STUDIES ON MICROBIAL DEGRADATION OF CARBENICILLIN

MINORU NISHIDA, YOSHIKO YOKOTA and TADAO MATSUBARA

Research Laboratories,
Fujisawa Pharmaceutical Co., Ltd.
Osaka, Japan

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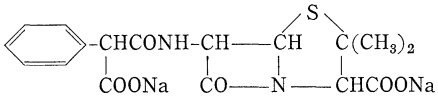
Carbenicillin is a new semi-synthetic penicillin with a broad spectrum of activity against Gram-positive and Gram-negative bacteria.[1,2,3] The most significant feature of carbenicillin is its activity against *Pseudomonas aeruginosa* which is resistant to ampicillin. Both penicillins differ in activity to a certain extent against many kinds of bacteria. In view of this, some clinically isolated strains of *Staphylococci* and *Proteus* which are susceptible to carbenicillin but not to ampicillin were selected, and studied for the correlation bewteen the microbial degradation and the antibacterial activity of both penicillins.

Materials and Methods

1. Organisms: *Pr. morganii* No. 900 and No. 901 were isolated from patients at Kyoto Municipal Hospital, and *Staphylococci* were supplied by the Institute for Medical Science. *Pseudomnas aeruginosa* No. 721 and No. 723 were isolated from patients at Toho University.

2. Penicillins: Carbenicillin and ampicillin used were pure crystalline compounds.

Fig. 1. Chemical structure of carbenicillin (α-Carboxybenzyl-penicillin)



3. Preparation of enzyme fractions: Fractionation of the disrupted bacteria was carried out according to SALTON's method[4]. The bacteria were grown in Brain-heart infusion broth (Difco) with shaking for 6 hours at 37°C, then centrifuged at 6,000 rpm for 20 minutes. The supernatant was used as the extracellular enzyme fraction. The cells collected were washed twice with 0.1 M phosphate buffer (pH 6.8) and suspended in the buffer equal in volume to the original culture broth. The cell suspensions were then sonicated for 60 minutes (*Staph. aureus*) or 20 minutes (*Pr. morganii* and *Ps. aeruginosa*). The supernatant from the centrifugation (10,000 rpm, 20 minutes) was used as the cell-bound enzyme.

4. Penicillin inactivation by enzymes: The extracellular or the cell-bound enzyme fractions, were incubated for 1 hour at 37°C with carbenicillin or ampicillin at a final concentration of 500 mcg/ml, then the enzymatic activity was terminated by heating the incubation mixture in boiling water.

5. Microbioassay: The residual activities of carbenicillin or ampicillin in the incubation mixtures were assayed by a disc method using *B. subtilis* ATCC 6633 as a test organism.

Results

1. Inactivation of carbenicillin and ampicillin by *Pr. morganii*:

Clinically isolated strains of *Pr. morganii* No. 900 and No. 901 showing different sensitivities to carbenicillin and ampicillin, were used. As shown in Table 1, both strains were highly resistant to all antibiotics tested except carbenicillin, streptomycin and gentamicin. Carbenicillin inhibited completely the growth of these strains at concentrations of 1 mcg/ml (No. 901)~2.5 mcg/

Table 1. Susceptibilities of standard or some clinically isolated strains of *Pr. morganii* to common antibiotics. (Agar dilution method)

Antibiotics	Minimal inhibitory concentration (mcg/ml)		
	NIHJ	No. 900	No. 901
Carbenicillin	1.0	2.5	1.0
Ampicillin	0.5	100	100
Penicillin G	5.0	>500	>500
Cephaloridine	2.5	>100	>100
Cephalothin	2.5	>100	>100
Streptomycin	2.5	10	2.5
Chloramphenicol	10	250	25
Tetracycline	2.5	25	25
Erythromycin	50	500	250
Polymyxin B	2.5	>500	>500
Gentamycin	2.5	2.5	2.5

ml (No. 900), while ampicillin was less active against these strains (MIC; 100 mcg/ml). As shown in Fig. 2, the antibacterial activity of carbenicillin showed a 14 % decrease, and that of ampicillin a 14 % decrease when the penicillins were incubated with the extracellular enzyme from *Pr. morganii* No. 900. With the cell-bound enzyme from *Pr. morganii* No. 900, the activity of carbenicillin decreased by only 9 %, but that of ampicillin decreased by 15 %. With *Pr. morganii* No. 901, ampicillin was degraded 26 % by the cell-bound enzyme and carbenicillin 8 %.

Fig. 2. Inactivation of carbenicillin and ampicillin by enzyme fractions from clinically isolated strains of *Proteus morganii* resistant to ampicillin but not to carbenicillin.

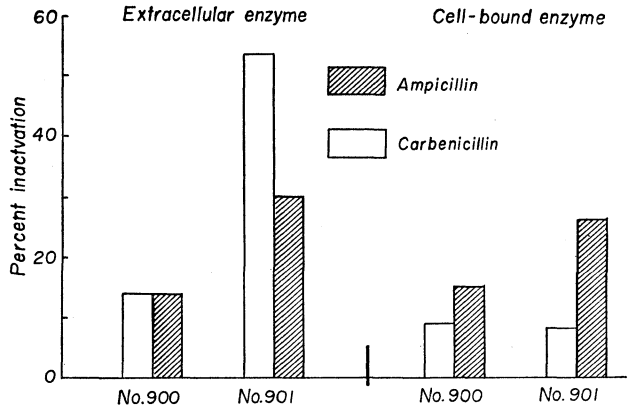
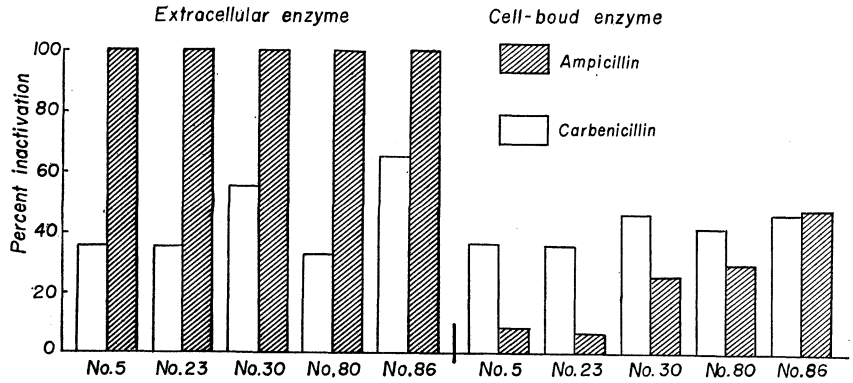


Fig. 3. Inactivation of carbenicillin and ampicillin by enzyme fractions from clinically isolated strains of *Staph. aureus* resistant to ampicillin but not to carbenicillin.



However with the extracellular enzyme from *Pr. morganii* No. 901, carbenicillin was more extensively degraded than was ampicillin (53 % to 30 %).

2. The inactivation of carbenicillin and ampicillin by *Staph. aureus*:

Five strains isolated from patients were used with the antibacterial spectra of which are shown in Table 2. All of these strains, carbenicillin was more stable than ampicillin to the action of the extracellular enzymes.

On the other hand, the opposite results were observed with the cell-bound enzyme. Ampi-

Fig. 4. Inactivation of carbenicillin and ampicillin by enzyme fractions from clinically isolated strains of *Pseudomonas aeruginosa* resistant to ampicillin but not to carbenicillin.

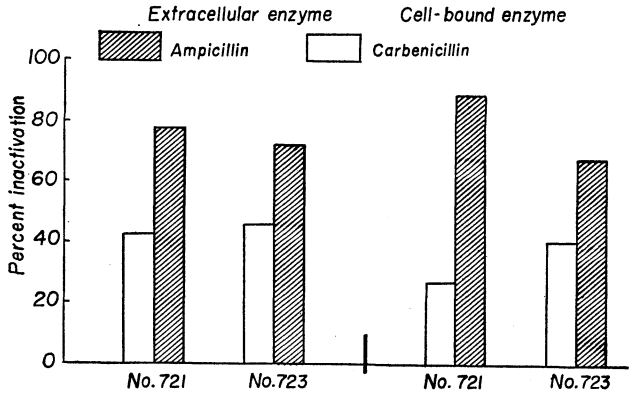


Table 2. Susceptibilities of some clinically isolated strains of *Staph. aureus* to common antibotics (Agar dilution method).

Antibiotics	MIC; mcg/ml				
	No. 5	No. 23	No. 30	No. 84	No. 86
Carbenicillin	125	25	25	25	50
Ampicillin	500	250	250	>500	500
Penicillin G	>500	>500	500	500	500
Cephaloridine	5.0	5.0	2.5	5.0	2.5
Cephalothin	0.6	1.2	1.2	1.2	0.6
Streptomycin	>100	>100	>100	>100	>100
Chloramphenicol	10	10	10	50	50
Tetracycline	>100	>100	>100	>100	>100
Erythromycin	0.6	>100	>100	>100	>100
Polymyxin B	250	250	250	250	500
Gentamicin	0.5	0.5	1.2	5.0	1.2

Table 3. Susceptibilities of some clinically isolated strains of *Ps. aeruginosa* (Agar dilution method).

Antibiotics	MIC; mcg/ml		
	IAM	Ps-721	Ps-723
Carbenicillin	125	1.25	125
Ampicillin	>250	>500	>500
Penicillin G	>500	500	500
Cephaloridine	>100	>500	>500
Cephalothin	>100	>500	>500
Streptomycin	100	50	>500
Chloramphenicol	250	250	>250
Tetracycline	100	50	100
Erythromycin	250	500	500
Polymyxin B	5.0	5.0	5.0
Gentamicin	25	12.5	12.5

cillin was more stable to the cell-bound enzymes than was carbenicillin except for the enzyme from strain No. 86 (Fig. 3).

These results suggest that the antibacterial activity of carbenicillin against the clinically isolated *Staphylococci* relates to the stabililty against the extracellular enzymes from these strains, rather than the cell-bound enzymes.

3. Inactivation of carbenicillin and ampicillin by *Pseudomonas aeruginosa*:

Enzyme from two strains of *Pseud. aeruginosa* were tested for their activity against penicillins by the above procedures. These strains were sensitive to carbenicillin, *i. e.* the growth of *Pseud. aeruginosa* No. 721 was inhibited at a concentration of 1.25 mcg/ml, and No. 723 at 125 mcg/ml, but both strains were not inhibited by 500 mcg/ml of ampicillin (Table 3). Ampicillin was more sensitive than carbenicillin to both the cell-bound and the extracellular enzymes from these strains, as shown in Fig. 4.

For instance, in the case of *Pseud. aeruginosa* No. 721, carbenicillin lost by 43 % of the initial activity with the extracellular enzyme, but ampicillin lost 78 %. With the cell-bound enzyme, the loss of carbenicillin was 27 % and that of ampicillin was 89 % (Fig. 4).

These results show that some strains of *Pseud. aeruginosa*, highly resistant to ampicillin, but not to carbenicillin, can degrade ampicillin more rapidly than carbenicillin.

Some pathogenic bacteria sensitive to carbenicillin but not to ampicillin were selected and the effect of enzyme preparation from them on these penicillins was studied. The antibacterial activity of ampicillin was completely degraded by incubating with the extracellular enzymes from each of the five strains of *Staph. aureus* used, but that of carbenicillin was only partially lost. The inverse result was obtained with enzyme fractions from *Pr. morganii*, the antibacterial activity of ampicillin was highly degraded by the cell-bound enzyme rather than by the extracellular enzyme. The activity of enzyme fractions from *Pseud. aeruginosa* differed from those from *Staph. aureus* or *Pr. morganii*. In each case carbenicillin was more resistant than ampicillin.

Acknowledgement

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