ANTIBACTERIAL ACTIVITY OF COMBINATIONS OF CEFAZOLIN AND SEMISYNTHETIC PENICILLINS

TOYOJI OKUBO, MATSUHISA INOUE and SUSUMU MITSUHASHI

Department of Microbiology, School of Medicine, Gunma University
Maebashi, Japan

(Received for publication July 7, 1975)

The antibacterial activity of cephalosporin (CS) and semisynthetic penicillins was studied using CS-resistant strains of *Escherichia freundii* and *Proteus morganii*. A synergistic growth inhibitory action toward these microorganisms was demonstrated by a qualitative method and confirmed by a quantitative determination.

Cephalosporin β-lactamase (CSase), one of the main mechanisms of resistance to cephalosporin antibiotics, is distributed among most genera of the family *Enterobacteriaceae* including *Escherichia coli*, *Escherichia freundii*, *Aerobacter aerogenes*, *Proteus morganii*, *Proteus rettgeri*, *Arizona*, *Providence* and *Pseudomonas aeruginosa*[10,11]. Owing to the wide-spread use of cephalosporin (CS) antibiotics, the frequency of isolation of CS-resistant strains is now increasing among clinical isolates. It is known that the V_{max} values for cephalosporins are different among CSases from various species of gram-negative bacteria and the high affinity for synthetic penicillins is a common character in CSase from Gram-negative bacteria[11]. Based on these findings, we examined the synergistic action of cephalosporins and semisynthetic penicillins against CS-resistant strains of bacteria. This paper deals with an attempt to find an improved method of treatment of CS-resistant bacteria using combinations of cephalosporins and semisynthetic penicillins.

Materials and Methods

Bacterial strains used. *E. freundii* GN99, GN346 and GN1706, and *P. morganii* GN926 were used. They are all of clinical origin and presented to our laboratory. The minimum inhibitory concentrations (MIC) of cefazolin, cloxacillin (MCI) and dicloxacillin (MDI) against these organisms are shown in Table 1.

Drugs. Cefazolin (CEZ) was kindly supplied by the Fujisawa Pharmaceutical Co., Osaka. Cloxacillin (MCI) and dicloxacillin (MDI) were provided by the Bristol Laboratories, Inc., Syracuse, N.Y.

Drug resistance. Drug resistance was assayed by the method described previously[6].

Media. Brain heart infusion (BHI, Difco) broth was used for liquid culture. Heart

Table 1. Bacterial strains used and their resistance to β-lactam antibiotics.

Bacterial strain	Minimum inhibitory concentration (mcg/ml)		
	CEZ	MCI	MDI
E. freundii GN99	>800	400	>800
GN346	>800	800	>800
GN1706	>800	>800	>800
P. morganii GN926	200	800	>800

One tenth ml of overnight BHI broth culture of each strain was inoculated in 100 ml of medium B containing various concentrations of each drug. After 18-hour incubation at 37°C, the minimum inhibitory concentration was scored by observing the visible growth of bacteria.

infusion (HI, Difco) agar was used for the determination of synergistic effects by the plate technique. Medium B was used for the determination of synergism in liquid culture. Medium B consisted of 2 g yeast extract (Difco), 10 g peptone, 7 g Na_2HPO_4, 2 g KH_2PO_4, 1.2 g $(NH_4)_2SO_4$, 2 g glucose, 0.4 g $MgSO_4$, and 1,000 ml of distilled water (pH 7.2).

Qualitative demonstration of synergism. A slight modification of the crossed paper strip method reported by CHABBERT[2] was used for the demonstration of synergism. The method was reported previously[3,8]. Quantitative determination of synergism and statistical methods were reported previously[1,3,5,9].

Results

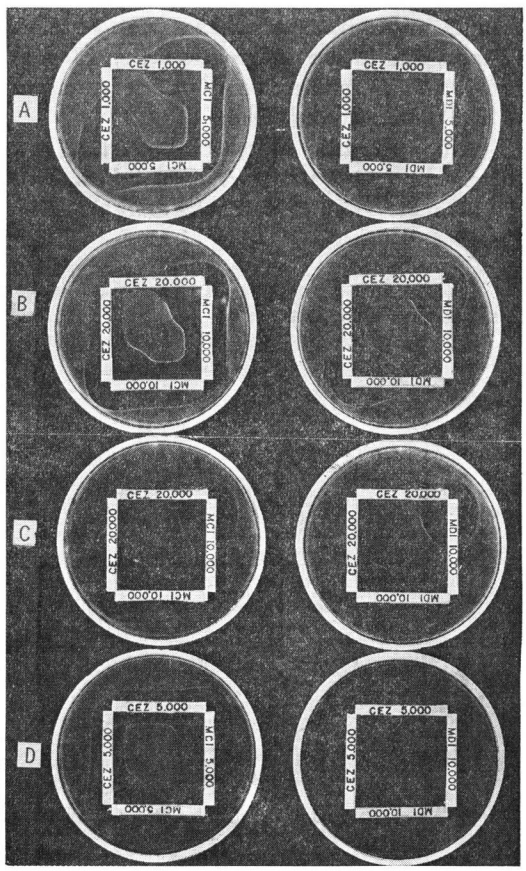
Synergistic Effect

Synergistic growth inhibitory effects of combined drugs were demonstrated by the crossed paper strip method. As shown in Fig. 1, the growth inhibitory effects were seen at the corner of both paper strips which contained CEZ and MCI (or MDI). The synergistic effects were also demonstrated by the checker board method using liquid cultures (Fig. 2).

Fig. 1. Synergistic action of CEZ and synthetic penicillins.

The number on each paper strip represents the concentration of drugs in mcg per ml.

A, *E. freundii* GN99; B, *E. freundii* GN346; C, *E. freundii* GN1706; D, *P. morganii* GN926.



Quantitative Determination of Synergism

About 10^5 bacteria in 10 ml medium B containing various concentrations of single and combined drugs were incubated at 37°C. The optical density was measured and recorded automatically by photometer. Dose-response effect of single and combined drugs is shown in Fig. 3. There was a reciprocal relationship between growth rate and drug concentration at ED_{70}, and the regression lines were found to fit in well with the plotted points of each group by a X^2 test (P=0.05). ED_{70} and its confidence limits calculated from this line are shown in Table 2. The experimentally determined ED_{70} of combined drugs was much smaller than hypothetical ED_{70} assuming an additive effect of the combined drugs.

As shown in Table 3, the synergistic ratios at ED_{70} of combined drugs, *i.e.*, (CEZ + MCI) and (CEZ + MDI), were 26.7 and 27.8, respectively, when *E. freundii* was used as a test organism. When *P. morganii* GN926 was used, the synergistic ratios at ED_{70} of combined drugs, *i.e.*, (CEZ + MCI) and (CEZ + MDI),

Table 2. Effective dose of single and combined drugs.

Strain	Drug	CEZ/MCI (or MDI)	Regression constants (log)		ED_{70} mcg/ml	95 % Confidence
			a	*b*		
E. freundii GN346	CEZ[c]		2.36	1.06	814.0	515.2~ 1286.1
	MCI		2.27	2.37	3231.6	1798.4~ 5806.9
	CEZ + MCI	1 : 1	1.53	0.31	48.8	48.3~ 49.3
	CEZ + MCI[d]	1 : 1			1300.5	815.3~ 2074.4
E. freundii GN346	CEZ[c]		2.36	1.06	814.0	515.2~ 1286.1
	MDI		2.43	2.81	7924.8	1141.4~55021.2
	CEZ + MDI	1 : 1	1.55	0.33	53.0	51.7~ 54.4
	CEZ + MDI[d]	1 : 1			1476.4	803.4~ 2713.0
P. morganii GN926	CEZ[c]		1.26	1.59	122.2	56.4~ 264.8
	MCI		2.87	0.83	2029.5	787.6~ 5229.6
	CEZ + MCI	1 : 10	1.55	0.36	54.9	31.0~ 97.4
	CEZ + MCI[d]	1 : 10			839.0	366.6~ 1920.1
P. morganii GN926	CEZ[c]		1.26	1.59	122.2	56.4~ 264.8
	MDI		2.89	0.87	2230.5	823.9~ 6038.7
	CEZ + MDI	1 : 10	1.32	0.18	64.9	50.5~ 83.3
	CEZ + MDI[d]	1 : 10			868.4	375.7~ 2007.2

a, *b* The resultant working regression equation can be denoted by the next equation[9], *i.e.*, $Y = a + bX$. Y is log (dose) and X is log (1/growth rate). Namely, *a* and *b* show the point of intercept and slope, respectively.

c) Experimentally determined values.

d) A hypothetical value assuming an additional effect of the combined drugs[3,9].

Table 3. Synergistic action of CEZ and synthetic penicillins.

Strain	Drug	Ratio	S. R. (at ED_{70})	95 % Confidence
E. freundii GN346	CEZ + MCI	1 : 1	26.7	16.7~42.5
	CEZ + MDI	1 : 1	27.8	15.1~51.2
P. morganii GN926	CEZ + MCI	1 : 10	15.3	5.6~41.8
	CEZ + MDI	1 : 10	13.4	5.6~32.1

ED_{70}, 70 % effective dose; S. R., synergistic ratio[9].

combination against gram-negative bacteria[4] have already been reported. Our results demonstrate the synergistic action of CEZ/synthetic penicillins against gram-negative bacteria. It should be further noted that the CEZ/MCI and CEZ/MDI combinations exhibited the synergistic action toward CEZ-resistant strains of *E. freundii* and *P. morganii*. This fact is consistent with the result that the enzymatic hydrolysis of CEZ by CSases derived from CEZ-resistant strains of *P. morganii* and *P. aeruginosa* is inhibited by synthetic penicillins, especially MCI and MDI[10,11].

CSases are widely distributed among most genera of gram-negative bacteria isolated from clinical specimens and the frequency of isolation of highly resistant bacteria is now increasing owing to the wide-spread use of cephalosporin antibiotics. Accordingly, the combination of cephalosporins and synthetic penicillins will be useful in medicine due to the broadness of antibacterial spectrum and the synergistic action toward gram-negative bacteria, especially cephalosporin-resistant bacteria.

Acknowledgement

We are greatly indebted to the Cefazolin Research Group at the Central Research Laboratory, Fujisawa Pharmaceutical Co., Osaka for their data of CEZ pharmacokinetics.

References

1) Bushby, S. R. M. & G. H. Hitchings: Trimethoprim, a sulphonamide potentiator. Brit. J. Pharmacol. Chemotherapy 33: 72~90, 1968
2) Chabbert, Y.: Une technique nouvelle d'etude de l'action bacteriocide des associations d'antibiotiques: le transfert sur cellophane. Ann. Inst. Pasteur 93: 289~299, 1957
3) Kawakami, M.; Y. Nagai, S. Shimizu & S. Mitsuhashi: Anti-microbial effect of combinations of colistin methanesulfonate and chloramphenicol. I. *In vitro* effect. J. Antibiotics 24: 884~891, 1971
4) Klastersky, J.; G. Swings, L. Vandenborre, D. Weerts, & V. de Maertelaer: Effectiveness of the carbenicillin/cephalothin combination against gram-negative bacilli. Amer. J. Med. Sci. 265: 45~53, 1973
5) Litchfield, J. T. & F. Wilcoxon: A simplified method of evaluating dose-effect experiments. J. Pharmacol & Exp. Therap. 96: 99~113, 1949
6) Mitsuhashi, S.; H. Oshima, U. Kawaharada & H. Hashimoto: Drug resistance of staphylococci. I. Transduction of tetracycline resistance with phage lysate obtained from multiply resistant staphylococci. J. Bact. 89: 962~976, 1965
7) Miyake, A.; H. Sagai, T. Saito, T. Ando & S. Goto: Synergistic effect of ampicillin and dicloxacillin on pathogenic bacteria. I. Chemotherapy (Tokyo) 21: 1235~1240, 1973
8) Mouton, R. P. & A. Koelman: Bacteriostatic and bactericidal action of combined antibacterial agents *in vitro*. Antimicr. Agents & Chemoth.1965: 261~266, 1966
9) Waitz, J. A. & A. J. Dresner: Selected statistical methods in chemotherapy. Adv. Chemotherapy 3: 1~37, 1968
10) Yaginuma, S.; T. Sawai, H. Ono, S. Yamagishi & S. Mitsuhashi: Biochemical properties of a cephalosporin β-lactamase from *Pseudomonas aeruginosa*. Jap. J. Microbiol. 17: 141~149, 1973
11) Yaginuma, S.; T. Sawai, S. Yamagishi & S. Mitsuhashi: β-Lactamase formation and resistance of *Proteus morganii* to various penicillins and cephalosporins. Jap. J. Microbiol. 18: 113~118, 1974