Susceptibility of Intra- and Extracellular *Mycobacterium avium-intracellulare* to Cephem Antibiotics

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Intra- and extracellular susceptibility of 35 clinically isolated *Mycobacterium avium-intracellulare* strains to cefotaxime (CTX), ceftizoxime (CZX), and cefoperazone was studied. MICs for 50% of the isolates in vitro were 6.25 µg/ml for CTX and CZX and 25 µg/ml for cefoperazone. A strain susceptible to CTX (MIC, 0.78 µg/ml) and CZX (MIC, 1.56 µg/ml) infected human peripheral blood mononuclear cells in the presence of 20% autologous plasma. The mycobacteria replicated exclusively in monocytes under the above culture condition. Concentrations of CZX 1- to 16-fold higher than its in vitro MIC had little effect on intracellular replication of the strain. A concentration of CTX 16-fold higher than its in vitro MIC was bacteriostatic to the mycobacteria, but CTX of lower concentrations showed no effect on intracellular replication. Thus, ineffectiveness of the cephems on the therapy of *M. avium-intracellulare* infection was suggested.

Since *Mycobacterium avium-intracellulare* is resistant to most antitubercle agents, the current therapy for this infection is disappointing (2–4, 9–11). We look for agents effective against this organism among nonantitubercle drugs. Third-generation cephem antibiotics were studied in this report. The susceptibility of 35 clinically isolated *M. avium-intracellulare* strains to cefoperazone (CPZ), cefotaxime (CTX), and ceftizoxime (CZX) was tested in FST medium (8) (Fig. 1). MICs of CPZ, CTX, and CZX for 90% of the isolates were 25, 6.25, and 6.25 µg/ml, respectively. It was reported that five strains of *M. avium-intracellulare* isolated from acquired immune deficiency syndrome patients were all resistant to 10 µg of CPZ per ml (3), coinciding with our result. Latamoxef, another third-generation cephem, was found to be the least effective (MIC, 200 µg/ml) compared with the three cephems and was omitted from Fig. 1. Thus, CZX and CTX were evaluated as possible useful drugs for *M. avium-intracellulare* infection after in vitro testing.

Mycobacteria are primarily intracellular parasites, and it is essential to test the susceptibility of intracellularly replicating *M. avium-intracellulare* to these cephems. Recently, we developed a simple culture method for determining intracellular replication of mycobacteria in human monocytic cells. *M. avium-intracellulare* infected monocytic cells in the presence of human plasma which inhibited extracellular replication of the mycobacteria, and monocytic cells were the permissive site for the mycobacterial replication (7). Strain J11 was the most susceptible among 35 isolates to CZX in vitro (MIC, 1.56 µg/ml). Mononuclear cells were isolated after the centrifugation of heparinized blood on Mono-Poly resolving medium (Flow Laboratories, McLean, Va.) and seeded at 2×10^5 cells in each well of a flat-bottomed 96-well tray with 0.15 ml of MCDB 103 medium (5) supplemented with 20% autologous plasma (7). Then, 10 µl of J11 cell suspension (1×10^3 to 10×10^3 CFU) infected the mononuclear cells, and the tray was incubated at 37°C in the presence of 5% CO_2. On day 1, the mycobacteria were

treated with various concentrations of CZX. On day 5, half of the medium was removed and replaced with fresh medium containing each concentration of CZX. On day 8, mononuclear cells in each well were lysed with 10 µl of each of 1 N NaOH and 5% Tween 80, vigorously pipetted, neutralized
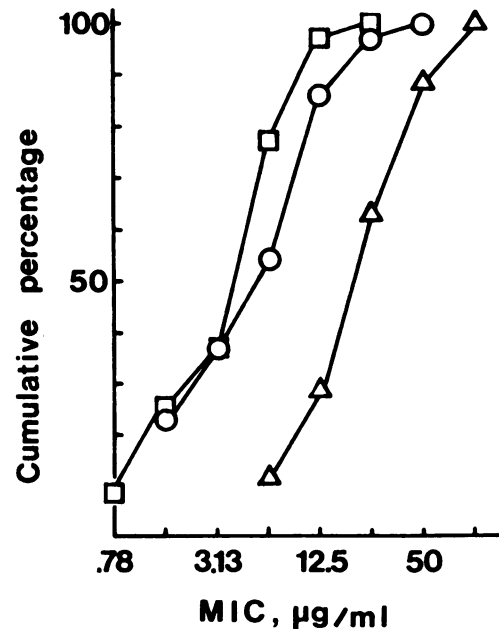

FIG. 1. In vitro susceptibilities of *M. avium-intracellulare* to cephem antibiotics CPZ (△), CTX (□), and CZX (○). A total of 35 freshly cultured strains of the mycobacteria in FST medium (F12 medium supplemented with 5% heat-inactivated fetal bovine serum and 0.05% Tween 80) were inoculated at 10^4 to 10^5 CFU in each well of a 96-well tray with 0.1 ml of FST medium containing twofold serially diluted cephems. Mycobacterial growth was estimated by the naked eye after a 5-day incubation at 37°C in the presence 5% CO_2 (8).

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with 10 μl of 1 N HCl, and appropriately 10-fold diluted with Tween-free FST medium. Each cell suspension (0.1 ml) was plated in a 96-well tray. Resulting colonies of mycobacteria were counted under a microscope after a 5-day incubation (7). In the absence of CZX, $5.2 \pm 0.5 \times 10^3$ CFU of J11 cells on day 1 increased to $2.5 \pm 0.1 \times 10^4$ CFU on day 8 (Fig. 2). With 1.56 to 6.25 μg of CZX per ml (1 to 4× MIC), J11 cells replicated to almost the same extent as without the antibiotic. With 12.5 to 25 μg of CZX per ml (8 to 16× MIC), J11 cells also proliferated, although the growth rate slightly decreased. Thus, even a 16-times-higher concentration of CZX than its in vitro MIC failed to inhibit the replication of M. avium-intracellulare in human monocytes. This experiment also indicated that infecting mycobacteria had replicated intracellularly but not extracellularly under the culture condition, confirming our previous result (7). Similarly, strain T11, which was most susceptible to CTX (MIC, 0.78 μg/ml), infected mononuclear cells were treated with indicated concentrations of CTX on day 1 and cultivated as above. With 0.78 to 6.25 μg of CTX per ml (1 to 8× MIC), intracellular replication of T11 was not greatly inhibited (Fig. 3). With 12.5 μg of CTX per ml, however, viable cells of T11 on day 8 decreased below that on day 1, indicating that a concentration of CTX 16 times higher than its in vitro MIC was bacteriostatic to intracellular mycobacteria. Since the in vitro MIC of CZX and CTX for 90% of 35 strains of clinically isolated M. avium-intracellulare was 12.5 μg/ml (Fig. 1), we suspect that these cephems are therapeutically effective on the mycobacterial infection.

Of four cephems tested for M. avium-intracellulare infection, two (latamoxef and CPZ) were excluded after the in vitro susceptibility testing. The survived cephems were further tested for their effect on two mycobacterial strains resided in human monocytes. However, even 10-times-higher concentrations of the cephems than their in vitro MICs exhibited no bacteriostatic effect on intracellular mycobacteria. Such a characteristic of the cephems was
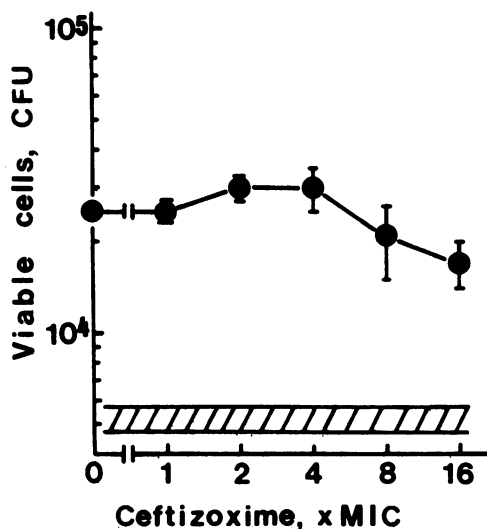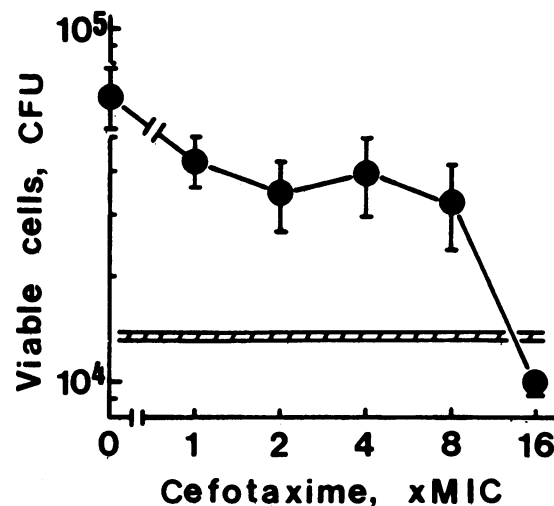

FIG. 3. Effect of CTX on intracellular replication of M. avium-intracellulare. Mononuclear cells were infected with strain T11 on day zero. On day 1 when the number of intracellular mycobacterial cells was $1.35 \pm 0.03 \times 10^4$ CFU (shaded zone), mononuclear cells were treated with 0.78 to 12.5 μg of CTX per ml (1 to 16× MIC) and further incubated for 7 days. Each symbol represents the average of three determinations. Bar represents standard deviation.

quite different from that of aminoglycoside antibiotics; their intracellular MICs are comparable to their extracellular MICs (7). A possible reason that cephems had no effect on intracellular parasites is that they are poorly permeated into mononuclear phagocytes. CZX and CTX reversibly binds to human plasma (as much as 30 and 70%, respectively) (1, 6). It is not known whether plasma affects the permeability of the cephems. Another possibility is that human monocytic cells have enzymes with some cephalosporinase activity. It is essential to elucidate those possibilities for the chemotherapeutical use of cephems in mycobacterial infection.

LITERATURE CITED

1. Aratani, H., H. Tateishi, S. Negita, and Y. Yamanaka. 1980. Studies on phamacokinetics of cefotaxime. Chemotherapy 28(Suppl. 1):65–72.
2. Davidson, P. T. 1981. Treatment of Mycobacterium intracellulare complex disease. Semin. Respir. Dis. 2:233–239.
3. Greene, J. B., G. S. Sidhu, S. Lewin, J. F. Levine, H. Masur, M. S. Simberkoff, P. Nicholas, R. C. Good, S. B. Zolla-Pazner, A. A. Pollock, M. L. Tapper, and R. S. Holzman. 1982. Mycobacterium avium-intracellulare: a cause of disseminated life-threatening infection in homosexuals and drug abusers. Ann. Intern. Med. 97:539–546.
4. Heifets, L. B. 1982. Synergistic effect of rifampin, streptomycin, ethinamide and ethambutol on Mycobacterium intracellulare. Am. Rev. Respir. Dis. 125:43–48.
5. McKeehan, W. L., W. G. Hamilton, and R. G. Ham. 1976. Selenium is an essential trace nutrient for growth of WI-38 diploid human fibroblasts. Proc. Natl. Acad. Sci. U.S.A. 73:2023–2027.
6. Murakawa, T., H. Sakamoto, S. Fukada, S. Nakamoto, T. Hirose, N. Ito, and M. Nishida. 1980. Pharmacokinetics of ceftizoxime in animals after parenteral dosing. Chemotherapy 28(Suppl. 5):111–118.
7. Nozawa, R. T., H. Kato, and T. Yokota. 1984. Intra- and extracellular susceptibility of Mycobacterium avium-intracellulare complex to aminoglycoside antibiotics. Antimicrob. Agents Chemother. 26:841–844.

FIG. 2. Effect of CZX on intracellular replication of M. avium-intracellulare. Mononuclear cells were infected with strain J11 on day zero. On day 1 when the number of intracellular mycobacterial cells was $5.2 \pm 0.5 \times 10^3$ CFU (shaded zone), mononuclear cells were treated with 1.56 to 25 μg of (1 to 16× MIC) CZX per ml and further incubated for 7 days. Each symbol represents the average of three determinations. Bar represents standard deviation.

8. **Nozawa, R. T., and T. Yokota.** 1983. Rapid drug susceptibility testing of mycobacteria in tissue culture medium. Antimicrob. Agents Chemother. **24:**268–272.
9. **Wolinsky, E.** 1979. Nontuberculous mycobacteria and associated diseases. Am. Rev. Respir. Dis. **119:**107–159.
10. **Yeager, H., Jr., and J. W. Raleigh.** 1973. Pulmonary disease due to *Mycobacterium intracellulare*. Am. Rev. Respir. Dis. **108:**547–552.
11. **Zimmer, B. L., D. R. DeYoung, and G. D. Roberts.** 1982. In vitro synergistic activity of ethambutol, isoniazid, kanamycin, rifampin, and streptomycin against *Mycobacterium avium-intracellulare* complex. Antimicrob. Agents Chemother. **22:**148–150.