NOTES

EFFECT OF PH UPON THE ACTIVITY OF CEFOXITIN

KENSUKE TOBE*, TAKESHI NISHINO, YOSHIMI HIRAI and SHOZO NAKAZAWA

Department of Microbiology, Kyoto College of Pharmacy, Kyoto, Japan

(Received for publication October 1, 1977)

Cefoxitin is a new semisynthetic cephamycin antibiotic, effective against gram-positive and gram-negative bacteria. It is of particular interest because of its activity against cephalosporin-resistant strains of *Escherichia coli*, indole-positive *Proteus, Klebsiella* and *Serratia*[6] and because of its high resistance to hydrolysis by β-lactamases[3].

Antibacterial activities of penicillins and cephalosporins are generally known to increase with decreasing pH of test medium. However, WALLICK and HENDLIN[6] reported that there was no appreciable effect of the initial pH of medium on the activities of cefoxitin and cephalothin.

We have studied the effect of pH on the antibacterial activity of cefoxitin, and it is apparent that cefoxitin exhibits different characteristics from the other cephalosporins in this respect.

Materials and Methods

Minimal inhibitory concentration (MIC) determined by the broth dilution method: A loopful of overnight broth culture was inoculated to tubes containing 1 ml of antibiotic diluted in twofold increments in Heart Infusion (HI) broth (Nissan), adjusted with either HCl or NaOH to pH 5, 6, 7, 8 and 9. The tubes were incubated overnight at 37°C, and the MIC was determined. MIC was defined as the lowest concentrations of antibiotic at which no visible growth was observed after an overnight incubation.

MIC determined by the agar dilution method: Each culture was grown in Tryptosoya broth (Nissan) overnight at 37°C. Antibiotics were added aseptically in twofold increments to HI agar (Nissan) which had been autoclaved, cooled to 50°C and adjusted with either HCl or NaOH to pH 5~9. Agar with the test compounds was distributed into sterile agar plates. When the agar had solidified, the agar plates were streaked with an inoculator so that the number of organisms was approximately 5×10^6 colony forming units (CFU). Then plates were incubated at 37 °C for 18 hours and examined for the presence of growth.

Survival kinetics: *E. coli* No. 29 was preincubated in HI broth, 0.5 ml of this culture (cell density being 1×10^9 cells/ml), was inoculated into flasks containing 9 ml of pH adjusted HI broth and 0.5 ml of HI broth containing 125 μg of cefoxitin, cefazolin or cephalothin per ml, respectively. These mixtures were incubated at 37°C without shaking. Growth was followed by sampling each tube at 1.5, 3.0 and 4.5 hours. Appropriate dilutions were plated in Nutrient agar (Nissan) to obtain CFU.

Results and Discussion

The effect of pH of the medium upon MICs for cefoxitin, cefazolin, cephalothin and cephamycin C with 4 strains selected from *Escherichia coli, Proteus, Klebsiella* and *Staphylococcus* as determined by the broth dilution method is presented in Table 1. The antibacterial activities of the control drugs, cefazolin and cephalothin, increased as the pH decreased. On the contrary, the antibacterial activity of cefoxitin against the first three of the above organisms increased with increasing pH.

Effect of pH upon MICs assayed by the agar dilution method is indicated in Table 2. These data are the averages of the MIC values of cefoxitin, cefazolin, cephalothin and cephamycin C against 49 strains of clinical isolates, including *E. coli, Shigella, Salmonella, Klebsiella, Proteus* and *Staphylococcus*. The table shows that the antibacterial activity of cefoxitin against all genera except *Staphyloccocus* is enhanced as the pH increases, and the activities of cefazolin and cephalothin against *Proteus* and *Klebsiella* are not affected by the hydrogen ion concentration. The pH dependence on MIC of cephamycin C is similar to that of cefoxitin, but of lesser degree.

The effect of pH on survival or growth rates is illustrated in Fig. 1. The MICs for cefoxitin,

* Present address: Research Institutes Daiichi Seiyaku Co., Ltd., 2810 Minami Funabori, Edogawa-ku, Tokyo 132, Japan.

Table 1. Effect of pH upon the antibacterial activities of cefoxitin (CFX), cephalothin (CET) and cefazolin (CEZ) by the broth dilution method.

Test organisms	pH	MIC (μg/ml)		
		CFX	CET	CEZ
Staphylococcus aureus FDA 209P	5.0	—	—	—
	6.0	0.78	0.045	0.045
	7.0	1.56	0.09	0.09
	8.0	1.56	0.09	0.09
	9.0	1.56	0.09	0.09
Escherichia coli NIH JC-2	5.0	12.5	6.25	1.56
	6.0	6.25	12.5	3.13
	7.0	6.25	12.5	3.13
	8.0	3.13	25.0	6.25
	9.0	0.39	50.0	12.5
Proteus mirabilis 1287	5.0	3.13	1.56	6.25
	6.0	3.13	1.56	6.25
	7.0	3.13	1.56	6.25
	8.0	3.13	1.56	6.25
	9.0	3.13	3.13	12.5
Klebsiella pneumoniae	5.0	6.25	3.13	0.78
	6.0	3.13	3.13	1.56
	7.0	3.13	3.13	1.56
	8.0	3.13	3.13	1.56
	9.0	1.56	3.13	3.13

Tests were conducted in HI broth by the broth dilution method. Approximately 10^6 cells of each culture were used to inoculate into each tube.

cefazolin and cephalothin for *E. coli* No. 29, by the broth dilution tests were all 1.56 μg/ml. Fig. 1a shows the growth curves of untreated controls, and killing rate of 6.25 μg/ml of cefoxitin-treated cultures in HI broth. The initial pH of each culture was adjusted to 5.5, 7.0 and 8.5, respectively. In the beginning of incubation, the killing rate of the cefoxitin-treated culture at pH 5.5 is greater than that at pH 8.5, but during 1.5~3 hours of incubation the latter exceeded the former. On the contrary, in the case of cefazolin and cephalothin, as shown in Fig. 1b and 1c, the killing rates of the cultures at pH 5.5 are uniformly greater than those at pH 8.5 from beginning to end of the incubation.

The effect of pH of the medium on the activities of cefoxitin, cefazolin, cephalothin and cephamycin C was studied as the variations of MICs obtained by the broth dilution method with 4 representative strains, and by the agar dilution method with 49 clinically isolated strains. The effect of pH of medium on the killing rate of these four drugs was also studied. It is apparent from the data presented in this paper that the antibacterial activities of cefazolin and cephalothin against gram-negative bacteria are enhanced as the pH decreases. Conversely, the activity of cefoxitin is enhanced, as the pH increases.

As for the effect of pH on the activity of cefoxitin, our result is different from the WALLICK and HENDLIN's report[6] which described that

Fig. 1. Effect of pH of medium upon antibacterial activities of cefoxitin, cephalothin and cefazolin against *E. coli* No. 29.

MIC values for cefoxitin, cefazolin and cephalothin with *E. coli* No. 29 were all 3.13 μg/ml.
Drug concentrations used were all 6.25 μg/ml.

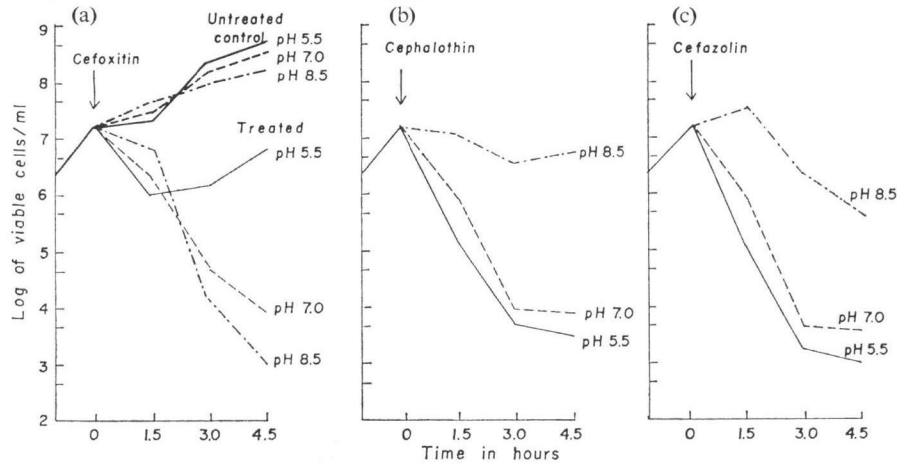

Table 2. Effect of pH upon the antibacterial activities of cefoxitin, cephalothin, cefazolin, and cephamycin C by the agar dilution method.[a)]

Drugs[c)]	Organisms	No.[b)]	Average MIC (μg/ml)						
			pH 5.0	pH 5.5	pH 6.0	pH 7.0	pH 8.0	pH 8.5	pH 9.0
CFX	*E. coli*	15	12.9		7.34	3.54	2.81		2.45
	Shigella	4	4.69		3.91	3.52	2.74		2.35
	Salmonella	8		36.91		1.86		1.71	
	Proteus	8		21.80		9.38		5.86	
	Klebsiella	7		16.4		7.59		4.91	
	Staphylococcus	7		0.96		2.00		2.68	
CET	*E. coli*	15	8.02		10.80	12.60	14.59		42.08
	Shigella	4	4.69		9.38	12.50	21.88		21.88
	Salmonella	8		20.12		2.88		4.30	
	Proteus	8		7.03		7.30		7.80	
	Klebsiella	7		12.70		12.05		13.30	
	Staphylococcus	7		0.30		0.60		0.64	
CEZ	*E. coli*	15	1.25		2.24	2.92	3.44		9.19
	Shigella	4	2.15		2.54	3.52	4.30		8.60
	Salmonella	8		13.09		1.56		5.86	
	Proteus	8		10.90		11.72		11.72	
	Klebsiella	7		12.95		7.59		12.00	
	Staphylococcus	7		1.19		1.51		2.62	
CM-C	*E. coli*	15	11.38		8.83	7.81	7.10		6.78
	Shigella	4							
	Salmonella	8							
	Proteus	8		43.75		10.47		11.72	
	Klebsiella	7		28.57		11.61		9.82	
	Staphylococcus	7		41.00		71.40		78.57	

a) Tests were conducted in HI agar by the agar dilution method. Approximately 10^6 cells of each culture were deposited on the surface of the plates.

b) Numbers indicate the numbers of isolates used.

c) Abbreviations of drugs are as follows; CFX: cefoxitin, CET: cephalothin, CEZ: cefazolin, CM-C: cephamycin C.

there was no appreciable effect of pH on the activity of cefoxitin and cephalothin, but agreed with ONISHI's report[3)] which described that the binding rate of ^{14}C-cefoxitin to the gram-negative bacterial strains decreased with the increasing hydrogen ion concentration.

With regard to the pH dependence of the penicillin action, RETSEMA and RAY[4)] proposed that the increase in the rate of penicillin binding to *S. aureus* with decreasing pH is perhaps due to the increasing in the surface concentration of penicillin caused by electrostatic attraction.

We suppose that the relation between *S. aureus* and penicillin proposed by RETSEMA and RAY is similar to the relation between gram-positive bacteria and cefoxitin, but not applicable to that between gram-negative bacteria and cefoxitin.

On the other hand, antibiotic-induced killing is supposed to be due to both factors, a direct action of the antibiotic and an action of autolytic enzymes[5)], whose optimum pH of gram-negative bacteria is reported to consist in alkali region[1,2)].

It is one of the possible reasons for the strong bactericidal action of cefoxitin that the optimum pH on the action of cefoxitin coincides with that of autolytic enzymes of gram-negative bacteria.

References

1) ELMROS, T.; L. G. BURMAN & G. D. BLOOM: Autolysis of *Neisseria gonorrhoeae*. J. Bacteriol. 126: 969~976, 1976
2) IZAKI, K. & J. L. STROMINGER: Biosynthesis of the peptidoglycan of bacterial cell wall. XIV. Purification and properties of two D-alanine carboxypeptidases from *Escherichia coli*. J. Biol. Chem. 242: 3193~3201, 1968
3) ONISHI, H. R.; D. R. DAOUST; S. B. ZIMMERMAN; D. HENDLIN & E. O. STAPLEY: Cefoxitin, a semisynthetic cephamycin antibiotic: Resistance to beta-lactamase inactivation. Antimicr. Agents & Chemoth. 5: 38~48, 1974
4) RETSEMA, J. A. & V. A. RAY: Correlation between the binding of β-lactam antibiotics to *Staphylococcus aureus* and their physical-chemical properties. Antimicr. Agents & Chemoth. 2: 173~180, 1972
5) TERNOV, E. H. & L. DANEO-MOORE: The autolytic enzymes in bactericidal effect of cell wall antibiotics. Published in 16th Interscience Conference on Antimicrobial Agents and Chemotherapy in Chicago, 1976
6) WALLICK, H. & D. HENDLIN: Cefoxitin, a semisynthetic cephamycin antibiotic: Susceptibility studies. Antimicr. Agents & Chemoth. 5: 25~32, 1974