radioactive precursors of DNA, RNA, and proteins; however, the drug has not been shown to inhibit macromolecular synthesis.[5] It has been suggested that lidocaine disrupts bacterial membrane potential through depolarization of the cytoplasmic membrane.[4] Infections after epidural and spinal blocks are rare, which might be related to the antibacterial effect of local anesthetics such as lidocaine.[6,7]

The pH of local anesthetics is increased by coadministration of sodium bicarbonate. Using this method, the nonionized portion of a local anesthetic may be increased, resulting in the onset of anesthesia at a lower serum-drug concentration, greater spread of the drug, and a longer duration of anesthesia; in short, improvement in the quality of anesthesia.[8–10] However, studies of the antibacterial effect of alkalinized lidocaine using sodium bicarbonate are limited.[11,12] Studies have reported conflicting findings of the antibacterial effectiveness of sodium bicarbonate at different doses: sodium bicarbonate 100 mEq/L was found to be effective,[11] while sodium bicarbonate 100 mEq/mL was not effective.[12]

The normal flora of human epidermis includes *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, all of which are responsible for nosocomial infections and are also frequently isolated from spinal and epidural abscesses.[2,13,14] Therefore, the aim of this study was to assess the antibacterial effects of alkalinized lidocaine on these 3 microorganisms in vitro.

MATERIALS AND METHODS

All tests were performed in an accredited laboratory by experienced scientists using approved materials. Review of the study by an institutional review board (IRB) was waived because this was an in vitro study. However, the researchers were aware of the Inonu University IRB's policies and procedures as they related to this study. Copies of the IRB's written procedures were distributed to all individuals involved in the study who had research responsibilities.

Solutions of lidocaine 2% and alkalinized lidocaine* were used in this study. Lidocaine was alkalinized with sodium bicarbonate (1 mL 8.4% sodium bicarbonate/10 mL lidocaine 2%). Sample pH was measured using a PHM 84 Research pH meter (Radiometer, Copenhagen, Denmark) calibrated to a pH of 7.000 with a certified standard buffer (Fisher Scientific, Fair Lawn, New Jersey). The pH of lidocaine was 6.3 before alkalinization and 7.2 after alkalinization.

S aureus, *E coli*, and *P aeruginosa* (American Type Culture Collection [ATCC] numbers 25923, 25922, and 27853, respectively [ATCC, Manassas, Virginia]) were

*Trademark: Aritmal® 2% (Biosel, Istanbul, Turkey).

grown on standard blood agar and incubated at 37°C for 24 hours. Fresh bacterial cultures were prepared to match the turbidity of a McFarland 0.5 scale (10^8 colony-forming units [CFUs]/mL) with sterile saline 0.9%, and each solution was further diluted in Mueller-Hinton broth to obtain standard inoculum (10^5 CFU/mL).

The test solutions and the control solution (physiologic saline) were added to 2 mL of standard bacterial preparations. The final concentration of lidocaine was 10 mg/mL (1%).

At baseline and at 3 and 6 hours after incubation at 37°C, 3-mL aliquots were vortexed and pipetted into sterile polystyrene spectrophotometer cuvettes. Bacterial growth was measured as the absorption of light at a wavelength of 540 nm (absorption A540) (Ultrospec Plus, Pharmacia LKB Biochrom Ltd., Cambridge, United Kingdom). *Baseline* refers to the end of the period used to prepare the solution (≤20 minutes).

Each experiment was repeated 5 times and each test was performed in duplicate.

Statistical Analysis

The results are expressed as mean (SD). All results were shown to be abnormally distributed according to the Shapiro-Wilks test. Differences between groups were evaluated using the Mann-Whitney test. The Wilcoxon signed rank test was used for intragroup comparisons. $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using SPSS for Windows, version 10.0 (SPSS Inc., Chicago, Illinois).

RESULTS

Compared with the control solution, lidocaine significantly inhibited the growth of *S aureus* at baseline and after 3 and 6 hours of incubation (mean [SD] absorption, 0.01 [0.01]; $P = 0.001$, $P < 0.001$, and $P = 0.004$, respectively). Alkalinized lidocaine significantly inhibited the growth of *S aureus* at baseline and 3 hours (both, 0.02 [0.01]; $P = 0.005$ and $P = 0.002$, respectively) and at 6 hours (0.01 [0.01]; $P < 0.001$) (**Figure 1**).

Compared with the control solution, lidocaine significantly inhibited the growth of *E coli* at baseline and after 3 and 6 hours of incubation (all, 0.02 [0.01]; $P = 0.006$, $P = 0.001$, and $P < 0.001$, respectively). Alkalinized lidocaine significantly inhibited the growth of *E coli* only at 6 hours (0.04 [0.04]; $P < 0.001$). Compared with alkalinized lidocaine, lidocaine significantly inhibited the growth of *E coli* at baseline and 3 hours (both, 0.02 [0.01]; $P = 0.036$ and $P = 0.015$, respectively) (**Figure 2**).

Compared with the control solution, lidocaine significantly inhibited the growth of *P aeruginosa* at baseline and 3 and 6 hours after incubation (0.01 [0.01], 0.01 [0.00], and 0.02 [0.02]; $P = 0.004$, $P < 0.001$, and $P = 0.002$, respectively). Alkalinized lidocaine significantly inhibited the growth of *P aeruginosa* at 6 hours (0.02 [0.01]; $P < 0.001$) (**Figure 3**).

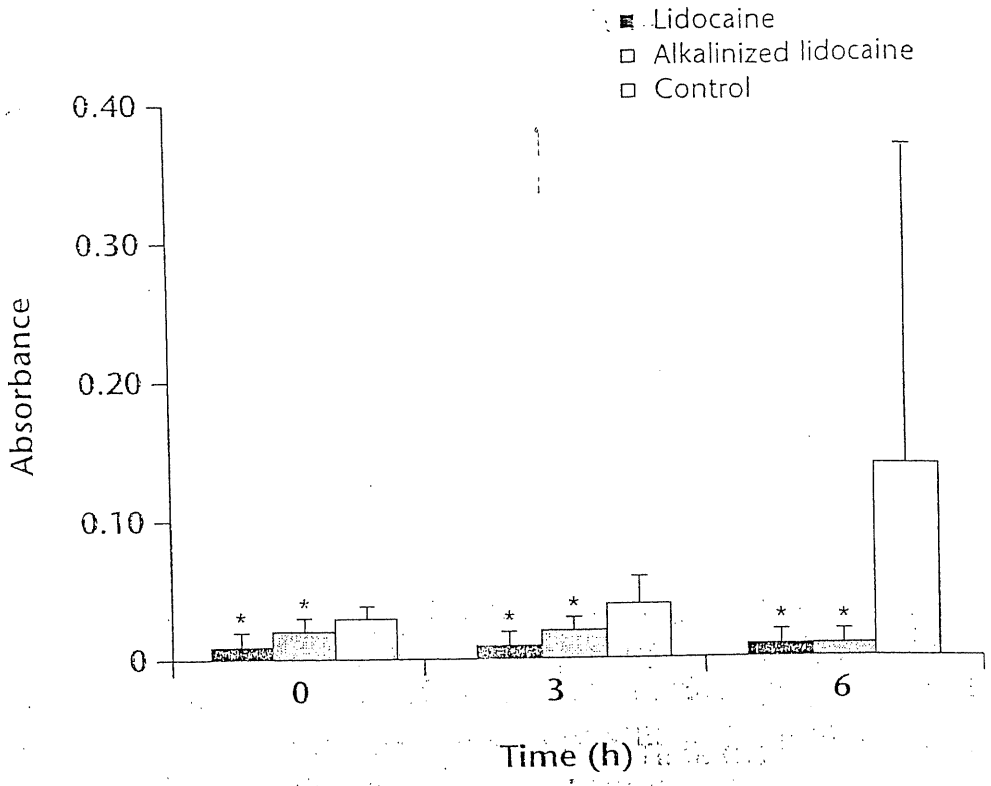


Figure 1. The (mean [SD]) effect of lidocaine 1% and alkalinized lidocaine on *Staphylococcus aureus*. Lower absorption of light at 540 nm indicates greater inhibition of growth. *$P < 0.05$ versus control.

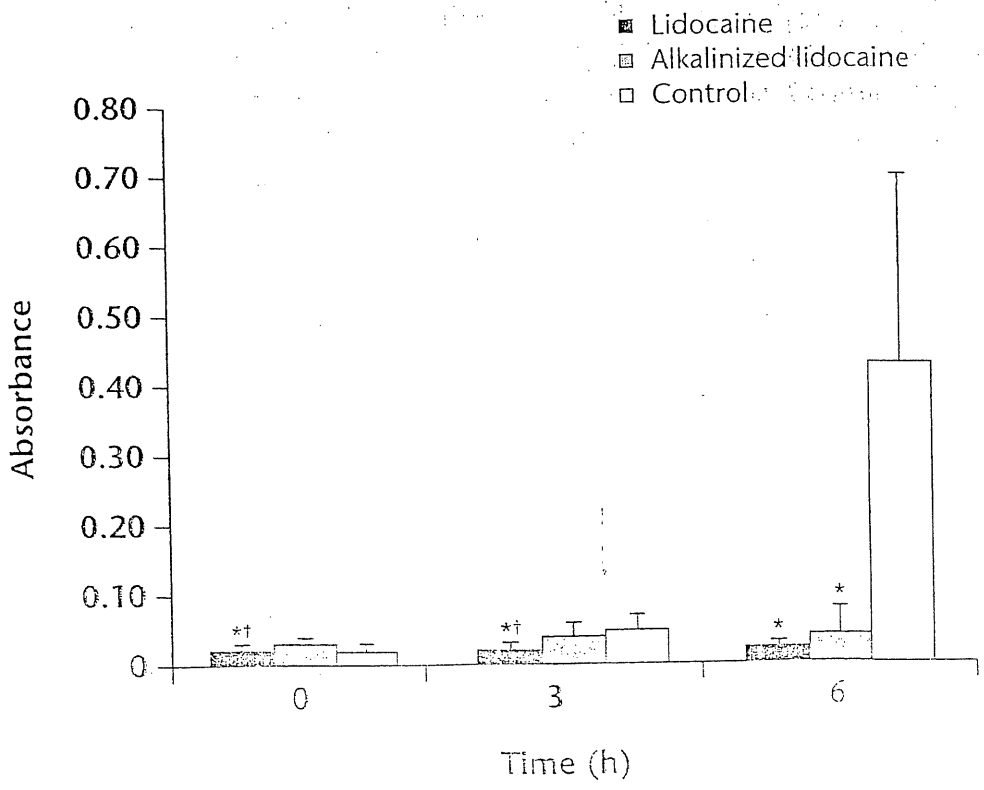


Figure 2. The (mean [SD]) effect of lidocaine 1% and alkalinized lidocaine on *Escherichia coli*. Lower absorption of light at 540 nm indicates greater inhibition of growth. *$P < 0.05$ versus control; †$P < 0.05$ versus alkalinized lidocaine.

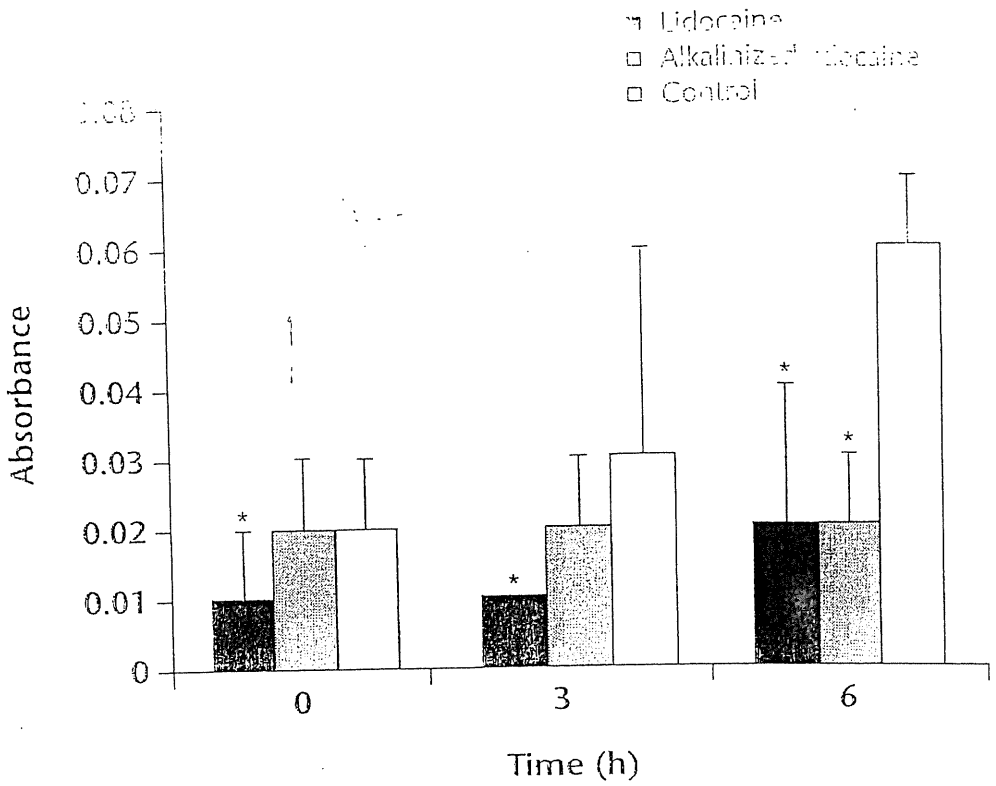


Figure 3. The (mean [SD]) effect of lidocaine 1% and alkalinized lidocaine on *Pseudomonas aeruginosa*. Lower absorption of light at 540 nm indicates greater inhibition of growth. *$P < 0.05$ versus control.

DISCUSSION

Both lidocaine and alkalinized lidocaine significantly inhibited the growth of *S aureus* at all study points (baseline and after 3 and 6 hours of incubation; all, $P < 0.05$). Lidocaine also significantly inhibited the growth of *E coli* and *P aeruginosa* at all study points, but alkalinized lidocaine inhibited the growth of these bacteria only at 6 hours ($P < 0.05$). The absorption of lidocaine, alkalinized lidocaine, and the control solution differed at the start of the study because adding sodium bicarbonate to lidocaine significantly enhances absorption. It has been stated that conditions of temperature, pH, and ionic strength of medium affect the precipitin reaction while the control solution included only bacteria, broth, and physiologic saline.[15]

Lidocaine is used clinically at various concentrations (0.5%–5.0%).[2–5] Schmidt and Rosenkranz[5] found that concentrations as low as lidocaine 0.5% reduced the number of viable bacteria in a solution of *E coli*. They also found that the MIC of lidocaine to *S aureus* was between 1% and 2%. Aydın et al[2] found that lidocaine 5% and 2% reduced the number of CFUs of *S aureus* ($P = 0.007$ and $P = 0.006$, respectively), *E coli* ($P = 0.04$ and $P = 0.01$), *P aeruginosa* ($P = 0.009$ and $P = 0.003$), and *Candida albicans* ($P = 0.001$ and $P = 0.019$), while lidocaine 1% reduced only the number of CFUs of *P aeruginosa* ($P = 0.009$). We found that lidocaine 1% inhibited *S aureus*, *E coli*, and *P aeruginosa*. The differences between our findings and those of other studies may be attributable to

the differences in the experimental conditions, including the bacterial strains used, the methods of evaluating bacterial growth, and drug dilutions. The absorption method used to assess bacterial growth has enhanced sensitivity, measuring subnanograms per milliliter of bacteria.[15] The samples used in this study had specific turbidity levels, and the levels changed when bacterial growth occurred. Growth was measured as the absorption of light at a wavelength of 540 nm, with lower absorption indicating greater inhibition of growth.

Few data are available on the antibacterial activity of local anesthetics mixed with sodium bicarbonate. In their investigation of the antibacterial effectiveness of lidocaine, sodium bicarbonate, and epinephrine individually against 2 types of bacteria and 7 fungi often encountered in immunosuppressed patients, Williams et al[12] reported that the use of sodium bicarbonate and epinephrine should be avoided when taking biopsy specimens for culture. Their study differed from ours in that they used sodium bicarbonate in 12.5-, 25-, and 100-mEq/mL concentrations, while we used sodium bicarbonate 100 mEq/L in combination with lidocaine.

Thompson et al[11] reported that sodium bicarbonate combined with lidocaine had greater antibacterial effectiveness than lidocaine monotherapy. The study was performed with the solution of 4% lidocaine containing 0.1% methylparaben and epinephrine using 3 concentrations of sodium bicarbonate (25, 50, and 100 mEq/L), 6 microorganisms, and the colony counting method. A low concentration of lidocaine (1%) was used in our study, while methylparaben and epinephrine were not used. The alkalinization rate was constant (100 mEq/L), only 3 microorganisms were used, and the absorption method was used to assess bacterial growth. All of these differences in methodology may have resulted in the differences in the findings between our study and that of Thompson et al. Methylparaben, a preservative often found in lidocaine, has also been reported to have antibacterial effects.[16] Further studies of the antibacterial effects of alkalinized lidocaine using different drug dilutions and different microorganisms are needed.

We hold the opinion that because the alkalinized lidocaine did not inhibit growth of bacteria studied at baseline and 3 hours after incubation, it should be preferred only under certain circumstances.

CONCLUSION

The antibacterial effect of lidocaine 1% on *S aureus* was not changed after alkalinization. The effect of alkalinized lidocaine on *E coli* and *P aeruginosa* was significant only at 6 hours. Lidocaine significantly inhibited the growth of these 3 microorganisms at all study periods.

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