STUDIES ON MICROBIAL DEGRADATION OF CEPHALOSPORIN C DERIVATIVES. II

ON THE DEGRADATION OF SEVERAL DERIVATIVES AND THE ACTIVITIES OF SOME STRAINS ISOLATED FROM PATIENTS

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Several cephalosporin derivatives were classified into three groups based on their degradation by bacteria. Different properties of *m*-Br-PACA and *α*-Ph-POCA with respect to microbial degradations were also found with other clinically isolated strains of *Staph. aureus* and *E. coli*. The antibacterial activity of cephalosporin derivatives is related to degradation by *Staph. aureus* No. 33, but not that by *E. coli* No. 11.

The authors reported previously that *m*-bromophenylacetamido-cephalosporanic acid (*m*-Br-PACA) was highly degraded by a strain (No. 11) of *E. coli*, but less degraded by a strain (No. 33) of *Staph. aureus*, and that *α*-phenylphenoxyacetamido-cephalosporanic acid (*α*-Ph-POCA) gave opposite results with these bacteria[1]. These results suggest that *m*-Br-PACA had a high affinity for *β*-lactamase from *E. coli* No. 11, while *α*-Ph-POCA had a high affinity for acylesterase from *Staph. aureus* No. 33.

The present paper reports the degradation of some other cephalosporins with different substituents at C-7 by these bacteria, shows the correlation between inactivation of cephalosporins and antibacterial activities *in vitro*, and reports on the degradation of cephalosporins by some clinically isolated strains.

Methods and Materials

(1) Organisms: *Staph. aureus* No. 33 was isolated from a patient at the Institute for Medical Science. *E. coli* No. 11 was isolated from a patient at Kyoto Municipal Hospital. Other strains were isolated from patients at the Hospital of Tōhō University.

(2) Cephalosporin C derivatives used: Cephalosporin derivatives used in this experiment were synthesized from 7-acetaminocephalosporanic acid in our laboratories.

(3) Incubation of cephalosporin derivatives with bacteria: The bacteria were cultured in nutrient broth with shaking for 6 hours at 37°C. After centrifugation, the bacterial cells were washed 2 times with KREBS-RINGER solution and suspended in it to give a cell count of 3×10^9 cells/ml. For the incubation at 37°C, five ml of the bacterial suspension was added to the cephalosporin solution (10 ml) at a concentration of 750 mcg/ml. The reaction mixture was centrifuged ($5,000\times g$, 10 min.) and mixed with an equal volume of 95% ethanol. The resulting mixture was used as the starting material for various determinations.

(4) Microbioassay: The antibacterial activity in the incubation mixture was assayed by a disk method with *Bacillus subtilis* ATCC 6633 as the test organism.

(5) Determination of minimum inhibitory concentration: One loopful of an overnight broth culture of bacteria was streaked on nutrient agar containing graded concentrations of cephalosporin derivatives. The minimal inhibitory concentration (MIC) was determined as the lowest concentration preventing the growth of bacteria after 20 hours incubation at 37°C.

Results

1. Microbial degradations of *m*-Br-PACA by some other strains of staphylococci and *E. coli* isolated from patients

(1) Degradation by Staphylococci

The antibacterial activity of *m*-Br-PACA showed a decrease of 4.7 % (strain No. 39)~19.1 % (strain No. 30), when incubated with four strains of staphylococci for 60 minutes. On the other hand, *α*-Ph-POCA was more sensitive to these staphylococci (43.3~57.5 %). These results showed that *m*-Br-PACA was considerably resistant to the enzymatic attack by staphylococci but *α*-Ph-POCA was less resistant. Thus the degradation of *m*-Br-PACA and *α*-Ph-POCA by these strains of staphylococci corresponded to the results observed previously in a case of *Staph. aureus* No. 33[1].

(2) Degradation by *Escherichia coli*

Microbial degradation of *m*-Br-PACA and *α*-Ph-POCA was compared by incubating them with the clinically isolated strains (K-10, T-13 and T-16) and a standard strain (NIHJ). As shown in Table 1, *m*-Br-PACA was highly sensitive to enzymatic

Table 1. Inactivation of cephalosporin derivatives by *Staph. aureus* and *E. coli* isolated from patients

	Strain No.	C7 :  (*m*-Br-PACA)		C7 :  (*α*-Ph-POCA)	
		MIC ; mcg/ml	% Inactivated	MIC ; mcg/ml	% Inactivated
Staph. aureus	30	1	19.1	10	43.3
	39	1	4.7	25	43.3
	40	1	8.3	10	57.5
	84	0.25	10.2	10	49.4
E. coli	NIHJ	1	99.9	10	31.2
	K-10	100	98.7	>100	13.9
	T-13	100	94.6	>100	27.1
	T-16	>100	57.7	>100	10.6

The bacterial suspension (5 ml) was added to the cephalosporin solution (10 ml) at a concentration of 750 mcg/ml, and incubated for one hour at 37°C. It was then centrifuged (5,000×*g*, 10 min.) and mxied with an equal volume of 95% ethanol. The antibacterial activity in the resulting mixture was assayed by a disk method with *B. subtilis* ATCC 6633 as the test organism.

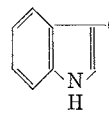
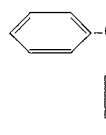
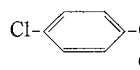
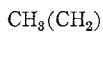
degradation by these strains (57.7~99.9 % decreases in NIHJ, K-10, T-13 and T-16 strains), while *α*-Ph-POCA was comparatively resistant to enzymatic attack by these strains (10.6~31.2 %). The degradation of both derivatives by the four strains of *E. coli* corresponded to the results obtained previously with *E. coli* No. 11.[1]

2. Enzymatic degradations of several cephalosporin C derivatives by the clinically isolated strains of *Staph. aureus* and *E. coli*

(1) Degradation by *Staph. aureus* No. 33

Since it was found that some clinically isolated strains of *Staph. aureus* and *E. coli* gave a different result on the enzymatic degradation of *m*-Br-PACA and *α*-Ph-POCA, several cephalosporin derivatives, differing at the C7-side chain activity[2], were incubated with *Staph. aureus* No. 33 and assayed for residual antibacterial activity. As shown in Table 2, *α*-Ph-POCA was degraded the most of the seven derivatives used and *α*,*β*-dichloro-*β*-(*p*-chlorophenyl)-propionyl derivative was considerably degraded by this strain. The other derivatives, *m*-Br-PACA, *β*-naphthylacetyl, thienylacetyl, 3-indolylmethylthioacetyl and *α*-bromoctanoyl derivatives were more resistant to *Staph. aureus* No. 33.

Table 2. Inactivation of cephalosporin derivatives by *Staph. aureus* No. 33 and *E. coli* No. 11

C7-side chain		*Staph. aureus* No. 33			*E. coli* No. 11		
		MIC ; mcg/ml	% Inactivated		MIC ; mcg/ml	% Inactivated	
			1 hr	3 hrs		1 hr	3 hrs
m-Bromophenylacetyl		6.25	4.8	4.8	>100	79.5	100
β-Naphthylacetyl		25.0	4.3	1.1	>100	44.0	88.5
2-Thienylacetyl		25.0	0	0	>100	31.0	81.0
3-Indolylmethylthio-acetyl		10.0	0	4.3	>100	15.8	29.1
α-Phenylphenoxy-acetyl		>100.0	36.1	96.9	>100	4.0	23.6
α,*β*-Dichloro-*β*-(*p*-chlorophenyl)-propionyl		50.0	10.7	23.5	>100	9.6	25.2
α-Bromoctanoyl		25.0	8.4	8.4	>50	9.1	14.6

Experimental details similar to those of Table 1.

(2) Degradation by *E. coli* No. 11

Similarly, when the same cephalosporins were incubated with *E. coli* No. 11 (Table 2), *β*-naphthylacetyl and thienylacetyl derivatives were as highly degraded as *m*-Br-PACA. 3-Indolylmethylthioacetyl, *α*,*β*-dichloro-*β*-(*p*-chlorophenyl)-propionyl and *α*-bromoctanoyl derivatives were resistant similar to *α*-Ph-POCA.

From the results of this investigation, these cephalosporin derivatives could be divided into three groups as following

(a) *m*-Br-PACA type (sensitive to enzymatic degradation of *E. coli*, but resistant to that of *Staph. aureus*).

(b) *α*-Ph-POCA type (sensitive to enzymatic degradation of *Staph. aureus*, but resistant to that of *E. coli*).

(c) Moderately resistant group (moderately resistant to enzymatic degradation of *Staph. aureus* and *E. coli*).

3. Correlation between microbial degradation and antibacterial activity *in vitro*

As shown in Table 2, seven cephalosporins have different antibacterial activities *in vitro*. Five derivatives were active (MIC : 6.25~25 mcg/ml) and less degraded by this *Staphylococcus* (0~8.4 % in a 3-hr incubation).

On the other hand, α,β-dichloro-β-(*p*-chlorophenyl)-propionyl derivative and α-Ph-POCA were less active against *Staph. aureus* No. 33 (MIC : 50 and >100 mcg/ml) and highly degraded by this *Staphylococcus* (23.5 and 96.9 % in 3-hr incubation).

As shown in Table 2, these cephalosporins gave different results from enzymatic degradation by *E. coli* No. 11, although all these derivatives have no antibacterial activities against this strain.

It may be concluded that there is a relationship between the antibacterial activity of these derivatives against *Staph. aureus* No. 33 and the degradation by this *Staphylococcus*, while such correlation was not found with *E. coli* No. 11.

Discussion

It was found that the two cephalosporin derivatives, *m*-Br-PACA and α-Ph-POCA gave different results in the microbial degradation by *Staph. aureus* No. 33 and *E. coli* No. 11. These results are based on the fact that the activities of β-lactamase and acylesterase[3,4] differ considerably in the two strains, and the affinities of *m*-Br-PACA and α-Ph-POCA for both enzymes differ greatly. Such differences in the microbial degradation of *m*-Br-PACA and α-Ph-POCA, observed in *Staph. aureus* (No. 33) and *E. coli* (No. 11), were also found in the other strains of both bacteria. It is considered that the degradation of cephalosporins by the bacteria depends on the C7-side chain of the cephalosporins.

It may be interesting to study the correlation of the microbial degradation of cephalosporins, used in the experiment, and their activities as the inhibitor for cephalosporin β-lactamase[5,6]. The result may explain the fact that there is some relationship between antibacterial activity and microbial degradation in *Staph. aureus* No. 33, but not in *E. coli* No. 11.

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Bibliography

1) NISHIDA, M.; Y. YOKOTA, M. OKUI, Y. MINE & T. MATSUBARA : Studies on microbial degradation of cephalosporinc derivatives. I. The role of β-lactamase and acylesterase in the enzymatic degradation of cephalosporins. J. Antibiotics 21 : 165~169, 1968
2) CHAUVETTE, R. R. ; E. H. FLYNN, B. G. JACKSON, E. R. LAVAGNINO, R. B. MORIN, R. A. MUELLER, R. P. PIOCH, R. W. ROESKE, C. W. RYAN, J. L. SPENCER & E. VAN HEYNINGEN : Structure-activity relationships among 7-acylamidocephalosporanic acids. Antimicr. Agents & Chemoth. 1962 : 687~694, 1963
3) HAMILTON-MILLER, J.M.T. : Penicillinacylase. Bacteriol. Reviews 30 : 761~771, 1966
4) SJÖBERG, B.; L. NATHORST-WESTFELT & B. ÖRTENGREN : Enzymatic hydrolysis of some penicillins and cephalosporins by *Escherichia coli* acylase. Acta Chim. Scand. 21 : 547~551, 1967
5) O'CALLAGHAN, C. H. ; P. W. MUGGLETON, S. M. KIRBY & D. M. RYAN : Inhibition of β-lactamase decomposition of cephaloridine and cephalothin by other cephalosporins. Antimicr. Agents & Chemoth. 1966 : 337~343, 1967
6) CROMPTON, B.; M. JAGO, K. CRAWFORD, G.G.F. NEWTON & E. P. ABRAHAM : Behaviour of some derivatives of 7-aminocephalosporanic acid and 6-aminopenicillanic acid as substrates, inhibitors and inducers of penicillinases. Biochem. J. 83 : 52~63, 1962