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Rate of Bactericidal Activity for *Streptococcus faecalis* of a New Quinolone, CI-934, Compared with That of Amoxicillin

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The rate of bactericidal activity of a new quinolone, CI-934, was compared with that of amoxicillin for 20 strains of *Streptococcus faecalis*. At 10 and 100 μg/ml, the bactericidal activity of CI-934 was more rapid at 6 h than that of amoxicillin. A paradoxical effect (a killing rate higher at 1 μg/ml than at 100 μg/ml at 6 h) was observed for 19 of the 20 strains with amoxicillin and for 1 of the 20 strains with CI-934. It remains to be demonstrated whether in vivo studies will confirm the results obtained in vitro.

CI-934 is a new quinolone carboxylic acid exhibiting particular strength against gram-positive bacteria. Even notably resistant organisms such as *Enterococcus* spp. and methicillin-resistant *Staphylococcus aureus* are susceptible to CI-934 (1).

In this investigation, we evaluated with killing curves the rate of bactericidal activity of CI-934 for *Streptococcus faecalis* compared with that of amoxicillin.

MATERIALS AND METHODS

Bacterial strains. Twenty strains of *S. faecalis* isolated from blood cultures were identified by the methods recommended by Facklam and Carey (3). These group D streptococci were hippurate positive, L-pyrrolidonyl aminopeptidase positive, and bile-esculin positive and grew in 6.5% NaCl. The identification of these strains was confirmed by the API 20 Strep system (Analytab Products, Plainview, N.Y.).

Antibiotic. Amoxicillin was obtained from Beecham Research Laboratories, Brockham Park, Betchworth Surrey, United Kingdom, and CI-934 (lot W) was from Warner Lambert, Morris Plains, N.J. Manipulations were performed in glass tubes protected from light by metal foil to avoid the possible degradation of CI-934 by light.

Antimicrobial susceptibility testing. MICs were determined by the macrodilution broth method prescribed by Jones et al. (4). The tubes were incubated at 35°C for 24 h. The mean MICs and ranges (in micrograms) for amoxicillin and CI-934 were 0.4 (0.2 to 0.8) and 0.24 (0.01 to 0.4), respectively.

Killing curves. (i) Preparation of inocula. Portions of five colonies growing on Iso-Sensitest agar were suspended in 10 ml of Iso-Sensitest broth. The tube was placed on a test tube rotator (Cenco; Breda Scientific B.V., Tilburg, The Netherlands), stopped at turbidity 0.5 McFarland standard (HF Instrument turbidometer; Shaban Manufacturing Inc., Fredonia, N.Y.), and then diluted 1/100 in Iso-Sensitest broth medium containing different antibiotic concentrations. The initial bacterial density was approximately 10^6 CFU/ml.

(ii) Incubation. All inoculated tubes (final volume, 2 ml) were incubated in plastic racks in a water bath at 35°C.

(iii) Sampling. Immediately after inoculation and vortexing and then after 3, 6, and 24 h, a petri dish (diameter, 16 cm) containing 30 ml of Iso-Sensitest agar medium was inoculated (before and after dilution, 1/10 and 1/100, respectively) with 0.05 ml of the culture which was distributed on the agar surface by the spiral inoculator system (Spiral System Instruments, Inc., Bethesda, Md.) (6, 10). In this system, a variable cam-activated syringe dispenses the culture from the center to the edge of the plate in a logarithmically decreasing quantity in the form of an Archimedes spiral. The Spiral 500 laser colony counter and the Casba 800 microprocessor (Spiral System Instruments, Inc.) were used for the CFU count. Antibiotic carry-over could be detected by the absence of colonies in the center of the petri dishes.

RESULTS

The killing curves (mean CFU ± the standard deviation) for 20 strains of *S. faecalis* exposed to 1, 10, and 100 μg of CI-934 or amoxicillin per ml are shown in Fig. 1.

With CI-934, the bactericidal activity was more rapid as the concentration was increased, except for 1 of the 20 strains. For this strain, the killing was more rapid with 1 μg/ml than with 10 and 100 μg/ml.

With amoxicillin, a paradoxical effect (manifested by 1 $\log_{10}$ CFU/ml or more) was observed for 19 of the 20 strains. At 6 h, there was a significant difference (Mann-Whitney test) between the CFU per milliliter of *S. faecalis* tested with 1 and 10 μg of amoxicillin per ml ($P < 0.00003$) or with 1 and 100 μg/ml ($P < 0.00003$).

Overall comparison of these two antimicrobial agents shows a more rapid killing rate at 6 h by CI-934 than by amoxicillin at 10 μg/ml ($P < 0.00003$) and 100 μg/ml ($P < 0.00003$).

DISCUSSION

CI-934 is a new azaquinolone possessing an increased antimicrobial activity against gram-positive bacteria (1). Its activity against various species of streptococci, and *S. faecalis* in particular, appears better than that of other quinolones. Cohen et al. reported that all *S. faecalis* strains were inhibited by ≤0.8 μg of CI-934 per ml (1). We showed in the present study that the bactericidal action of CI-934 against *S. faecalis* is more rapid than that of amoxicillin at 10 and 100 μg/ml.

Amoxicillin (alone or in combination with an aminoglycoside) is considered the reference antibiotic for treatment of

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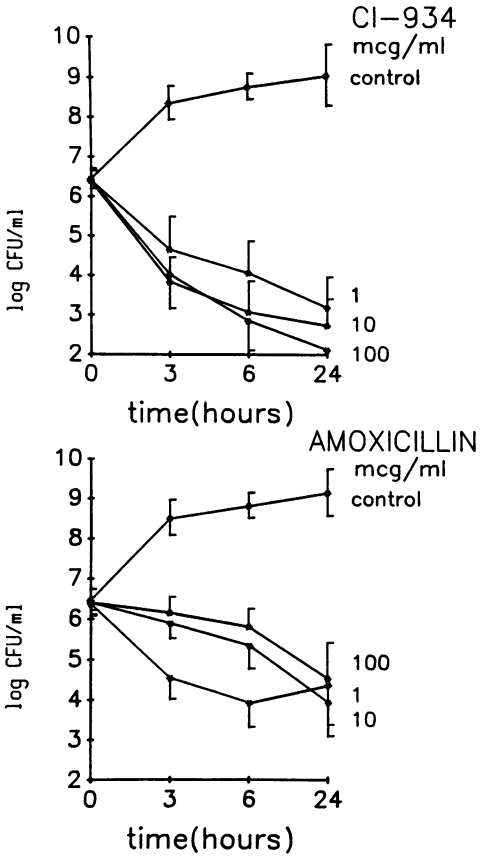

FIG. 1. Killing curves (mean CFU ± the standard deviation) for 20 strains of *S. faecalis* exposed to CI-934 and amoxicillin.

S. faecalis infections. A paradoxical effect or Eagle effect (2) of beta-lactams on *S. faecalis* has been described (7, 8), in which the bactericidal activity of beta-lactams decreases with increasing beta-lactam concentrations. We were surprised by the extent of the paradoxical effect of amoxicillin on *S. faecalis*.

Many technical factors influence the determination of bactericidal activity (9). In this study, we used log-phase inocula and excluded the presence of carry-over, which would have been well detected in the spiral inoculator system by the absence of growth in the center of the petri dish. The spiral plater distributes 0.05 ml from the center to the edge of the dish in a logarithmically decreasing quantity. With a 16-cm petri dish, there is more than a 100:1 change in the volume plated per millimeter of radial advance from the start (center) to the end (edge) of the plating cycle (6, 10). With amoxicillin, carry-over was excluded, because the killing curves for *S. faecalis* exposed to amoxicillin did not change when a penicillinase was added to the agar.

The effect of penicillin on *S. faecalis* is a matter for discussion. Tolerant strains, for example, may not be clearly distinguishable from nontolerant strains on the basis of MBC-to-MIC ratios in Mueller-Hinton broth. A penicillin gradient and replicate plate was proposed for the demonstration of tolerance in streptococci groups B and D (5). However, the paradoxical effect is a different phenomenon. In a previous study using a triple-layer method and ampicillin neutralization by a beta-lactamase, we found a paradoxical effect for 10 of 10 *S. faecalis* strains (11).

Pharmacological studies will define the concentrations of CI-934 attained in the urine, blood, and tissues and should predict whether the effects observed in vitro can be expected (at least theoretically) in vivo.

It can be concluded that the in vitro bactericidal activity of CI-934 for *S. faecalis* appears to be more rapid at 6 h than that of amoxicillin, an antibiotic for which a major paradoxical effect has been observed.

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LITERATURE CITED

1. **Cohen, M. A., T. J. Griffin, P. A. Bien, C. L. Heifetz, and J. M. Domagala.** 1985. In vitro activity of CI-934, a quinolone carboxylic acid active against gram-positive and -negative bacteria. Antimicrob. Agents Chemother. **28**:766–772.
2. **Eagle, H., and A. D. Musselman.** 1984. The rate of bactericidal action of penicillin in-vitro as a function of its concentration, and its paradoxically reduced activity at high concentrations against certain organisms. J. Exp. Med. **88**:99–131.
3. **Facklam, R. R., and R. B. Carey.** 1985. Streptococci and aerococci, p. 154–175. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
4. **Jones, R. N., A. L. Barry, T. L. Gavan, and J. A. Washington II.** 1985. Susceptibility tests: microdilution and macrodilution broth procedures, p. 972–977. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
5. **Kim, K. S., and B. F. Anthony.** 1983. Use of penicillin-gradient and replicate plates for the demonstration of tolerance to penicillin in streptococci. J. Infect. Dis. **148**:488–491.
6. **Kramer, J. M., M. Kendall, and R. J. Gilbert.** 1979. Evaluation of the spiral plate and laser counting techniques for the enumeration of bacteria in foods. Appl. Microbiol. Biotechnol. **6**:289–299.
7. **Shah, P.** 1982. Paradoxical effect of antibiotics. I. The "Eagle effect." J. Antimicrob. Chemother. **10**:259–260.
8. **Stille, W., and H. Uffelmann.** 1973. Untersuchungen über den paradoxen Effekt von Penicillin auf Enterokokken (Eagle-Effekt). Dtsch. Med. Wochenschr. **98**:611–613.
9. **Taylor, P. C., F. D. Schoenknecht, J. C. Sherris, and E. C. Linner.** 1983. Determination of minimum bactericidal concentrations of oxacillin for *Staphylococcus aureus*: influence and significance of technical factors. Antimicrob. Agents Chemother. **23**:142–150.
10. **Walsh, T. J., W. E. Venanzi, and D. M. Dixon.** 1985. Quantification of medically important *Candida species* and *Torulopsis glabrata* by a spiral inoculation system: correlation with pour plate and spread plate methods. J. Clin. Microbiol. **22**:745–747.
11. **Yourassowsky, E., M. P. Van der Linden, M. J. Lismont, and E. Schoutens.** 1978. Qualitative study of the paradoxical zone phenomenon of penicillins against 17 bacterial species of clinical importance. Chemotherapy (Basel) **24**:92–96.