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In-vitro activities of trospectomycin, cefpodoxime, and second-generation cephalosporins against *Haemophilus influenzae* type b

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The in-vitro activities of trospectomycin, cefpodoxime, cefamandole, cefonicid, and cefuroxime against β-lactamase-negative and -positive invasive clinical isolates of *Haemophilus influenzae* type b were determined by the agar dilution method. Trospectomycin and cefpodoxime inhibited 90% of the strains at concentrations of 5 and 0·06 mg/l, respectively, and no differences between the susceptibilities of the β-lactamase-negative and -positive strains were noted. The activity of cefpodoxime was minimally affected by increased inoculum size, but significant inoculum effects were noted with cefamandole, cefonicid, and cefuroxime with β-lactamase positive strains.

Introduction

The increasing incidence of resistance among clinical isolates of *Haemophilus influenzae* type b to ampicillin and other β-lactam antibiotics has underscored the need to identify new agents which may be effective in the therapy of *H. influenzae* infections (Doern *et al.*, 1988). Trospectomycin sulphate, a novel spectinomycin analogue, and cefpodoxime, an oral third generation cephalosporin have both demonstrated activity against *Haemophilus* species *in vitro* (Jones & Barry, 1988; Zurenko *et al.*, 1988). The purpose of our study was to evaluate the susceptibilities of invasive clinical isolates of type b *H. influenzae* to these antimicrobial agents. For comparison, we examined the in-vitro activities of ampicillin and chloramphenicol, as well as cefamandole, cefonicid, and cefuroxime, second generation cephalosporins which are active against *H. influenzae*. Because certain cephalosporins have lower activity against β-lactamase producing organisms (Bulger & Washington, 1980), we examined the sensitivity of both β-lactamase-positive and -negative *H. influenzae*. As the sensitivity of *H. influenzae* to certain agents is markedly affected by inoculum size (Simard & Bergeron, 1982), we also undertook an examination of sensitivity using two different inocula.

Materials and methods

Thirty-seven type b *H. influenzae* clinical isolates, recovered from cerebrospinal fluid or blood, were examined. Nineteen strains produced β-lactamase, and 18 strains did not, as determined by screening for nitrocefin hydrolysis (O'Callaghan *et al.*, 1972). Isolates were recovered from stocks maintained in skim milk at −70°C. Brain heart infusion (Difco Laboratories, Detroit, MI) agar or broth, supplemented with 10 mg/l haemia and 10 mg/l β-NADH (sBHI), was used for growth and MIC determinations.

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Ampicillin and chloramphenicol were obtained from Sigma Chemical Co. (St. Louis, MO); cefuroxime from Glaxo Inc. (Research Triangle Park, NC); cefamandole from Eli Lilly and Co. (Indianapolis, IN); cefonicid from SmithKline & French (Philadelphia, PA), and cefpodoxime and trospectomycin from Upjohn Co. (Kalamazoo, MI).

MICs were determined by an agar dilution technique (Syriopoulou *et al.*, 1979). In brief, bacteria were grown to midlog phase in broth at 37°C, diluted to a known concentration in phosphate buffered saline (pH 7·0), and inoculated with a Steers replicator on to sBHI agar containing various concentrations of antibiotics. The susceptibility of each isolate was tested using inocula of 10^5 and 10^7 cfu. The agar plates were examined after 20–24 h of incubation at 37°C in 5% CO_2 and the MIC was defined as the lowest concentration of antibiotic that prevented visible growth of bacteria. Cultures inoculated on to antibiotic-free media were used as controls.

Results

All isolates that were positive for β-lactamase were resistant to ampicillin (MIC_{90} = 20 mg/l), and all the β-lactamase-negative isolates were susceptible to ampicillin (MIC_{90} = 0·5 mg/l). The activities of chloramphenicol, trospectomycin, cefpodoxime, cefamandole, cefonicid, and cefuroxime against 10^5 cfu of both the β-lactamase-negative and -positive isolates are shown in Table I. Predictably, chloramphenicol and trospectomycin were unaffected by β-lactamase production. Among the cephalosporins, cefpodoxime demonstrated the greatest activity (MIC_{90} = 0·06 mg/l) and no difference was found in the sensitivities of the β-lactamase-negative and -positive isolates. Cefamandole, cefonicid, and cefuroxime demonstrated comparable activities against the β-lactamase-negative strains (MIC_{90} = 0·25, 0·125, and 0·25 mg/l respect-

Table I. In-vitro susceptibilities of *H. influenzae* type b

Antibiotic	10^5 cfu		10^7 cfu	
	MIC_{50}[a]	MIC_{90}[b]	MIC_{50}	MIC_{90}
β-Lactamase negative				
Ampicillin	0·25	0·5	0·5	5·0
Chloramphenicol	0·125	0·25	0·25	0·25
Trospectomycin	0·5	5·0	5·0	5.0
Cefpodoxime	0·06	0·06	0.06	0·125
Cefamandole	0·125	0·25	0·25	1·0
Cefonicid	0·125	0·125	0·125	0·25
Cefuroxime	0·125	0·25	0·25	0·25
β-Lactamase positive				
Ampicillin	20	20	>20	>20
Chloramphenicol	0·125	0·25	0·5	0·5
Trospectomycin	0·5	5·0	1·0	5·0
Cefpodoxime	0·06	0·06	0·125	0·125
Cefamandole	0·25	0·25	>5·0	>5·0
Cefonicid	0·125	0·25	>5·0	>5·0
Cefuroxime	0·25	0·5	1·0	>5·0

[a]Minimal inhibitory concentration (mg/l) for 50% of the strains.
[b]Minimal inhibitory concentration (mg/l) for 90% of the strains.

ively); very little difference was found between the susceptibilities of the β-lactamase-negative and -positive isolates with this inoculum.

To examine the effect of increasing inoculum size upon the susceptibility to the cephalosporin antibiotics, we also determined the activity of these agents against 10^7 cfu of both the β-lactamase-negative and -positive isolates (Table I). Cefpodoxime demonstrated little change in activity; the MIC_{90} was one dilution higher (0·125 mg/ml) against 10^7 cfu than against 10^5 cfu of both β-lactamase-negative and positive strains. The susceptibility of the β-lactamase-negative strains to cefonicid and cefuroxime changed little with an increased inoculum, but cefamandole demonstrated a four-fold decrease in activity (MIC_{90} = 1·0 mg/ml) against the higher inoculum. Cefamandole, cefonicid, and cefuroxime, all demonstrated significant decreases in activity (MIC_{90} > 5·0 for all three antibiotics) against the β-lactamase-positive organisms at an inoculum at 10^7 cfu.

Discussion

Trospectomycin is a novel spectinomycin analogue with broad-spectrum antibiotic activity. Zurenko *et al.* (1988) reported a MIC_{90} of 2 mg/l trospectomycin against 10^4 cfu of β-lactamase negative and positive *H. influenzae* in an agar-dilution assay. Our results extend those of Zurenko *et al.* (1988); trospectomycin was active *in vitro* against clinical isolates of type b *H. influenzae* at inocula of 10^5 and 10^7 cfu and we found no difference in susceptibility between β-lactamase-producing and -nonproducing isolates. Although the clinical relevance of these data is difficult to ascertain in the absence of clinical tests of cure, pharmacokinetic studies, demonstrating that high levels of trospectomycin (mean maximum level in plasma of 81·2 mg/l) are attainable in humans receiving doses that are well tolerated (Novak *et al.*, 1987), suggest that this agent will be effective in the treatment of *H. influenzae* infections.

Cefpodoxime, a new oral broad-spectrum cephalosporin antibiotic, has been found to be active *in vitro* against a variety of Gram-positive and Gram-negative bacteria (Knapp, Sierra-Madero & Washington, 1988). The activity against *H. influenzae* observed in our study is somewhat greater than that reported by Chin & Neu (1988) (MIC_{90} = 0·25 mg/ml) and similar to that demonstrated by Jones & Barry (1988) who observed MIC_{90}s of ≤ 0·06 mg/ml and no differences between the susceptibilities of β-lactamase-positive and β-lactamase-negative *H. influenzae*.

Previous investigations have demonstrated the importance of inoculum size in determination of the susceptibility of *H. influenzae* to β-lactam antibiotics (Simard & Bergeron, 1982). Further, the density of *H. influenzae* in certain tissues may exceed 10^7 cfu/ml during the course of human infection by this bacterium (Feldman, 1976). Thus, we investigated the susceptibility of both β-lactamase-producing and -non-producing isolates using inocula of 10^5 and 10^7 cfu. Whereas Knapp *et al.* (1988) reported moderate (two- to eight-fold) increases in the MICs of cefpodoxime against four β-lactamase-producing strains with an increase in inoculum from 10^5 to 10^7 cfu/ml in broth dilution assays, we demonstrated a minimal (two-fold) effect of increased inoculum upon the activity against β-lactamase-positive strains. We found a four-fold increase in the MIC_{90} of cefamandole among β-lactamase-negative isolates and a greater than ten-fold increase in the MIC_{90} among β-lactamase-positive isolates as the inoculum was increased from 10^5 to 10^7 cfu. Our data also demonstrate that although cefonicid and cefuroxime remain active against β-lactamase-negative isolates at an

inoculum of 10^7 cfu, there is a marked decrease in activity against β-lactamase-positive isolates at the higher inoculum. Feldman (1976) reported that the persistence of positive CSF culture following the initiation of antimicrobial therapy was correlated with an initial CSF bacterial density of $\geq 10^7$ cfu/ml. The failure of cefamandole in the treatment of meningitis due to *H. influenzae* type b (Chartrand *et al.*, 1981), and the persistence of positive CSF cultures during treatment with cefuroxime (Marks *et al.*, 1986), may result from an inoculum effect such as that demonstrated *in vitro*.

Whereas previous studies of the antimicrobial activities of trospectomycin and cefpodoxime have examined uncharacterized strains of *H. influenzae*, our investigations focused on type b encapsulated strains obtained from clinical specimens. Our study demonstrates that trospectomycin is active *in vitro* against both β-lactamase-negative and -positive isolates of *H. influenzae* type b. Cefpodoxime also showed excellent in-vitro activity against *H. influenzae*, demonstrating no decrease in activity against β-lactamase-producing isolates. Further, in contrast to several other cephalosporin antibiotics, the activity of cefpodoxime against β-lactamase-producing *H. influenzae* was only minimally affected by inoculum size. The utility of these new agents in the treatment of invasive infections due to type b *H. influenzae* has yet to be determined in clinical trails.

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