Comparison of the In Vitro Activities of Fentonazole, Other Imidazoles, Metronidazole, and Tetracycline against Organisms Associated with Bacterial Vaginosis and Skin Infections

BRIAN M. JONES,* IAN GEARY, MARGARET E. LEE, AND BRIAN I. DUERDEN

Department of Medical Microbiology, University Medical School, Sheffield S10 2RX, United Kingdom

Received 5 December 1988/Accepted 6 March 1989

The in vitro antibacterial activity of the antifungal compound fentonazole was compared with those of clotrimazol, miconazole, tetracycline, and metronidazol against 177 strains of bacterial species associated with either bacterial vaginosis (BV) or skin infections by agar dilution MIC determinations. BV-associated Bacteroides isolates of the Bacteroides melaninogenicus-B. oralis group, Gardnerella vaginalis, Mobiluncus spp., and anaerobic, gram-positive cocci were highly susceptible to fentonazole, clotrimazol, and miconazole; but Bacteroides spp. not associated with BV, Bacteroides ureolyticus and the Bacteroides fragilis group, were resistant. All Bacteroides strains were susceptible to metronidazol, but the susceptibility of G. vaginalis and Mobiluncus spp. varied. Among the skin bacteria, Staphylococcus aureus, coryneforms, and streptococci were highly susceptible to the imidazoles; but Staphylococcus epidermidis strains were generally resistant. This antibacterial activity may give fentonazole a useful role in the topical treatment of vaginal discharge and in mycotic skin infections that are superinfected with bacteria.

Fentonazole is a new imidazole derivative that was developed for the topical treatment of fungal infections by Recordati S.p.A. (Milan, Italy). It is reported to be a safe, well-tolerated compound with good in vitro and in vivo activities against dermatophyte pathogens such as Microsporum, Epidermophyton, and Trichophyton spp. and against Candida albicans in patients with vaginal candidiasis (May and Baker Ltd., personal communication).

In initial studies it was reported that fentonazole is also active against some bacteria, particularly staphylococci, and it was thought that it may have useful roles in mycotic infections superinfected with bacteria and in vaginal infections, in which activity against C. albicans and the organisms associated with bacterial vaginosis (BV), Gardnerella vaginalis, Mobiluncus spp., Bacteroides spp., and anaerobic gram-positive cocci, could be appropriate for empiric treatment.

Therefore, we undertook this study to compare, in vitro, (i) the activities of fentonazole, clotrimazol, miconazole, and metronidazol against the organisms associated with BV and (ii) the activity of the imidazole compounds and tetracycline against organisms associated with skin infections.

Fentonazole [1-(2,4-dichlorophenyl)-2-(N-imidazolyl) ethyl 4-phenylthiobenzyl ether nitrate; batch N860/2/115] and metronidazol (batch WN404) were supplied by May and Baker Ltd. (Dagenham, United Kingdom). Miconazole, clotrimazol, and tetracycline were obtained from Sigma Ltd. (Poole, United Kingdom). All antibiotics were used without a need for adjustment of concentrations, as all the preparations had 100% (wt/wt) activity.

At the outset of the study, we experienced considerable difficulties with the protein-binding properties of fentonazole. The activity of the compound was adversely affected by horse blood and by the medium-enriching additives Proteose Peptone (Difco Laboratories, Detroit, Mich.), hemin, and menadione. Wilkins-Chalgren agar, the standard reference medium, was unsuitable as it contained these ingredients. Thus, we had the problem of finding a suitable medium lacking blood, serum, or the additives mentioned above which would support the growth, in an agar dilution technique, of all the fastidious organisms that were the targets of the study. We did not find one that would support all the organisms and were, therefore, obliged to select only those organisms which would grow on a basic medium. Two hundred such strains were chosen from those isolated from clinical specimens submitted to our department and the Departments of Bacteriology at the Royal Hallamshire and Children's Hospitals (Sheffield, United Kingdom). All strains were stored in liquid nitrogen vapor at −135°C before use. The collection was made up of Staphylococcus aureus (20 isolates), Staphylococcus epidermidis (20 isolates), coryneforms (aerobic diphtheroids; 15 isolates), Streptococ cus pneumoniae (5 isolates), beta-hemolytic streptococci (20 isolates), gram-positive, anaerobic cocci (10 isolates), G. vaginalis (20 isolates), Mobiluncus spp. (10 isolates), and Bacteroides spp. (80 isolates). The reference strains included in this study were Staphylococcus aureus NCTC 6571, coagulase-negative Staphylococcus sp. strain NCTC 7292, Bacteroides fragilis NCTC 9343, and Bacteroides melaninogenicus NCTC 9338. Staphylococci, streptococci, coryneforms, and anaerobic cocci were identified by standard methods (1). G. vaginalis, Bacteroides spp., and Mobiluncus spp. were identified by the methods of Jones (4), Duerden et al. (2), and Sprott et al. (6), respectively. The culture medium used throughout the study for initial cultures and for MIC tests was DST agar (Oxoid Ltd., Basingstoke, United Kingdom) without enrichment.

With the exception of tetracycline, which was dissolved and diluted in distilled water, the antimicrobial agents were weighed and then dissolved and diluted in polyethylene glycol 200. This compound was the only one found that effectively dissolved the imidazoles and was recommended for use in MIC studies by May and Baker Ltd. (personal communication). A range of doubling dilutions was made in 10-ml volumes, and these were added to 90 ml of molten DST agar to produce plates containing 16 to 0.03 μg of each
agent per ml. Cultures on control plates containing polyethylene glycol 200 (10% vol/vol) showed that the solvent and diluent itself did not inhibit the growth of the test strains.

The test strains were grown on DST agar for 24 to 48 h (the longer period was used for slow-growing strains), and the growth was harvested into sterile saline. These suspensions were then diluted to a concentration of 10⁶ CFU/ml, which was determined by comparison with match-opacity tubes (Wellcome Diagnostics, Dartford, United Kingdom) that were previously calibrated in this laboratory (3). A multipoint inoculator (Denley Instruments Ltd., Billinghamurst, United Kingdom) was used to deliver 0.001-ml inocula (10⁶ CFU) to the surface of the plates. Cultures were incubated appropriately, i.e., streptococci, staphylococci, and coryneforms for 18 h at 37°C in 5% CO₂ in air; G. vaginalis for 48 h at 37°C in 5% CO₂ in air; and anaerobic organisms for 48 h at 37°C in an anaerobic cabinet containing 10% H₂, 10% CO₂, and 80% N₂ (Don Whitley Scientific, Shipley, United Kingdom). MICs were taken as the lowest concentration that either prevented growth or produced a barely visible haze.

In all, satisfactory MIC results were obtained for 177 of the strains tested. The results obtained with the species associated with BV are given in Tables 1 and 3. The Bacteroides spp. associated with BV, the B. melaninogenicus-B. oralis group, were highly susceptible to fenticonazole, clotrimazole, and miconazole. Among the Bacteroides spp. not associated with BV, the B. fragilis group from intestine-related abdominal sepsis was mainly resistant to these compounds, and B. ureolyticus strains from superficial necrotizing lesions were even more resistant. Metronidazole was very active against all Bacteroides strains tested. G. vaginalis, Mobiluncus spp., and gram-positive, anaerobic cocci were highly susceptible to fenticonazole, clotrimazole, and miconazole; but their susceptibilities to tetracycline and metronidazole varied.

The results obtained with the organisms associated with skin infections are shown in Tables 2 and 3. Generally, Staphylococcus aureus, coryneforms, and streptococci were highly susceptible to fenticonazole, clotrimazole, and miconazole; the exception was that the MICs of clotrimazole against Staphylococcus aureus were as follows: range, 2 to 4 μg/ml, MICs for 50% and 90% of strains tested, 2 and 4 μg/ml, respectively. Staphylococcus epidermidis strains were generally more resistant to all agents tested.

Fenticonazole had high in vitro activity against the Bacteroides spp. associated with BV, with MICs comparable to those of metronidazole. It was superior in activity to metronidazole against G. vaginalis, Mobiluncus spp., and the anaerobic, gram-positive cocci. It was highly active against Staphylococcus aureus, diphtheroids, and streptococci but not against many strains of Staphylococcus epidermidis.

Fenticonazole has proven activity against dermatophyte infections and vaginal candidiasis. With this additional range of antibacterial activity, it may have a useful role in the topical treatment of BV, particularly in the empiric treatment of vaginal discharge when it may be thought that it is appropriate to use an agent that has combined antibacterial and antifungal activities. If it were clinically effective, some patients would prefer topical treatment to systemic treatment with metronidazole. Although standard treatment with metronidazole is safe and effective (5), some patients find it undesirable because it may cause an unpleasant metallic taste and because of its interaction with alcohol. There may be a similar role for fenticonazole in mycotic skin infections.
superinfected with bacteria which may not be readily accessible to systemic antimicrobial agents.

The in vitro results indicate that clinical studies of fenticonazole in these mixed infections would be appropriate.

We are grateful to the Bacteriology Departments of the Royal Hallamshire and Children’s Hospitals, Sheffield, for providing many of the strains tested and thank H. Storer for excellent secretarial assistance.

The financial support of Rhone-Poulenc Ltd. is gratefully acknowledged.

LITERATURE CITED


