Antimicrobial activity of local anesthetics

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Abstract

Purpose: Continuous epidural analgesia is increasingly used to manage acute and chronic pain. Epidural analgesia affords profound pain relief, and, in certain high-risk patients, may decrease morbidity, mortality, and treatment costs. However, some investigators have reported that this form of treatment has resulted in the development of epidural abscesses.

Some investigators have described the antimicrobial properties of local anesthetic agents that have been used in continuous epidural analgesia. It has been suggested that the use of these agents may significantly alter the microbial population.

The purpose of this study was to determine the risk of growth of *Staphylococcus epidermidis* in local anesthetics commonly used in clinical practice, specifically 0.5%, 1%, and 2% lidocaine; 0.125%, 0.25%, and 0.5% bupivacaine; and 0.5%, 1%, and 2% mepivacaine.

Methods: Diluted bacterial suspension of *Staphylococcus epidermidis* was added to the 0.9% sterile non-bacteriostatic saline or the different local anesthetics. After the organisms were added, a sample from each vial was put into one plate of trypticase soy agar at 0, 0.5, 2 and 4 h intervals for a total of three plates per solution per sampling period. All were stored and incubated at 37°C in an atmosphere of 5% CO₂ for 24 h. Each plate medium was read and the number of colony-forming units (CFUs) was counted and recorded by the same investigator.

Results: The number of CFUs declined in proportion to time incubated in all three anesthetics, indicating that all the local anesthetics inhibited the growth of *Staphylococcus epidermidis*, with the antimicrobial activity of bupivacaine being strongest, followed by lidocaine and then mepivacaine. Effects were proportional to concentration and time: higher concentrations and longer times led to a reduced number of CFUs.

Conclusions: We conclude that lidocaine, mepivacaine and bupivacaine all inhibit the growth of *Staphylococcus epidermidis*.

Key words: *Staphylococcus epidermidis*, antimicrobial activity, bupivacaine, lidocaine, mepivacaine

INTRODUCTION

The antimicrobial properties of local anesthetic agents were originally described by Jonnesco¹⁾. He

indicated that, due to their antiseptic characteristics, there was no need to sterilize the local anesthetics used for spinal anesthesia. The earliest work documenting the *in vitro* antimicrobial activity of anes-

Table 1 CFUs after incubating in all solutions: lidocaine, bupivacaine, mepivacaine

	NS	Lidocaine (%)			Bupivacaine (%)			Mepivacaine (%)		
		0.5	1	2	0.125	0.25	0.5	0.5	1	2
I (0)	1011	809	809	644	907	833	351	1067	933	904
II (0.5 h)	700	349	164	32	350	163	9	512	485	313
III (2 h)	88	27	5	1	3	3	0	108	174	58
IV (4 h)	1	1	2	0	0	1	0	1	31	0

NS: normal saline

thetic agents was reported by Schlegel and Swan²⁴), who described the inhibition by 0.2% benoxinate of the growth of *Micrococcus* and *Pseudomonas*. Similar effects of anesthetic agents have been observed on microorganisms found in clinical specimens^{7,12,13,16}. Each of these studies concluded that local anesthetics reduce microbial growth. The use of continuous epidural analgesia to control chronic pain is increasingly common; however, some investigators have reported that this form of treatment has resulted in the development of epidural abscesses^{1,2,27,28}). The purpose of this study was to determine the risk of growth of *Staphylococcus epidermidis* in local anesthetics commonly used in clinical practice, specifically 0.5%, 1%, and 2% lidocaine; 0.125%, 0.25%, and 0.5% bupivacaine; and 0.5%, 1%, and 2% mepivacaine. Non-bacteriostatic 0.9% isotonic sodium chloride solution was used as the control.

MATERIALS

1) *Bacterium*

Staphylococcus epidermidis ATCCr12228 (SE)

2) *Local anesthetics*

0.5%, 1%, 2% lidocaine (L)

0.5%, 1%, 2% mepivacaine (M)

0.125%, 0.25%, 0.5% bupivacaine (B)

METHODS

Staphylococcus epidermidis at 2×10^6 /ml (ATCCr12228) was diluted 1:50 with sterile 0.9% saline. A 0.2 ml aliquot of this diluted bacterial suspension was then added to a 9.8 ml solution of the 0.9% sterile nonbacteriostatic saline or the different local anesthetics. All experiments were carried out in

triplicate. After the organisms were added, a sample from each vial was put into one plate of trypticase soy agar, using a calibrated precision 1- μ l loop at 0, 0.5, 2 and 4 h intervals for a total of three plates per solution per sampling period. All were stored and incubated at 37°C in an atmosphere of 5% CO₂ for 24 h. Each plate medium was read and the number of colony-forming units (CFUs) was counted and recorded by the same investigator. For each solution, the number of CFUs per plate was averaged for each sample period.

Data are presented as mean $\pm$ SEM of three replicated assays. For each anesthetic, Student's T-Test was applied to comparisons among time period and with 0.9% saline and among the different concentrations. The probability of $p < 0.05$ was assumed to be statistically significant.

RESULTS

As seen in **Table 1**, the number of CFUs declined in proportion to time incubated in all three anesthetics, indicating that all the local anesthetics inhibited the growth of *Staphylococcus epidermidis*. The effect of bupivacaine was the strongest.

With lidocaine (**Fig. 1**), a significant decline ($p < 0.05$) in CFUs in 0.9% saline and 0.5%, 1%, and 2% lidocaine was observed over the baseline. The 0.5%, 1%, and 2% lidocaine showed a significant reduction in CFUs compared with 0.9% saline at 0, 0.5 h and 2 h. Of the different concentrations of lidocaine, the number of CFUs showed a significant difference at 0.5 h and 2 h. Effects were proportional to concentration and time: higher concentrations and longer times led to a reduced number of CFUs. With bupivacaine (**Fig. 2**),

Fig. 1 Number of colony-forming units of *Staphylococcus epidermidis* counted versus time after inoculation in various concentrations of lidocaine.

*: significantly different from baseline (time 0) ($p < 0.05$)

+: significantly different from ns at each time ($p < 0.05$)

Fig. 2 Number of colony-forming units of *Staphylococcus epidermidis* counted versus time after inoculation in various concentration of bupivacaine.

*: significantly different from baseline (time 0) ($p < 0.05$)

+: significantly different from ns at each time ($p < 0.05$)

Fig. 3 Number of colony-forming units of *Staphylococcus epidermidis* counted versus time after inoculation in various concentration of mepivacaine.

+: significantly different from ns at each time ($p < 0.05$)

the number of CFUs showed a significant decline in 0.125%, 0.25%, and 0.5% bupivacaine compared with the baseline. The 0.125%, 0.25%, and 0.5% bupivacaine demonstrated a significant decline compared with 0.9% saline at 0, 0.5 and 2 h. As with lidocaine, higher concentrations and longer times incubated in bupivacaine led to a reduced number of CFUs. With mepivacaine (**Fig. 3**), 2% mepivacaine showed a significant reduction ($p < 0.05$) in CFUs compared with 0.9% saline at 0.5 and 2 h, but there was no significant difference with 0.5% and 1% mepivacaine ($p < 0.05$). At 2 h, 2% mepivacaine showed a rapid reduc-

tion ($p < 0.05$) compared with 0.9% saline. However, results for 0.5% and 1% mepivacaine showed no significant decline compared with 0.9% saline. At 4 h, the CFUs in 1% mepivacaine were significantly more numerous than those in 0.9% saline and 0.5% mepivacaine. Apart from 1% mepivacaine, the CFUs had declined in all the local anesthetic solutions and in 0.9% saline at 4 h.

DISCUSSION

Continuous epidural analgesia is increasingly used to manage acute and chronic pain such as that caused by cancer and sick cell vaso-occlusive crises, and traumatic and post-operative pain resulting from surgery. Epidural analgesia affords profound pain relief, and, in certain high-risk patients, may decrease morbidity, mortality, and treatment costs^{3,15}. However, studies in which epidural catheter tips were cultured after removal demonstrate that epidural catheters placed in adults are at risk of being contaminated with skin flora after only short-term use^{17,26}. In our hospital, we also found infections after continuous epidural analgesia (**Table 2**).

Other investigators have described the antimicrobial properties of local anesthetic agents containing lidocaine^{1,2,27,28}. It has been suggested that the use of these agents may significantly alter the microbial

Table 2 Cases of infection after continuous epidural at our hospital

Age (y)	Sex	Time of continuous epidural (day)	Bacteria	Anesthetic (continuous)	Anesthetic (intermittent)
46	M	25	<i>S. aureus</i>	0.75%M 5 ml/h	0.25%M
25	F	24	(-)	0.125%B 5 ml/h	0.125%B
39	F	10	(-)	0.5%M 5 ml/h	0.75%M

M: mepivacaine, B: bupivacaine

population on the surfaces that they contact, especially the bronchi during bronchial tree procedures using topical anesthetics²¹. The purpose of this study was to compare the potential for antimicrobial activity of the most commonly used local anesthetics (lidocaine, bupivacaine and mepivacaine) employed during epidural analgesia.

Staphylococcus is the most common infecting organism (83%) in catheter-related epidural abscesses. It reaches the epidural space via hematogenous spread^{5,22} from a remote infection site or by direct implantation in the organism of contaminated needles, catheters, or drugs¹⁹. A recent study²³ examining human skin flora as a potential source of epidural abscess revealed *Staphylococcus epidermidis* from excised human back skin to be predominant. Therefore, in our study, we used *Staphylococcus epidermidis* (SE).

The results of our study demonstrate that lidocaine, bupivacaine and mepivacaine all act against *Staphylococcus epidermidis*, with bupivacaine showing the strongest effect, followed by lidocaine and then mepivacaine. This result agrees with other reports¹⁸. The molecular structure of bupivacaine is different from mepivacaine: where mepivacaine has a piperidyl group, bupivacaine has a butyl group. It is possible that the piperidyl group has a stronger effect than the butyl group against lipophilic substances, making bupivacaine the more potent bactericide. Gajraj⁸ reported that bacterial colony counts progressively fell with rising concentration of lidocaine. Our study indicated that bupivacaine and lidocaine have concentration-related and time-related effects against SE: higher concentrations and longer incubation times led to more marked bactericidal effects.

Several hypotheses have been proposed to ex-

plain the effects of anesthetic agents on the survival of microorganisms. Pugsley et al.²⁰, in a study using *Escherichia coli* k-12, have reported that anesthetics inhibit the synthesis of a major outer membrane pore protein, causing an increase in the molecular packing of lipid molecules in the membrane, which might in turn result in sufficient alteration of membrane fluidity to cause selective inhibition of the processing of precursors of exported proteins¹⁴. Schmidt and Rosenkranz²⁵ described potential mechanisms of action for lidocaine and procaine when tested with over 1,200 different strains of microorganisms. In cultures exposed to lidocaine, protein synthesis is somewhat more sensitive to inhibition than DNA or RNA production. Procaine affects both DNA and protein production, with higher concentrations of the drug required to inhibit RNA synthesis. Hiraoka¹⁰ reported that the effect of mepivacaine was the most effective against RNA synthesis, followed by protein synthesis and DNA synthesis. Since neither lidocaine nor procaine selectively inhibits the synthesis of DNA, RNA or proteins, the basis of action of these agents may involve the cell wall or cytoplasmic membrane. These studies suggest that the mechanisms of action of local anesthetics vary according to type and concentration. The results of our study show that the CFUs after incubation in 1% mepivacaine to be, surprisingly, more numerous than those in 2% and 0.5% mepivacaine at both 0.5 h and 2 h, and more numerous than in 0.9% saline at 2 h. To confirm this result, we repeated the test but gained the same data. It is possible that the mechanisms of antimicrobial activity of mepivacaine are different at different concentrations. Except for 1% mepivacaine, all the solutions showed fewer CFUs at 4 h. We think this is due to the nutrition of the solution we stored has a time dependent decline. However, overall, there

was a significant decline in CFU count after incubation in local anesthetic solutions compared with the 0.9% saline controls.

Local anesthetics have been demonstrated to influence membrane-dependent responses to surface stimuli in a variety of cells: they inhibit phagocytosis of human leucocytes⁶⁾, inhibit the adhesion, spreading and locomotion of macrophages²¹⁾, inhibit lysosomal enzyme release in human granulocytes⁹⁾, inhibit macrophage-mediated cytotoxicity, and enhance neutrophil-mediated cytotoxicity⁴⁾. These results suggest that local anesthetics affect immunity. Further studies are needed to determine whether local anesthetics injected into the epidural or spinal space will immunologically compromise the patient. We conclude that lidocaine, mepivacaine and bupivacaine all inhibit the growth of *Staphylococcus epidermidis*, with the antimicrobial activity of bupivacaine being strongest, followed by lidocaine and then mepivacaine.

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