Alkalinisation does not enhance the antimicrobial properties of local anaesthetic solutions

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SAJAA 2008; 14(4): 25-28

ABSTRACT

Background: The purpose of the study was to examine the previously reported finding that the addition of bicarbonate to lignocaine enhanced the antimicrobial effect of the local anaesthetic agent on a range of bacteria implicated in epidural infections and to determine if this would also hold true for bupivacaine.

Methods: Bupivacaine at a concentration of 0.25% and lignocaine at a concentration of 1% with and without an alkaline buffer were inoculated with suspensions of *Staphylococcus epidermidis, Staphylococcus aureus* or *Escherichia coli.* The mixtures were plated on 2% blood agar plates immediately (T = 0) and after 24 hours at room temperature (T = 24), and numbers of colonies were recorded.

The difference in the number of colonies between T = 0 and T = 24 was expressed as a fraction of the T=0 value, giving a percentage inhibition of colony growth. Statistical testing was performed using Kruskal-Wallis non-parametric One Way Analysis of Variance (ANOVA) followed by multiple comparisons of mean ranks between all groups.

Results: At concentrations of 0.25% bupivacaine and 1% lignocaine, these findings suggest that 1% lignocaine has no antibacterial activity. The addition of bicarbonate has no influence.

Conclusions: Bupivacaine has significant antibacterial activity, but the addition of bicarbonate has no influence.

Introduction

Local anaesthetic infusion devices are becoming an increasingly popular method of administering postoperative analgesia. Although infectious complications are rare for the epidural route, their consequences can be devastating. The likely sources of these microbes are the patient's cutaneous flora, or the anaesthetist's ear, nose and throat flora. Certain patient populations may be at increased risk: for example those with indwelling axillary or femoral catheters, those with diabetes, or those with human immunodeficiency virus. Another high-risk group may be palliative care patients due to the prolonged nature of catheter placement and possible depressed immune response. Raedler et al. showed that the contamination rate of spinal and epidural needles might be as high as 18% even when full aseptic protocols are followed.[1] It has been postulated that the antibacterial properties of local anaesthetic solutions may play a role in the low clinical incidence of infection.[2] The antimicrobial properties of various local anaesthetic solutions have been well studied. It has been suggested that lignocaine in combination with a bicarbonate buffer has an enhanced antibacterial activity.[3] The effects of alkalinisation of other local anaesthetic solutions and its effect on the antimicrobial properties has not been studied. It remains to be shown whether this property can be enhanced and used to reduce the likelihood of clinical infection. This effect could be of some benefit in high-risk patient groups. The purpose of this study was to determine if bupivacaine, a more commonly used local anaesthetic than lignocaine for infusion devices in our clinical practice, would display similar properties to those described for lignocaine at clinically relevant concentrations. In addition, we attempted to confirm the findings of Thompson et al,[3] that the addition of bicarbonate enhanced the antimicrobial properties of lignocaine.

Methods

Preservative free solutions of 0.5% bupivacaine and 2% lignocaine used for this study were obtained from Micro Healthcare (Bethlehem, South Africa). Solutions were alkalinised with 4% sodium bicarbonate, Fresenius Kabi (Port Elizabeth, South Africa). Dilutions were made using sterile 0.9% sodium chloride solution (saline).

Table I: The solutions used for bacterial suspension inoculation

Saline is sterile 0.9% sodium chloride solution. LA is the volume of local anaesthetic added (either 0.5% bupivacaine or 2% lignocaine added). Bicarb is the volume of 4% bicarbonate added.

Solution	Saline (ml)	LA (ml)	Bicarb (ml)	Total Vol (ml)	Final LA conc (%)	pH (mean±SD)
Saline	4.95	-	-	4.95	-	6.20 ± 0.02
Saline + bicarbonate	4.90	-	0.05	4.95	-	8.70 ± 0.02
Lignocaine	2.45	2.50	-	4.95	1	6.13 ± 0.01
Lignocaine + bicarbonate	2.40	2.50	0.05	4.95	1	6.78 ± 0.03
Bupivacaine	2.45	2.50	-	4.95	0.25	5.45 ± 0.01
Bupivacaine + bicarbonate	2.40	2.50	0.05	4.95	0.25	7.33 ± 0.03

The solutions used for bacterial suspension inoculation consisted of local anaesthetic solutions (LA) made up to 4.95 ml with saline and/or bicarbonate as shown in Table I.

pH values for an identical, separate set of sterile solutions were measured using a Crison PH25 pH meter (Crison Instruments, Spain). Five pH readings were taken for each solution. The mean values are shown in Table I.

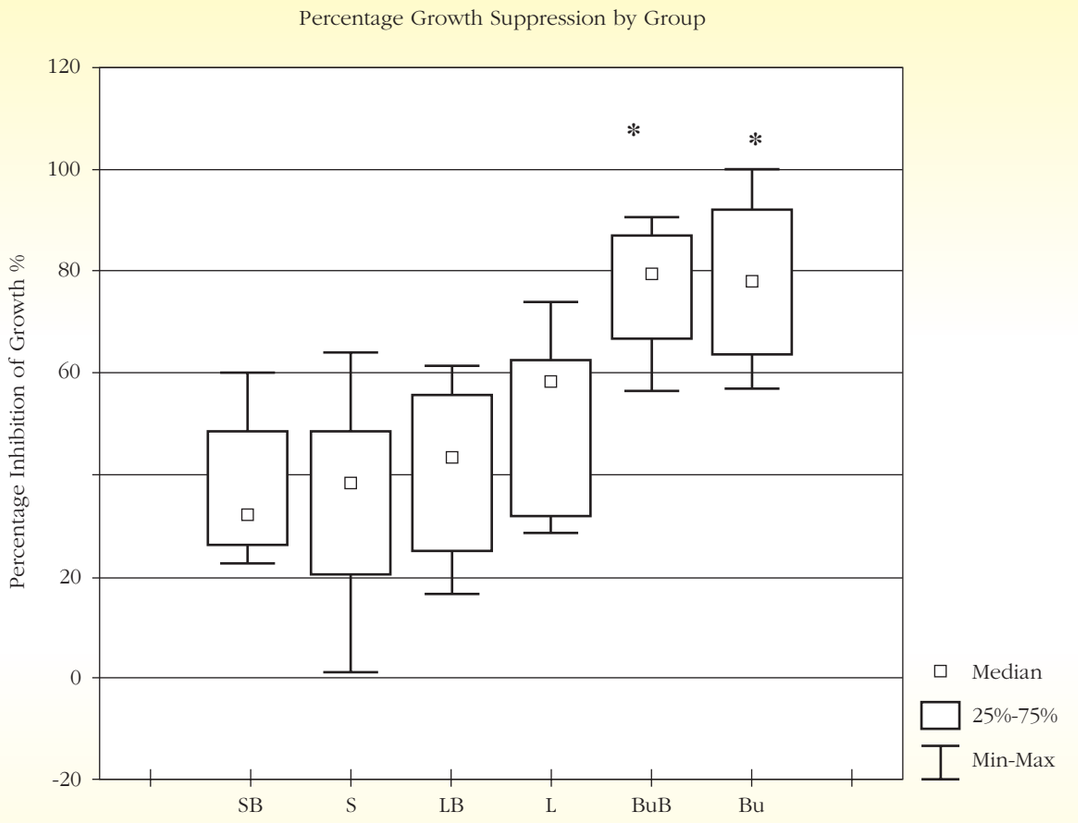
Microbiological assay
Bacteria tested were *Staphylococcus epidermidis, Staphylococcus aureus* and *Escherichia coli*. Densities of bacterial suspensions were prepared according to McFarland 0.5 using a Vitek Colorimeter (bioMerieux, Hazelwood, Missouri). McFarland standards are used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range. A 0.5 McFarland number equates to an approximate cell density of 1.5 x 108 colony forming units per millilitre. Fifty microlitres of this suspension was used to inoculate the local anaesthetic solutions described above. Control solutions consisted of 4.95 ml of 0.9% sodium chloride and 4.90 ml 0.9% sodium chloride with the addition of fifty microlitres of 4% bicarbonate (a total volume of 4.95 ml), as for local anaesthetic solutions. Immediately after inoculation, fifty microlitres of the solution was taken for dilution into 4.95 ml saline solution; equating to a 100-fold dilution. Fifty microlitres of this solution was then plated onto 2% blood agar plates and incubated at 35 degrees Celsius for 18–24 hours. Colonies were counted at this point to determine the initial number of colony forming units (T = 0) from which the percentage inhibition at 24 hours could be calculated. The inoculated solutions were then left in the laboratory, at room temperature. After 24 hours, fifty microlitres of the solution was taken for dilution into 4.95 ml saline solution, once again. Fifty microlitres of this solution was then plated onto 2% blood agar plates and incubated at 35 degrees Celsius for 18–24 hours. The number of colony forming units remaining now is defined as T = 24. The entire assay was performed on two separate occasions for each bacterial species and the results pooled.

In summary, each test solution was inoculated in turn with each of the three bacterial preparations. One sample was taken immediately and incubated for 24 hours on an agar plate. Another sample was obtained from each solution after being left in the laboratory, at room temperature, for 24 hours. This was then plated onto the agar and again incubated for 24 hours to count the number of colonies.

Statistical analysis
The difference in the number of colonies between T = 0 and T = 24 was expressed as a fraction of the T = 0 value, giving a percentage inhibition of colony growth, with the larger ratio indicating greater inhibition. This allows control for the different growth rates within the individual colonies and the different initial colony sizes. Statistical testing was performed using Kruskal-Wallis non-parametric ANOVA followed by multiple comparisons of mean ranks between all groups once a significant difference had been demonstrated in the ANOVA ($p < 0.0003$). The null hypothesis, that there would be no significant differences in bacterial growth between any of the solutions tested, was rejected if $p \leq 0.05$.
As there was no difference in percentage inhibition between bacterial colony types, all bacterial data were pooled by growth solution.

Results
Inhibition of bacterial growth was seen with bupivacaine, compared to saline in the pooled results for all bacterial colonies studied (Figure 1). There were no differences between the lignocaine-incubated samples compared with the saline solutions (Figure 1). The addition of bicarbonate to these solutions had no effect.

Figure 1: Percentage growth suppression in the various groups.



* - p<0.05 for differences from saline and saline with bicarbonate
Bu – plain bupivacaine
BuB – bupivne plus bicarbonate
L – plain lignocaine
LB – lignocaine plus bicarbonate
S – saline
SB – saline plus bicarbonate

Discussion

This study demonstrated that 1% lignocaine possesses no antibacterial properties greater than that of saline against the microorganisms tested, although there was a suggestion of some inhibition of colony growth in the plain lignocaine group. We cannot exclude the possibility that a larger study could have found a more subtle difference. This study had only a 30% power to detect a difference of this magnitude at an alpha value of 0.05. A sample size of 60 in each group would be needed for 80% power to detect an effect of this magnitude, the clinical significance of which would be debatable. The addition of sodium bicarbonate had no significant effect (see Figure 1). Conversely, 0.25% bupivacaine had a significant antibacterial effect, but the addition of sodium bicarbonate had no additional effect. Sterile 0.9% saline solution was used as a control, and the effect of sodium bicarbonate was found to be non-significant.

Clinically relevant, 'weaker', solutions were deliberately used in an attempt to determine subtle differences in a solution that would be expected to have only a weak antimicrobial effect. In particular, 0.25% bupivacaine was chosen as a commonly used concentration for infusion solutions.

Thompson and colleagues demonstrated that the addition of bicarbonate to lignocaine dramatically enhanced the rate of killing in the bacteria studied.[3] This study used a 4% lignocaine solution, which also contained a preservative, 0.1% methylparaben. Since a much stronger solution of lignocaine was used, this meant that a much larger amount of sodium bicarbonate could be added without precipitation occurring. Bacterial species included *Staphylococcus aureus* and *Escherichia coli*. This result could not be reproduced in our study using 1% lignocaine. The addition of bicarbonate to lignocaine or bupivacaine made no difference to the final bacterial counts. Their methodology was very different from ours, using Mueller-Hinton broth which is likely to have buffered the effect of the addition of bicarbonate. Mueller-Hinton broth is a liquid medium that utilises beef infusion solids, and casein to provide nitrogenous compounds, vitamins, carbon, sulphur and amino acids. Starch is added to absorb any toxic metabolites produced. No mention is made of the pH of these solutions. A control containing only additional bicarbonate was investigated and this appeared to have no effect on the growth of the organisms.

In concert with our study, Aydin et al also found 1% lignocaine to have non-significant antimicrobial properties against these bacteria,[2] whereas Feldman et al describes statistically significant reduction in bacterial colonies for this solution.[4] These varied results probably suggest only weak antimicrobial properties.

Previous studies have shown 0.25% bupivacaine to be bactericidal against bacteria commonly implicated in epidural catheter-associated infections.[5] Some studies have shown *S aureus* to be relatively resistant, requiring 0.5% bupivacaine for statistically significant results.[4,5] Our numbers do not allow us to draw conclusions in this regard.

The mechanism of the antimicrobial effect of local anaesthetics remains unknown. It is possible that osmolarity and pH differences have an effect,[6] although our data do not suggest a substantial effect. This may disrupt bacterial cell walls, but little is known about the precise mechanisms.

It has become common practice to decrease local anaesthetic concentrations with the addition of an opioid in infusion devices. This combination is known to have little antimicrobial effect.[4] The newer local anaesthetic solutions, such a ropivacaine and levobupivacaine, have also been shown to have minimal antimicrobial effects.[5,7] An increase in catheter related infections has not been noted, despite a probable increase in the frequency of immunocompromised patients. Either this has just not been studied, or, more likely, there are additional factors, not yet understood which lead to the low incidence of infection in the face of a high incidence of bacterial contamination. Bacterial loads are unlikely to have been as high as those used in this, and other, studies.

Our data show that 0.25% bupivacaine has only weak antimicrobial properties. One per cent lignocaine has no antimicrobial properties. The alkalinisation of these solutions does not alter their antimicrobial effects.

Acknowledgements

We are very grateful to Dr Andrew Whitelaw and the microbiology laboratory at Groote Schuur Hospital for their assistance, advice and cooperation. Special thanks to Prof Adrian Bösenberg and Prof Michael James for their advice and statistical analysis. **SAJAA**

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