In Vitro Effect of Local Anesthetics on Candida albicans Germ Tube Formation

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ABSTRACT

Objective: This study was planned to clarify the in vitro effect of lidocaine and bupivacaine on germ tube formation by Candida albicans isolates from cases of clinical vaginal candidiasis.

Methods: Fourteen C. albicans strains (clinical vaginal isolates) were grown on Sabouraud agar for 24 h at 37°C and tested as follows: 100 µl of a yeast suspension [10⁵ colony forming units (CFU)/ml of phosphate buffered saline (PBS)] was added to 500 µl of fresh human serum with lidocaine or bupivacaine (pure salts) in serial concentrations. The test was run in duplicate. Controls were prepared for each strain. After 4 h of incubation at 37°C, samples were taken from each vial and 200 yeasts were counted in a counting chamber. The pH of each suspension was measured.

Results: The results are given as the mean of the 2 readings and are expressed as the percentage of blastoconidia with germ tubes/total blastoconidia.

Conclusions: Our experiments show that both lidocaine and bupivacaine have a dose-dependent inhibitory effect, pH-independent, on germ tube formation by C. albicans and that both drugs seem to be promising in the treatment of genital candidiasis due to the combination of anesthetic and antifungal properties.

KEY WORDS
Local anesthetics, germ tube, antimicrobial activity, candidiasis

In 1909 Jonnesco first described the antibacterial effect of local anesthetic drugs and in 1955 Murphy et al. reported the toxicity of tetracaine in relation to Pseudomonas. Kleinfeld and Ellis could demonstrate a toxic effect of tetracaine, benoxinate, and cocaine to Candida albicans. Erlich showed that, while tetracaine at a 0.5% concentration inhibited C. albicans as well as Staphylococcus aureus; greater concentrations of lidocaine were required to obtain a similar effect. In 1970 Schmidt and Rosenkranz demonstrated the antimicrobial activity of lidocaine and procaine against several bacteria and yeasts. These authors did not study any genital specimens. Of 10 Cryptococcus neoformans and C. albicans studied (isolates from nongenital sources), all 5 Cryptococcus were inhibited by lidocaine and procaine in concentrations in excess of 1.0%. However, none of the 5 Candida strains were inhibited by 2.0% lidocaine or procaine. Meanwhile, all 10 isolates were inhibited by 0.5–1.0 µg/ml of amphotericin B. Therefore, the results of Schmidt and Rosenkranz regarding C. albicans are in disagreement with those of previous authors.

It is admitted that filamentation of Candida blastoconidia is a phenotypical change related to pathogenesis. The presence of hyphae on the bedside "wet smear" examination is supposed to indicate invasiveness and an abnormal stage not accepted as colonization. Some years ago, Taschdjian et al. used germ tube formation as a test for identification...
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Fig. 1. Effect of lidocaine.

of C. albicans. In addition to antifungal properties, local anesthetics could help in relieving the very stressful symptoms commonly present in genital candidiasis. The present work was performed to clarify the in vitro effect of 2 local anesthetics, lidocaine, a common and well-known agent available for topical use, and bupivacaine, a more recent and potent drug, on the germ tube formation by C. albicans.

MATERIALS AND METHODS

All 14 C. albicans strains used in this study were clinical isolates from microbiologically diagnosed cases of vaginal candidiasis, some of them from recalcitrant cases or cases resistant in vivo to several antifungal treatments. They were grown on Sabouraud agar (Difco, Detroit, MI) for 24 hours at 37°C and tested as follows: 100 μl of a yeast suspension [10^5 colony forming units (CFU)/ml of phosphate buffered saline (PBS)] was added to 500 μl of fresh human serum (pool) with lidocaine or bupivacaine in serial concentrations. Solutions of lidocaine and bupivacaine hydrochloride (pure salts, free of preservatives, purchased from Sigma, St. Louis, MO) were prepared in sterile PBS immediately before experiments in order to guarantee good stability. For each concentration of both local anesthetics, the test was run in duplicate. Duplicate controls, with plain serum, were prepared with each strain. After 4 hours of incubation at 37°C, the samples were taken from each vial and 200 yeasts were evaluated and counted using a Burke's chamber under a magnification of ×400 on a blind reading. The pH value of each suspension was measured. For the statistical analysis of data, we used the Wilcoxon signed rank tests.11

RESULTS

Results are given as the mean of the 2 readings, for each strain and concentration, and are expressed as the percentage of blastoconidia with germ tubes/
total blastoconidia. No significant pH variation was found between controls and local anesthetic solutions.

The effect of lidocaine is shown in Figure 1 and the effect of bupivacaine in Figure 2. The degree of germ tube inhibition of each local anesthetic at different concentrations is listed in Table 1.

**DISCUSSION**

The mechanisms involved in Candida pathogenesis are not yet well defined, even in *C. albicans*, which is commonly accepted as the most pathogenic species. In contrast to many bacteria, no single virulence factor can be identified as responsible for fungal infectivity. Yeast pathogenicity results from several different factors, the resultant being the overwhelming of the host defenses by the yeasts. Although not a crucial step, germ tube formation is believed to play an important pathogenic role in the initial phase of tissue invasion. In some studies, germ tubes were proved to be associated with better adhesion to epithelial cells in vitro, and it has been repeatedly shown in vitro that *C. albicans* germ tubes grow from macrophages as they do from neutrophils (PMNs) after phagocytosis, in this way preventing the intracellular killing of the yeast. These and several other experimental facts helped to create the concept of a differential behavior of *C. albicans* germ tubes and pseudohyphae in relation to blastoconidia. The reason why *C. albicans* is a common and persistent pathogen in the vaginas of a significant number of women still remains undetermined.

In our study, we decided to use serum for the germ tube test due to the more impressive germ tube production in this substrate when compared to others. In a previous study (unpublished data), we evaluated the different sensitivities for germ tube formation of several media, namely Eagle’s medium, RPMI medium, Sabouraud's dextrose broth, egg albumin, fetal calf serum, and human serum,
Our results show that both lidocaine and bupivacaine have a potent dose-dependent inhibitory effect on germ tube formation by *C. albicans*. This effect is pH-independent, as there is no significant pH variation when compared to controls. From the values we found, bupivacaine seems to be more potent on that action than lidocaine. In fact, when the results are compared for the 0.07% concentration (in which there is a significant effect of both drugs), bupivacaine seems to be 3 times more potent than lidocaine (91.8 ± 5.3% vs. 33.0 ± 20.0% of inhibition; \(P = 7.3 \times 10^{-6}\)) in achieving that effect. In very dilute concentrations such as 0.15% (compared to the ones used in clinical practice for several purposes), we found 100% inhibition of germ tube formation with bupivacaine and almost the same figure with lidocaine (91.6 ± 5.5%). When the 0.25% concentration is considered, both lidocaine and bupivacaine produced an inhibition of 100%. Similarly, low concentration values may be easily attainable in the vaginal milieu, in vivo, probably with similar effects. The mechanism by which local anesthetics exert their antimicrobial effects is still unclear to us. A plausible hypothesis is the impairment of protein synthesis necessary to germ tube formation, namely, the cell wall or the cytoplasmic membrane. Biochemical studies demonstrated that protein synthesis was more sensitive than RNA or DNA production to the inhibitory effect of lidocaine, although no distinct selective inhibition of macromolecular synthesis was found. Further testing is being carried out to clarify this effect. To our knowledge, this is the first report of the effect of local anesthetics, namely lidocaine and bupivacaine, on germ tube formation by *C. albicans*. From a theoretical point of view, these drugs seem to be promising in the treatment of genital candidiasis due to the combination of aesthetic and antifungal properties.

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