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Clarithromycin lacks bactericidal activity in cerebrospinal fluid in experimental pneumococcal meningitis

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Introduction

β-Lactam antibiotics have been the mainstay of therapy for pneumococcal meningitis. However, the number of isolates of *Streptococcus pneumoniae* which are resistant to penicillin is increasing rapidly and some of these are also resistant to broad-spectrum cephalosporins (Figueiredo *et al.*, 1992). There is therefore, a need to identify alternative agents for the treatment of patients with meningitis caused by these strains. Clarithromycin is a novel 14-membered macrolide antibiotic with excellent activity against Gram-positive bacteria, including *S. pneumoniae* against which it is bactericidal *in vitro* (Fernandes *et al.*, 1986; Barry, Thornsberry & Jones, 1987). Although many penicillin-resistant pneumococci are also resistant to erythromycin (Liñares *et al.*, 1992), strains which remain susceptible to the macrolides may respond favourably to drugs such as clarithromycin. We have therefore investigated the potential therapeutic role of clarithromycin in experimental pneumococcal meningitis, a model which represents a stringent test of the in-vivo bactericidal activity of an antibiotic; ceftriaxone was included for comparison.

Materials and methods

Bacterial strain

A strain of *S. pneumoniae* serotyped 3 was used in the study. The organism was stored at −70°C and was thawed and diluted in saline before use. The inoculum size was confirmed by quantitative culture.

Antimicrobial agents

Clarithromycin-lactobionate was provided by Abbott Laboratories (Abbott Park, IL, USA); ceftriaxone was purchased from the manufacturer (Hoffmann-LaRoche Inc., Nutley, NJ, USA).

Susceptibility testing

MICs were determined by the standard broth dilution method (National Committee for Clinical Laboratory Standards, 1985). The medium used was Todd-Hewitt broth

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(THB) (Difco Laboratories, Detroit, MI, USA) and the inoculum was $1–2 \times 10^5$ cfu/mL of a log phase culture. Tubes containing serial two-fold dilutions of the antibiotics (in concentrations ranging from 64–0·0075 mg/L) were incubated overnight at 37°C in room air with 5% CO_2. The MIC was defined as the lowest concentration of antibiotic which inhibited visible growth. The MBC was determined by subculturing 0·1 mL aliquots from all clear tubes on to blood agar plates which were then incubated overnight at 37°C. The MBC was taken as the lowest drug concentration which killed > 99·9% of the original inoculum.

Time-kill curves

Time-kill studies with clarithromycin were performed in 4 mL of THB (pH 7·4). The tubes were inoculated with 0·1 mL of an overnight culture of the pneumococcal strain and incubated at 37°C until suspensions of approximately 10^7 cfu/mL were obtained. Clarithromycin was added to give final concentrations of 0·5, 1, 5 and 10 × MIC. The tubes were incubated further at 37°C and samples were withdrawn at 0, 2, 4, 6 and 24 h and spread on to blood agar plates. The numbers of cfu were determined after overnight incubation. Control cultures without antibiotic were examined in parallel and each experiment was performed in duplicate.

Rabbit model of meningitis

The model originally described by Dacey & Sande (1974) and modified by Guerra-Romero *et al.* (1991) was used. New Zealand white rabbits were infected by instilling $1–3 \times 10^5$ cfu of the pneumococcal strain in 0·3 mL of saline through a 25 gauge butterfly needle into the cisterna magna. Eighteen hours after infection, baseline blood and CSF samples were obtained and antibiotic therapy was commenced. Each agent was initially administered as an iv bolus (10, 20 or 40 mg/kg of clarithromycin and 1·0 mg/kg of ceftriaxone), followed by a continuous infusion over 5 h of five times the dosage of the initial bolus; the total dosages, therefore, were 60, 120 and 240 mg/kg of clarithromycin and 6 mg/kg of ceftriaxone. Seven rabbits (controls) received saline, six received ceftriaxone and five, five, and seven received the three dosages of clarithromycin, respectively. The mean CSF bacterial titre before antibiotic therapy was initiated was $5·7 \pm 0·75$ $\log_{10}$ cfu/mL (range 4·5–7·0 $\log_{10}$ cfu/mL); the mean titres for the various treatment groups did not differ significantly.

Sample collection and processing

Blood samples were collected at 1 and 5 h and CSF samples at 1, 3 and 5 h after initiation of therapy. Broad-spectrum β-lactamases (β-lactamases I and II, Oxoid Ltd, Basingstoke, UK) were added to the CSF samples of animals treated with ceftriaxone, with subsequent correction to take account of the dilutional effect. Aliquots of CSF were spread on to Todd-Hewitt blood agar plates and quantitative colony counts were determined after overnight incubation; killing rates were calculated and expressed in terms of a reduction in bacterial titres ($\log_{10}$)/mL/h. The remaining CSF was used within 1 week for the bioassays.

Bioassays

Antibiotic concentrations were determined by an agar diffusion method with *Micrococcus luteus* ATCC 9431 and *Escherichia coli* ATCC 10536 as the indicator

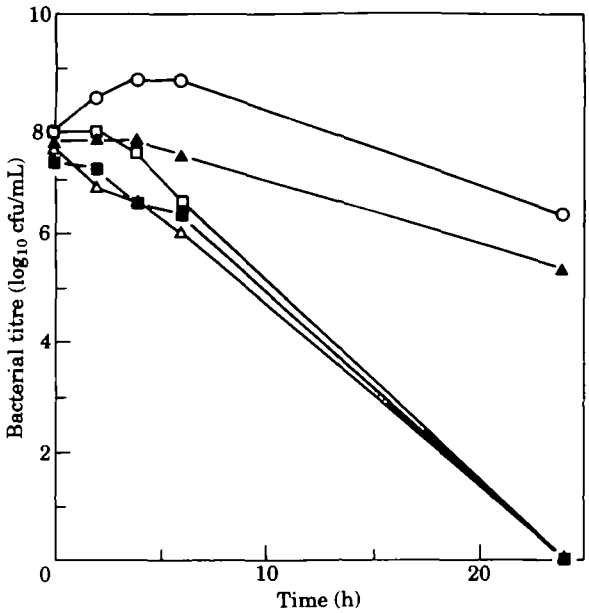

Figure. Time-kill curves for *S. pneumoniae* in the absence of antibiotic (control) (○) and in the presence of clarithromycin at 0·5 × MIC (▲), 1 × MIC (□), 5 × MIC (△) and 10 × MIC (■).

organisms for clarithromycin and ceftriaxone, respectively. The standards used in the determination of the serum concentrations were prepared in serum and those used in the determination of the CSF concentrations were prepared in saline. Inocula of 5×10^5 cfu/mL in antibiotic medium No. 11 for *M. luteus* and tryptic soy agar for *E. coli* (both media obtained from Difco Laboratories, Detroit, MI, USA) were used for the assays. The plates were incubated overnight at 37°C in room air with 5% CO_2. The lower limits of detection was 0·25 mg/L for ceftriaxone and 0·5 mg/L for clarithromycin. The percentage of antibiotic penetration into the CSF was calculated as follows: (CSF concentration ÷ serum concentration) × 100.

Statistical methods

The results are presented as means ± standard deviations (S.D.). Differences between the various treatment groups were analyzed by one-way analysis of variance.

Results

The MIC and MBC of clarithromycin for the pneumococcus were both 0·06 mg/L and of ceftriaxone, 0·02 and 0·06 mg/L, respectively. The time-kill studies confirmed the in-vitro bactericidal activity of clarithromycin; in the presence of concentrations as low as 1 × MIC, the cultures were sterile after 24 h of incubation (Figure). However, this bactericidal activity was relatively slow and even the highest concentration studied (10 × MIC) produced a decrease in the bacterial titres of only about 2 $\log_{10}$ cfu/mL after 6 h.

The serum and CSF concentrations at the end of the 5 h infusions are shown in Table I. Clarithromycin demonstrated excellent CSF penetration which was dosage-dependent. However, bactericidal activity *in vivo* was not detected in this model with

Table I. Clarithromycin and ceftriaxone concentrations in serum and CSF obtained from rabbits with pneumococcal meningitis at the end of a 5 h infusion

Antibiotic (dosage)	Antibiotic concentration (mg/L; mean ± S.D.) serum	CSF	Percent CSF penetration (mean ± S.D.)	Ratio of CSF concentration : MBC
Clarithromycin (60 mg/kg)	6·5 ± 2·4	1·6 ± 0·7	25 ± 5[a]	26
Clarithromycin (120 mg/kg)	16·4 ± 3·2	5·5 ± 1·6	34 ± 10	88
Clarithromycin (240 mg/kg)	40·8 ± 11·4	18·0 ± 5·9	45 ± 11[a]	288
Ceftriaxone (6 mg/kg)	9·9 ± 2·2	0·4 ± 0·3	5 ± 4	7

[a] $P < 0·05$.

the three dosages studied, despite CSF concentrations which substantially exceeded the MBC (Table II); the pneumococcus was isolated from the CSF of all of the rabbits after 5 h treatment with clarithromycin. On the other hand, ceftriaxone reduced the CSF bacterial titres by approximately 1 $\log_{10}$ cfu/mL/h and sterilized the CSF of five of the six animals during the same treatment period.

Discussion

The most noteworthy observation in this study was that clarithromycin demonstrated a complete lack of bactericidal activity in the CSF of rabbits with pneumococcal meningitis. This finding was unexpected since clarithromycin penetrated well into the CSF, achieved concentrations at this site which exceeded the MBC of the test strain and was shown to be bactericidal *in vitro* in time-kill studies. Clarithromycin might have exerted a more significant effect on CSF bacterial titres had treatment been continued for a longer period. This impression is supported by the results of the time-kill studies which showed that clarithromycin produced a slow bactericidal effect. In the short term, however, clarithromycin was clearly inferior to ceftriaxone, the results for which were in close agreement with those obtained in previous studies with the same model (Täuber *et al.*, 1984).

Table II. Effects of clarithromycin and ceftriaxone on CSF bacterial titres in experimental pneumococcal meningitis

Antibiotic	Dosage (mg/kg)	Number of animals	CSF killing rate ($\Delta\log_{10}$ cfu/mL/h; mean ± S.D.)
None (control)	—	7	+0·24 ± 0·35
Clarithromycin	60	5	−0·07 ± 0·07[a]
Clarithromycin	120	5	−0·02 ± 0·19[a]
Clarithromycin	240	7	+0·04 ± 0·19[a]
Ceftriaxone	6	6	−1·06 ± 0·32[b]

[a] No significant difference compared with control.
[b] $P < 0·01$ compared with all other groups.

We have made no attempt to identify precisely why clarithromycin was unable to produce in-vivo bactericidal activity in the CSF. Obviously, failure of the drug to reach the site of infection can be excluded. An inoculum effect is an equally unlikely explanation since clarithromycin was noted to be bactericidal in the time-kill experiments which involved the use of higher bacterial titres than those instilled into the CSF. Previous in-vitro studies have shown that the MBC of clarithromycin is substantially increased when the pH of the medium is acidified (Fernandes *et al.*, 1986). In meningitis, the CSF becomes acidic (Guerra-Romero *et al.*, 1992) and this may, at least in part, have contributed to the failure of clarithromycin to kill the pneumococcus *in vivo*.

We have previously described a similar discrepancy between the in-vitro and in-vivo activities of another new macrolide, azithromycin, in a model of *Staphylococcus aureus* osteomyelitis (O'Reilly *et al.*, 1992). Irrespective of the precise mechanisms involved, our results demonstrate that standard in-vitro investigations can fail to predict the efficacy of antibiotics *in vivo*. Animal models such as the one used in the present study will continued to play an important role in determining the therapeutic potential of new antibiotics.

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