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## Poor Antibacterial Effect of Ropivacaine

### Comparison with Bupivacaine

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ROPIVACAINE (1-propyl-2',6'-pipecoloxylidide) is a long-acting aminoamide local anesthetic that has been introduced into clinical use in the last few years,<sup>1</sup> largely as a replacement for bupivacaine. Bupivacaine has been reported to possess a significant antibacterial effect.<sup>2-4</sup> Because data regarding ropivacaine are not available, we compared the antibacterial effects of clinically appropriate concentrations of ropivacaine and bupivacaine in a laboratory setting.

### Materials and Methods

#### Drugs

Commercially available solutions of bupivacaine hydrochloride (7.5 mg/ml; Marcain, Astra, Södertälje, Sweden) and ropivacaine hydrochloride (7.5 mg/ml; Naropin, Astra) were diluted with growth media to produce the concentrations of 3.75, 1.875, and 0.938 mg/ml. None of the solutions contained preservatives.

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#### Bacterial Strains

Eight bacterial strains were included in the study. The strains were *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* ATCC13883, *Pseudomonas aeruginosa* ATCC27853, *Staphylococcus aureus* ATCC25923, *Staphylococcus epidermidis* ATCC12228, *Streptococcus pyogenes* ATCC19615, *Staphylococcus aureus* T-24171 (a clinical isolate), and *Staphylococcus aureus* T-24158 (a clinical methicillin and multiresistant isolate (MRSA)). The strains were grown on blood agar plates at 37°C before the antibacterial assays.

#### The Antibacterial Assay

The effect of the drugs on bacterial growth was determined by broth microdilution method.<sup>5</sup> Each determination was performed as five parallel assays. The test medium for *S. pyogenes* was Todd Hewitt broth that contained 30 g/l Todd Hewitt broth powder (BBL Microbiology Systems, Becton Dickinson and Co., Cockeysville, M.D.). The test medium for the other bacteria was a tryptone soya broth-based medium that contained 15 g/l tryptone soya broth powder (Oxoid CM129; Oxoid, Columbia, M.D.). The pH of the tryptone soya broth-based medium was adjusted to 7.2 and the pH of the Todd Hewitt broth medium was adjusted to 7.8. The inoculum was  $2.5 \times 10^4$  blood agar-grown cells per ml. Aliquots (100  $\mu$ l) of inoculated double-strength test media were pipetted into microplate wells that already contained increasing concentrations of the drug preparations in 100  $\mu$ l sterile purified water. After an incubation of 18 h at 37°C, the growth measured as the absorbance of light at the wavelength of 540 nm (absorbance A540) was measured with a Titertek Multiscan spectrophotometer (Labsystems, Helsinki, Finland). Uninoculated media were used to blank the spectrophotometer. Because a precipitate was formed when either bupivacaine or ropivacaine was added to Todd Hewitt broth at a concentration of 1.8 mg/ml, only the effect of the low

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concentration (0.938 mg/ml) of the local anesthetics on the growth of *S. pyogenes* could be studied.

### Statistical Analysis

The data on the effect of the different concentrations of bupivacaine and ropivacaine on the growth of the bacteria were compared using two-way analysis of variance for multiple comparisons. For comparison of the effect of the different concentrations of each drug with its control, the Scheffé F test was used. Statistical analysis was performed using StatView II (Brain Power, Calabasas, CA) and NCSS2000 (NCSS Statistical Software, Kaysville, UT) software. The results are given as mean and SD.  $P < 0.05$  was regarded as statistically significant.

## Results

With regard to all concentrations of the local anesthetics studied, bupivacaine was a stronger inhibitor of growth of all bacteria included in the study ( $P < 0.001$ , two-way analysis of variance) except *K. pneumoniae* (not significant; fig. 1). The highest concentration of bupivacaine, 3.75 mg/ml, fully inhibited the growth of *E. coli*, *P. aeruginosa*, and *S. epidermidis*. Even the lowest concentration of bupivacaine had a statistically significant inhibitory effect on the growth of *P. aeruginosa*, *S. epidermidis*, and *S. pyogenes*.

## Discussion

At clinically used concentrations, bupivacaine was statistically a significantly stronger inhibitor of growth of all bacteria included in the study, except *K. pneumoniae*, than was ropivacaine. The antibacterial effect of bupivacaine improved with increasing concentration.

Ropivacaine and bupivacaine have the same chemical structure except for the alkyl group:  $-C_3H_7$  in ropivacaine and  $-C_4H_9$  in bupivacaine. Even a small difference in the length of the alkyl chain is known to affect the antibacterial activity of hydrophobic inhibitors remarkably.<sup>6-8</sup> Unfortunately, mepivacaine, with the same basic pipecoloxylidide structure as ropivacaine and bupivacaine and with a still shorter alkyl chain ( $-CH_3$ ), has not been studied in a setting similar to ours using solutions without possibly neurotoxic preservatives. However, in a study by Sakuragi *et al.*<sup>9</sup> a very concentrated solution of mepivacaine 20 mg/ml had a similar inhibitory effect on the growth of two strains of methicillin-resistant *S. aureus* as did bupivacaine, 1.25 mg/ml, and

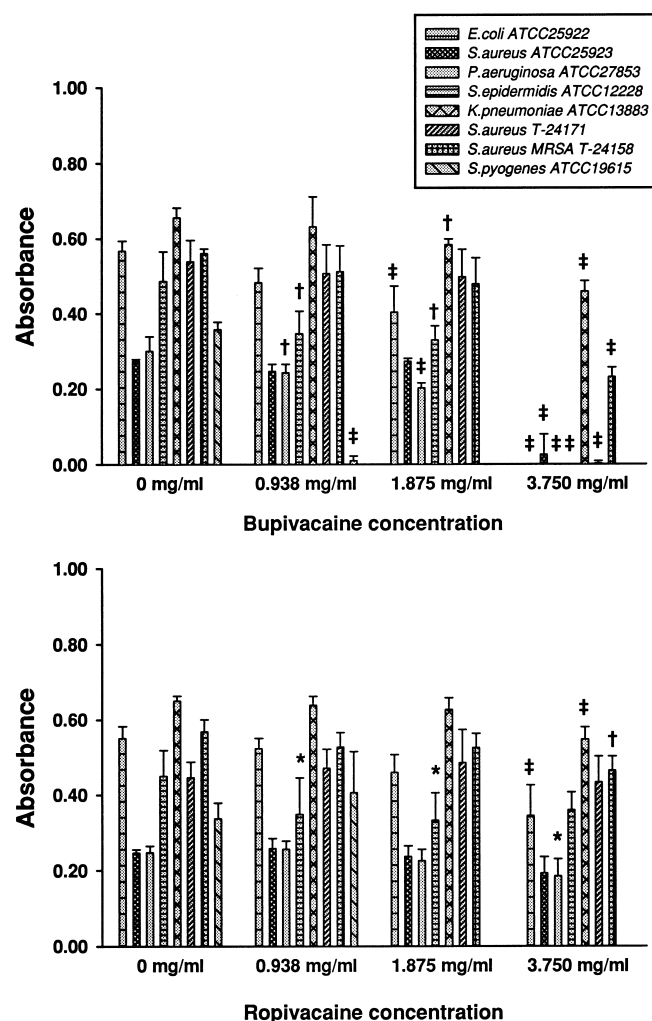


Fig. 1. The effect of different concentrations of bupivacaine and ropivacaine on the growth of eight strains of bacteria. Lower absorption of light at 540 nm (mean  $\pm$  SD) indicates greater inhibition of growth. \* $P < 0.05$ , † $P < 0.01$ , ‡ $P < 0.001$  in comparison with the control (0 mg/ml) of the same local anesthetic.

had a poorer effect than bupivacaine, 2.5 mg/ml, when solutions containing preservatives were used.

In addition to bupivacaine, many other local anesthetics have been shown to possess antibacterial activity. According to Nitescu *et al.*,<sup>10</sup> the order of the bactericidal potency from the strongest to the weakest would be dibucaine to tetracaine to bupivacaine to prilocaine to lidocaine to procaine (bupivacaine being approximately four times less effective than dibucaine).

In a recently published survey of 42 patients with catheter-related epidural abscesses,<sup>11</sup> *S. aureus* was identified as the infecting organism in 25 (60%), with

other *Staphylococci* identified in another 4 (10%), and *P. aeruginosa* in 3 (7%). A concentration of 5 mg/ml bupivacaine was necessary to inhibit the growth of *S. aureus* ATCC25923 in a previously published study,<sup>2</sup> but a 3.75-mg/ml solution of bupivacaine was effective against three different strains of *S. aureus* in our study, in which more sophisticated microbiological methodology was applied for the assessment.<sup>5</sup>

The enantiomers of local anesthetics may differ in terms of pharmacokinetic and pharmacodynamic properties, with resultant differences in clinical local anesthetic action and toxicity.<sup>12</sup> As the cardiotoxicity of d-bupivacaine is higher than that of the l-enantiomer,<sup>13</sup> the antibacterial properties of the enantiomers may also differ. This would warrant further study.

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