

Comparative Antibacterial Activities of 7 α -Methoxy Cephalosporins and 7 β -Methoxyiminoacetamido Cephalosporins Against *Bacteroides fragilis*

TADATAKA KESADO,* KUNITOMO WATANABE, YOSHINARI ASAHI, MIDORI ISONO, AND KAZUE UENO
Institute of Anaerobic Bacteriology, Gifu University School of Medicine, Gifu, Japan

Received 14 March 1983/Accepted 17 October 1983

The in vitro antibacterial activities of the newly developed 7 α -methoxy cephalosporins and 7 β -methoxyiminoacetamido cephalosporins against 67 clinical isolates of *Bacteroides fragilis* and their resistance to the hydrolytic action of a β -lactamase produced by *B. fragilis* were simultaneously compared. The minimal inhibitory concentrations that inhibited 90% of the 7 α -methoxy cephalosporins, cefoxitin, cefmetazole, moxalactam, and cefotetan, against the isolates were 4, 8, 8, and 16 μ g/ml, respectively, and these antibiotics were entirely resistant to hydrolysis by β -lactamases (0.10 μ mol/h per mg of protein) of the isolates. By contrast, 7 β -methoxyiminoacetamido cephalosporins represented by cefotaxime, ceftizoxime, and cefmenoxime were not effective, as indicated by the minimal inhibitory concentrations that inhibited 90%, 64, 32, and 128 μ g/ml, respectively. Their antibacterial activities clearly corresponded to their resistance to the hydrolytic action of the β -lactamase: namely, the correlation coefficients in regression curves of cefotaxime, ceftizoxime, and cefmenoxime, which were expressed by the antibacterial activity (x axis) and the β -lactamase activity (y axis) were 0.098, 0.034, and 0.163, respectively.

In recent years, anaerobic bacteria, especially *Bacteroides fragilis*, have been isolated from clinical specimens with increasing frequency (6, 8). Several investigators have reported that *B. fragilis* strains produce a β -lactamase which has cephalosporinase activity and that it plays a significant role in the resistance of these organisms to β -lactam compounds (2, 11, 13, 17, 22). On the other hand, several new β -lactam compounds have been developed in recent years for the treatment of infections caused by resistant organisms which produce a β -lactamase (1, 7, 9, 20). Prominent among such compounds are cefoxitin, cefmetazole, moxalactam, and cefotetan, which are grouped as 7 α -methoxy cephalosporins, and cefotaxime, ceftizoxime, and cefmenoxime, which are included in the 7 β -methoxyiminoacetamido cephalosporin group. Both groups are currently in clinical use. The present study was undertaken to compare the in vitro antibacterial activities of 7 α -methoxy cephalosporins and 7 β -methoxyiminoacetamido cephalosporins against 67 clinical isolates of *B. fragilis* and to investigate whether there exists a correlation between their antibacterial activity and the resistance to the hydrolytic action of β -lactamases produced by *B. fragilis*.

MATERIALS AND METHODS

Organisms. All of the 67 *B. fragilis* isolates tested were obtained from clinical specimens and were identified at the Institute of Anaerobic Bacteriology, Gifu University School of Medicine, Japan, according to the criteria of the Virginia Polytechnic Institute Anaerobe Laboratory Manual (21). Organisms were maintained at room temperature in tubes containing GAM semisolid medium (1% peptone, 0.3% soya peptone, 1% proteose peptone, 1.35% serum digest powder, 0.5% yeast extract, 0.22% beef extract powder, 0.12% liver extract powder, 0.3% glucose, 0.25% potassium dehydrogen phosphate, 0.3% sodium chloride, 0.5% soluble starch,

0.03% L-cystein hydrochloride, 0.03% sodium thioglycolate, 0.15% agar) (Nissui Seiyaku Co.) and were subcultured periodically onto fresh medium until the time of antibacterial activity determination.

Antibiotics. Standard antibiotic powders were kindly provided by the following manufacturers: cefoxitin by Merck & Co., Inc., Rahway, N.J.; cefmetazole by Sankyo Co., Tokyo; moxalactam by Shionogi & Co., Osaka; cefotetan by Yamanouchi Pharmaceutical Co., Tokyo; cefotaxime by Hoechst-Roussel Pharmaceutical Inc., Sommerville, N.J.; ceftizoxime by Fujisawa Pharmaceutical Co., Osaka; and cefmenoxime by Takeda Chemical Industries, Ltd., Osaka.

Determination of MICs. Antibacterial activity (expressed as minimum inhibitory concentration [MIC]) of all antibiotics tested was determined by the agar dilution technique as described previously (10). Antibiotic solutions were freshly prepared for each test, using sterilized distilled water as the diluent for cefoxitin, cefmetazole, moxalactam, and cefotetan and 10% sodium bicarbonate solution sterilized by filtering (MILLEX-GS; Millipore Corp., Bedford, Mass.) for cefotaxime, ceftizoxime, and cefmenoxime.

Assay for β -lactamase activities. The organism was subcultured overnight at 37°C in 30 ml of GAM broth (Nissui Seiyaku Co.) under an 80% N₂-10% H₂-10% CO₂ atmosphere in an anaerobic chamber (Forma Scientific) transferred to 300 ml of fresh GAM broth, and incubated for 4 h at 30°C under the same anaerobic conditions. The cells were harvested by centrifugation, washed once in 0.1 M phosphate buffer (pH 7.0), and suspended in 25 ml of the same buffer. Bacterial cells were sonicated in an ultrasonic cell disruptor (Branson Sonifier, model W-185; Branson Instruments, Inc., Stamford, Conn.) at full amplitude for three periods of 1 min each at 0°C. The remaining cell debris was removed by centrifugation at 15,000 \times g in a model RD-20II TOMY SEIKO refrigerated centrifuge for 1 h at 2°C. The clear supernatant fluid was used as the source of β -lactamase. β -Lactamase activity was determined by the iodometric method of Sawai et al. (18).

* Corresponding author.

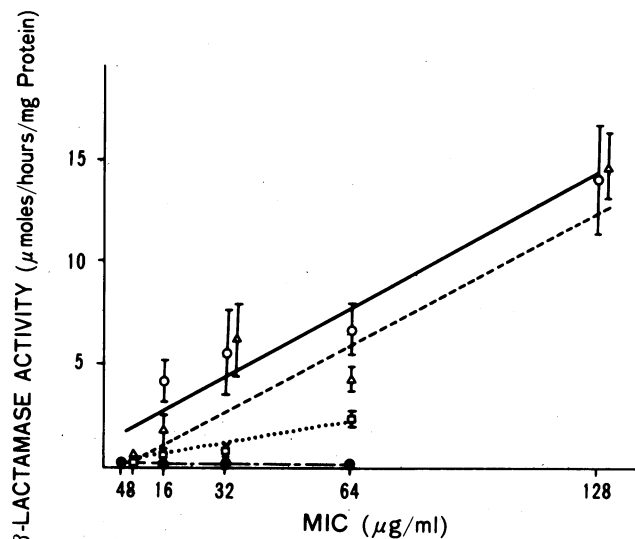


FIG. 1. Correlation between MICs and β -lactamase susceptibility of seven cephalosporins obtained with 67 *B. fragilis* strains and their enzymes. The MICs and β -lactamase activities were determined as described in the text. Symbols: ceftioxin, cefmetazole, moxalactam, and cefotetan, \bullet ; cefmenoxime, \circ ; cefotaxime, Δ ; ceftizoxime, \square .

RESULTS

The comparative *in vitro* activities of ceftioxin, cefmetazole, moxalactam, and cefotetan, which are grouped as 7 α -methoxy cephalosporins, against 67 isolates of *B. fragilis* are shown in Table 1, along with those for cefotaxime, ceftizoxime, and cefmenoxime, which belong to the 7 β -methoxyiminoacetamido cephalosporins. All of the isolates were susceptible to 16 $\mu\text{g/ml}$ or less of the 7 α -methoxy cephalosporins tested, except for cefmetazole. Only 4.5% of the *B. fragilis* isolates tested were not susceptible to cefmetazole at this concentration. Each of the 7 α -methoxy compounds gave a single mode in the sensitivity distribution pattern with MICs from 0.5 to 4 $\mu\text{g/ml}$. On the other hand, only 46.3, 56.8, and 48.7% of the *B. fragilis* isolates were susceptible to cefotaxime, ceftizoxime, and cefmenoxime at concentrations of 16 $\mu\text{g/ml}$, respectively. All members of this group tested showed a bimodal distribution pattern with MICs from 2 to 4 $\mu\text{g/ml}$ and from 32 to 64 $\mu\text{g/ml}$.

The correlation between the antibacterial activities of 7 α -methoxy and 7 β -methoxyiminoacetamido cephalosporins against 67 isolates of *B. fragilis* and their stabilities against hydrolysis by the *B. fragilis* β -lactamase is illustrated in Fig. 1. Our study shows that ceftioxin, cefmetazole, moxalactam, and cefotetan are stable for hydrolysis by β -lactamases produced by the *B. fragilis*. There were no significant differences between antibacterial activities against β -lactamase-producing and -nonproducing strains among the 7 α -methoxy cephalosporins tested. However, the antibacterial activities of cefotaxime, ceftizoxime, and cefmenoxime correlated with their stabilities to the hydrolytic action of the *B. fragilis* β -lactamases.

The regression curves between the antibacterial activities (x axis) and the β -lactamase activities (y axis) of cefotaxime, ceftizoxime, and cefmenoxime are expressed as $y = 0.098x - 0.1$ (correlation coefficient = 0.804), $y = 0.03x + 0.02$ (correlation coefficient = 0.856), and $y = 0.103x + 0.7$ (correlation coefficient = 0.693), respectively. The slope coefficients are approximately in close accord with the antibacterial activities represented by the MICs that inhibited 50% and the MICs that inhibited 90% (Table 1).

DISCUSSION

The production of β -lactamase by 91% of the 67 isolates of *B. fragilis* isolates in this study is very similar to the figure of 90% reported by Olsson et al. (13) for 231 *Bacteroides* isolates and 89% reported by Crosby et al. (2) for 100 *B. fragilis* isolates. Although other mechanisms such as impermeability (5, 12) can be associated with the resistance of *B. fragilis* against β -lactam compounds, the production of β -lactamase (4, 8, 19) seems to be the most important factor. Since 91% of the *B. fragilis* isolates elaborated a cephalosporinase, it is not surprising that 7 β -methoxyiminoacetamido cephalosporins such as cefotaxime, ceftizoxime, and cefmenoxime were not particularly effective against *B. fragilis*. Our figures on the correlation of the antibacterial activity and the stability of ceftioxin and cefotaxime to *B. fragilis* β -lactamase are consistent with those of Pechere et al. (15), who reported on the comparative hydrolysis of ceftioxin and cefotaxime by *B. fragilis* MULB-1008 β -lactamase. Thus, the antibacterial activities of both the 7 β -methoxy and the 7 β -methoxyiminoacetamido cephalosporins tested against *B. fragilis* correlate well with their stability to β -lactamase.

We have found in this study that the antibacterial activities of 7 α -methoxy cephalosporins against *B. fragilis* were unaf-

TABLE 1. Comparative *in vitro* activities of 7 α -methoxy and 7 β -methoxyiminoacetamido cephalosporins against *B. fragilis*

Antibiotics	No. of isolates tested	Mode MIC ($\mu\text{g/ml}$)	No. of isolates at the mode MIC	MIC ($\mu\text{g/ml}$)		
				Range	For 50% inhibition	For 90% inhibition
7α-Methoxy cephalosporins						
Ceftioxin	67	4	53	2-16	4	8
Cefmetazole	67	4	41	2-64	4	16
Moxalactam	67	0.5	25	0.25-16	2	8
Cefotetan	67	2	31	1-16	4	4
7β-Methoxyiminoacetamido cephalosporins						
Cefotaxime	67	4/64 ^a	16/19	1->128	32	64
Ceftizoxime	67	2/32 ^a	15/12	0.5-64	16	32
Cefmenoxime	67	4/64 ^a	19/12	2->128	32	128

^a Two numbers are given for 7 β -methoxyiminoacetamido cephalosporins because the MICs showed a bimodal distribution.

fectured by β -lactamase, whereas those of 7 β -methoxyiminoacetamido cephalosporins apparently were dependent on the production of β -lactamase. In our MIC determinations for the 7 β -methoxyiminoacetamido cephalosporins, we obtained bimodal distributions as shown in Table 1. Cuchural et al., however, have demonstrated that *B. fragilis* TAL 2480, a clinical isolate, inactivated cefoxitin and other β -lactam compounds (G. Cuchural, J. Mayhew, C. Denke, B. Goldin, M. Malamy, and F. P. Tally, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 22nd, Miami Beach, Fla., abstr. no. 717, 1982). The difference in our results and those of Cuchural et al. suggest that *B. fragilis* may elaborate a second β -lactamase which does not belong to the group proposed by Richmond and Sykes (16).

LITERATURE CITED

1. Birnbaum, J., E. O. Stapley, A. K. Miller, H. Wallick, D. Hendlin, and H. B. Woodruff. 1978. Cefoxitin, a semi-synthetic cephamycin: a microbiological overview. *J. Antimicrob. Chemother.* 4(Suppl. B):15-32.
2. Brown, J. E., V. E. Del Bene, and C. D. Collins. 1981. In vitro activity of *N*-formimidoyl thienamycin, moxalactam, and other new beta-lactam agents against *Bacteroides fragilis*: contribution of beta-lactamase to resistance. *Antimicrob. Agents Chemother.* 19:248-252.
3. Crosby, M. A., and D. Gump. 1982. Activity of cefoperazone and two β -lactamase inhibitors, sulbactam and clavulanic acid, against *Bacteroides* spp. correlated with β -lactamase production. *Antimicrob. Agents Chemother.* 22:398-405.
4. Darland, G., and J. Birnbaum. 1977. Cefoxitin resistance to beta-lactamase: a major factor for susceptibility of *Bacteroides fragilis* to the antibiotic. *Antimicrob. Agents Chemother.* 11:725-734.
5. Drasar, F. A., W. Farrell, A. J. Howard, C. Hinze, T. Leung, and J. D. Williams. 1978. Activity of HR756 against *Haemophilus influenzae*, *Bacteroides fragilis* and gram-negative rods. *J. Antimicrob. Chemother.* 3:445-460.
6. Finegold, S. M. (ed.). 1977. Anaerobic bacteria in human disease. Academic Press, Inc., New York.
7. Fu, K. P., and H. C. Neu. 1978. Beta-lactamase stability of HR-756, a novel cephalosporin, compared to that of cefuroxime and cefoxitin. *Antimicrob. Agents Chemother.* 14:322-326.
8. Gorbach, S. L., and J. G. Bartlett. 1974. Anaerobic infections. *N. Engl. J. Med.* 209:1177-1184.
9. Greenwood, D., N. Pearson, A. Eley, and F. O'Grady. 1980. Comparative in vitro activities of cefotaxime and ceftizoxime (FK749): new cephalosporins with exceptional potency. *Antimicrob. Agents Chemother.* 17:397-401.
10. Kesado, T., T. Hashizume, and Y. Asahi. 1980. Antibacterial activities of a new stabilized thienamycin, *N*-formimidoyl thienamycin, in comparison with other antibiotics. *Antimicrob. Agents Chemother.* 17:912-917.
11. Leung, T., and J. D. Williams. 1978. β -lactamase subspecies of *Bacteroides fragilis*. *J. Antimicrob. Chemother.* 4:47-54.
12. Nord, C. E., B. Olsson, and K. Dornbusch. 1978. Production of beta-lactamase by and susceptibility to beta-lactam antibiotics of *Bacteroides fragilis*, p. 489-492. In W. Siegenthaler and R. Luthy (ed.), *Current chemotherapy. Proceedings of the 10th International Congress of Chemotherapy*, vol. 1. American Society for Microbiology, Washington, D.C.
13. Olsson, B., C. E. Nord, and T. Wadstrom. 1976. Formation of beta-lactamase in *Bacteroides fragilis*: cell-bound and extracellular activity. *Antimicrob. Agents Chemother.* 9:727-735.
14. Olsson, B., K. Dornbusch, and C. E. Nord. 1979. Factors contributing to resistance to beta-lactam antibiotics in *Bacteroides fragilis*. *Antimicrob. Agents Chemother.* 15:263-268.
15. Pechere, J. C., R. Guay, J. Dubois, and R. Letarte. 1980. Hydrolysis of cefotaxime by a beta-lactamase from *Bacteroides fragilis*. *Antimicrob. Agents Chemother.* 17:1001-1003.
16. Richmond, M. H., and R. B. Sykes. 1973. The beta-lactamases of gram-negative bacteria and their possible physiological role. *Adv. Microbiol. Physiol.* 9:31.
17. Sato, K., Y. Matsuura, M. Inoue, and S. Mitsuhashi. 1982. Properties of a new penicillinase type produced by *Bacteroides fragilis*. *Antimicrob. Agents Chemother.* 22:579-584.
18. Sawai, T., and I. Takahashi. 1978. Assay methods for β -lactamase activity and their application. *Protein Nucleic Acid Enzyme* 23:391-400. (In Japanese.)
19. Tally, F. P., J. P. O'Keefe, N. M. Sullivan, and S. L. Gorbach. 1979. Inactivation of cephalosporins by *Bacteroides*. *Antimicrob. Agents Chemother.* 16:565-571.
20. Tsuchiya, K., M. Kondo, M. Kida, M. Nakao, T. Iwahi, T. Nishi, Y. Noji, M. Takeuchi, and Y. Nozaki. 1981. Cefmenoxime (SCE-1365), a novel broad-spectrum cephalosporin: in vitro and in vivo antibacterial activities. *Antimicrob. Agents Chemother.* 19:56-65.
21. Virginia Polytechnic Institute and State University Anaerobe Laboratory. 1976. Outline of clinical methods in anaerobic bacteriology. Virginia Polytechnic Institute and State University, Blacksburg, Va.
22. Weinrich, A. E., and V. E. Del Bene. 1976. Beta-lactamase activity in anaerobic bacteria. *Antimicrob. Agents Chemother.* 10:106-111.