Activity of Coumermycin against Clinical Isolates of Staphylococci

MARILYS N. GUILLEMIN,* HELEN M. MILES, AND MALCOLM I. MCDONALD

Microbiology Department, Royal Melbourne Hospital, Parkville, Victoria 3050, Australia

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Staphylococci, particularly methicillin-resistant strains of Staphylococcus aureus, are major nosocomial pathogens in large hospitals in eastern Australia. At present vancomycin is the drug of choice for the treatment of life-threatening methicillin-resistant S. aureus infections. A possible alternative drug is coumermycin, a bis-hydroxy coumarin which inhibits DNA gyrase. Coumermycin activity was determined against clinical isolates from the Royal Melbourne Hospital. MICs of 639 staphylococcal isolates were determined by agar dilution. MICs and MBCs of 100 staphylococcal isolates were also determined by microdilution methods. The results showed that coumermycin was bactericidal, with MBCs of ≤4 μg/ml against all isolates tested. The results indicate that coumermycin is a potential alternative to vancomycin in the treatment of severe staphylococcal infections.

Staphylococcus aureus and coagulase-negative staphylococci are major nosocomial pathogens. In 1977, methicillin-resistant Staphylococcus aureus emerged in large hospitals throughout eastern Australia. By 1979 at least 31 metropolitan hospitals in Melbourne were involved, and in six of these methicillin-resistant S. aureus accounted for 20 to 40% of all S. aureus isolates. Significant morbidity and mortality was reported, with methicillin-resistant S. aureus being a fully virulent organism (10). Over the last 6 years, methicillin-resistant S. aureus has been isolated from more than 17,000 hospitalized patients in the state of Victoria (population, 3 million) and has been responsible for significant nosocomial infection in more than 5,000 patients (2). Vancomycin is currently the drug of choice for the treatment of life-threatening methicillin-resistant S. aureus infections (3).

It is expensive and has potentially serious ototoxic and nephrotoxic effects (5). Traber and Levine (12) have reported a case of auditory toxicity that developed in a patient who was receiving vancomycin, with peak vancomycin levels of 46.5 and 49.2 μg/ml. At present there are no alternative bactericidal antibiotics available.

Coumermycin is an antibiotic derived from Streptomyces hazelensis. It is a bis-hydroxy coumarin compound which acts by inhibiting DNA gyrase (4). Coumermycin is suitable only for intravenous use; intramuscular administration has occasionally been associated with severe local reactions at the site of injection (Hoffman-La Roche Inc., Nutley, N.J., unpublished data). The drug is poorly absorbed from the gastrointestinal tract (9). When given intravenously, peak levels of 18 to 24 μg/ml per 100-mg dose in serum are achievable in adults. The drug has a slow elimination rate, with a half-life of 15 to 46 h (4).

When coumermycin was tested against methicillin-resistant S. aureus strains in the United States, MBCs ranging from 0.004 to 1.56 μg/ml were demonstrated (8). However, genetic analyses and susceptibility testing of methicillin-resistant S. aureus isolates from Australia, the United States, and Europe have shown significant strain differences between countries. These distinctions relate to penicillinase production, plasmid types, and susceptibilities to a range of antibacterial agents (11). For example, strains from eastern Australia are almost invariably resistant to gentamicin and cotrimoxazole (6). Therefore, it was considered necessary to determine independently the coumermycin susceptibility of Australian methicillin-resistant S. aureus strains.

MATERIALS AND METHODS

Antimicrobial agents. Coumermycin was provided by Hoffman-La Roche Inc. The powder was dissolved in methanol to give a stock solution of 16 mg/ml and kept at −20°C; the final methanol concentration in the test medium was 0.05%. For the microdilution method, coumermycin was serially diluted with normal saline in microtiter wells. The plates were stored at −20°C and thawed immediately prior to use. Vancomycin was obtained from Eli Lilly & Co., Indianapolis, Ind.; and fluclaxacillin was obtained from Beecham Laboratories, Bristol, Tenn.

Bacterial isolates. The isolates comprised methicillin-resistant S. aureus, methicillin-sensitive S. aureus, and coagulase-negative staphylococci. The organisms were recovered from clinical specimens of urine, blood, wounds, and sputum collected from patients at the Royal Melbourne Hospital in late 1984 and early 1985. After primary isolation, the isolates underwent coumermycin susceptibility tests together with routine antistaphylococcal susceptibility tests.

Agar dilution MICs. Serial twofold dilutions of coumermycin were set up with normal saline. Fractions were added to molten Iso-Sensitest agar (Oxoid Ltd., Basingstoke, United Kingdom) supplemented with 7% (vol/vol) lysed horse blood. Isolates were seeded into brain heart infusion broth and incubated for 3 to 4 h. Turbidity was adjusted with normal saline to match a 0.5 McFarland standard, and the broth was further diluted to obtain a suspension of approximately 10^7 CFU/ml. With a Steers replicator, agar plates were spot inoculated to give a final inoculum of approximately 10^8 CFU per spot and incubated at 35°C for 18 to 24 h in air. The MIC was read as the lowest antibiotic concentration inhibiting visible growth.

Microdilution MICs. Isolates were seeded into Iso-Sensitest broth (Oxoid Ltd.) and incubated at 35°C for 3 to 4 h. Cultures were then diluted in Iso-Sensitest broth and added to the antibiotic-containing wells to give a final inoculum of approximately 5 × 10^5 CFU/ml. This was confirmed by plate colony counts. After incubation at 35°C for 18 to 24 h in air, the MIC was read as the lowest antibiotic concentration showing no turbidity on visual inspection.

Microdilution MBCs. MBCs were determined by sampling...
the complete 100 μl of contents from each nonturbid well onto whole antibiotic-free horse blood agar plates. After incubation at 35°C for 24 h in air, the MBC was determined as the lowest antibiotic concentration to show 99.9% killing of the original inoculum.

RESULTS

By the agar dilution method, all 374 *S. aureus* isolates were inhibited by 0.125 μg of coumermycin per ml (Table 1). There was no significant difference between methicillin-resistant and -susceptible *S. aureus*. In contrast, the MIC for 100% of coagulase-negative staphylococci was 1.0 μg/ml. MICs for 50 and 90% of 265 coagulase-negative staphylococci were double those obtained with *S. aureus*. A total of 100 different staphylococcal isolates were tested by a microdilution method, and all were killed with 4.0 μg of coumermycin per ml (Table 2). No significant difference in activity against methicillin-resistant and -susceptible *S. aureus* was evident by this method. As only 11 strains of coagulase-negative staphylococci were tested by the microdilution method, comparisons cannot be made. When the results obtained by the two methods were compared, a 16-to 30-fold difference was evident in the MICs. For example, the MIC for 90% of methicillin-susceptible *S. aureus* by the agar dilution was 0.03 μg/ml, and by the microdilution method it was 0.5 μg/ml.

DISCUSSION

Results of in vitro studies demonstrated that coumermycin is active against *Streptococcus pneumoniae, Streptococcus pyogenes,* and *Staphylococcus aureus*; activity against enterococci was considerably poorer. When tested with gram-negative organisms, coumermycin showed moderate activity against *Pseudomonas aeruginosa* and members of the family *Enterobacteriaceae* and marked activity against *Haemophilus influenzae* (Hoffman-La Roche Inc., unpublished data).

In the case of life-threatening methicillin-resistant *S. aureus* infections, the drug of choice is vancomycin. Vancomycin is an expensive drug with potential toxicity problems. Moreover, there is growing concern about the possible emergence of vancomycin-resistant methicillin-resistant *S. aureus* strains (7). An alternative bactericidal drug against methicillin-resistant *S. aureus* may be valuable and timely. The results of this study indicate that coumermycin is bactericidal against hospital staphylococcal isolates in Melbourne. The MBC of coumermycin for 100% of staphylococci tested was ≤4 μg/ml. This is well within achievable coumermycin concentrations in serum of 18 to 24 μg/ml for a 100-mg adult dose.

Because of its slow elimination rate, coumermycin needs to be administered only once a day. The excretion profile for coumermycin has yet to be fully characterized. However, it appears to undergo complete transformation, as no intact drug is detectable in urine. The possibility of alternative routes of excretion has not been explored. In two preliminary safety and tolerance studies in human subjects, the administration of coumermycin was well tolerated, with no significant treatment-related adverse effects (Hoffman-La Roche Inc., unpublished data).

There are well-recognized discrepancies between the results obtained with different methods for determining the MICs and MBCs. Many factors affect the reproducibility of these tests, including inoculum size and preparation, time of incubation, pH, cation concentration, media variations, and manner of sampling for survivors (1). In this study, the broth microdilution MIC results were consistently higher than those obtained by the agar dilution method. The agar dilution MICs were determined with an inoculum of 105 CFU per spot. However, it was necessary to use an inoculum of 5 × 106 CFU/ml in the microdilution system for the MBC to be carried out. The higher inoculum that was used could account for the increase in MICs in the broth dilution over the agar dilution method.

Analyses of methicillin-resistant *S. aureus* strains from various countries show that there are differences in genetic composition and antibiotic susceptibility patterns. For this reason it was considered important that Australian methicillin-resistant *S. aureus* strains be tested. Data from coumermycin susceptibility tests previously performed in the United States may not be valid in Australia. The results indicate that coumermycin is a potential relatively nontoxic alternative to vancomycin for the treatment of life-threatening methicillin-resistant *S. aureus* infections. The in vivo activity of coumermycin has been evaluated in mice infected systemically with methicillin-sensitive and -resistant strains of *S. aureus*. Coumermycin was found to be highly active in these animal studies (Hoffman-La Roche Inc., unpublished data). It is now appropriate for the drug to be evaluated in a clinical setting.

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LITERATURE CITED


2. Bennett, N. M. 1985. Hospital outbreak of multiresistant *Staph-