# INDUCER ACTIVITY OF β-LACTAM ANTIBIOTICS FOR THE β-LACTAMASES OF *PROTEUS RETTGERI* AND *PROTEUS VULGARIS*

AKIRA YOTSUJI, SHINZABURO MINAMI, YASUHISA ARAKI, MATSUHISA INOUE* and SUSUMU MITSUHASHI

Laboratory of Drug Resistance in Bacteria, School of Medicine, Gunma University, Maebashi, Japan

(Received for publication July 12, 1982)

The β-lactam antibiotics can be classified into 3 groups on the basis of their inducer activity for β-lactamase in *Proteus rettgeri* and *Proteus vulgaris*.

In our previous paper[1], we reported that the β-lactam antibiotics could be classified into several groups on the basis of their inducer activity for β-lactamase in *Enterobacter cloacae*. There are a few reports concerned with the inducible formation of β-lactamase[2~6]. *Proteus rettgeri* and *Proteus vulgaris* also produce inducible cephalosporinases (CSase) which are species-specific in their substrate profiles[7~9]. This paper deals with the inducer activity of a number of β-lactam antibiotics for the production of β-lactamases by *P. rettgeri* and by *P. vulgaris*.

*P. rettgeri* GN4430 and *P. vulgaris* GN76 are clinical isolates maintained as stock cultures in this laboratory. For determination of inducer activity, various concentrations of each drug were added to a mid log phase culture and shaking of the culture continued. After a further 2 hours of incubation, the cells were harvested and washed once with 0.1 M phosphate buffer (pH 7.0). The drug concentrations added to the culture ranged from 10×MIC to 0.01×MIC. MIC (Minimal inhibitory concentration) was determined by the agar dilution method. A loopful (5 μl) of $10^8$/ml cells was inoculated on a heart infusion (HI) agar (Difco) plate containing a series of serial twofold dilutions of a drug. The MIC was scored after 18 hours of incubation at 37°C. Under these conditions most of the drugs showed little or no effect on the growth of *P. rettgeri* GN4430 when their concentrations were less than 100 μg/ml.

Antibacterial activity and inducer activity of various β-lactam antibiotics against *P. rettgeri* GN4430 are shown in Table 1.

*P. rettgeri* GN4430 was resistant to many β-lactam antibiotics except for the newly-introduced drugs. Both the cefuroxime type cephalosporins such as cefotaxime and ceftizoxime, and cephamycin derivatives such as latamoxef and cefotetan were highly active against *P. rettgeri* GN4430. Based on inducer activity, the β-lactam antibiotics can be classified into three groups, *i.e.*, with high (A), intermediate (B), and low (C) inducibility.

Cephaloridine, cephalothin, cephalexin and cefazolin which are easily hydrolyzed by the cephalosporinase, and the cephamycin derivatives such as cefoxitin, cefmetazole and cefotetan which are not hydrolyzed by the cephalosporinase, showed very high inducer activities for enzyme induction. Cefamandole, cefotiam, cefsulodin, cefuroxime, benzylpenicillin and ampicillin are also in the same group (A). Methicillin and cloxacillin[10] and clavulanic acid[11] also showed high inducer activity. However, latamoxef, carbenicillin and apalcillin showed intermediate inducer activity (group (B)). Cefoperazone, cefotaxime and piperacillin showed low inducer activity (group (C)).

Table 1. Antibacterial activity and inducer activity of various β-lactam antibiotics against *P. rettgeri* GN-4430.

| Drug | MIC[a] (μg/ml) | Enzyme induction[b] with MICs | | | | Relative[c] rate of hydrolysis (%) | Group |
|---|---|---|---|---|---|---|---|
| | | 1/100 | 1/10 | 1 | 10 | | |
| Cephaloridine | >800 | 0.163 | 0.198 | **0.267**[d] | — | 100 | |
| Cephalothin | >800 | 0.106 | 0.184 | **0.210** | — | 85 | |
| Cephalexin | >800 | 0.080 | 0.178 | **0.210** | — | 8 | |
| Cefazolin | >800 | 0.202 | **0.255** | 0.250 | — | 99 | |
| Cefamandole | 3.13 | 0.022 | 0.048 | 0.180 | **0.259** | 1 | |
| Cefotiam | 3.13 | 0.028 | 0.047 | 0.131 | **0.195** | 4 | |
| Cefsulodin | 25 | 0.012 | 0.035 | **0.561** | 0.355 | <1 | |
| Cefuroxime | 3.13 | 0.017 | 0.051 | 0.190 | **0.304** | 1 | A |
| Cefoxitin | 12.5 | 0.111 | 0.337 | **0.351** | 0.338 | <1 | |
| Cefmetazole | 6.25 | 0.124 | 0.321 | **0.506** | 0.224 | <1 | |
| Cefotetan | 0.39 | 0.117 | 0.126 | 0.354 | **0.675** | <1 | |
| Benzylpenicillin | 800 | 0.186 | 0.395 | **0.494** | — | 3 | |
| Ampicillin | 50 | 0.073 | 0.181 | **0.344** | 0.325 | <1 | |
| Methicillin | 200 | 0.013 | 0.047 | 0.240 | **0.460** | <1 | |
| Cloxacillin | 100 | 0.009 | 0.012 | 0.058 | **0.363** | <1 | |
| Clavulanic acid | 100 | 0.007 | 0.035 | **0.322** | 0.078 | — | |
| Latamoxef | 0.05 | 0.010 | 0.007 | 0.036 | **0.147** | <1 | |
| Carbenicillin | 0.2 | 0.007 | 0.015 | 0.012 | **0.105** | <1 | B |
| Apalcillin | 3.13 | 0.008 | 0.006 | 0.008 | **0.117** | <1 | |
| Cefoperazone | 3.13 | 0.009 | 0.010 | 0.015 | **0.031** | <1 | |
| Cefotaxime | 0.025 | 0.014 | 0.009 | 0.015 | **0.037** | <1 | C |
| Ceftizoxime | 0.006 | 0.012 | 0.012 | 0.018 | **0.036** | <1 | |
| Piperacillin | 1.56 | 0.007 | 0.012 | 0.016 | **0.038** | <1 | |

[a] MIC was determined by agar dilution method.
[b] β-Lactamase production is expressed as the specific activity of the induced enzyme (units/mg of protein) using cephaloridine as a substrate.
[c] Relative rate of hydrolysis of various β-lactam antibiotics by the enzyme is expressed in percent of the activity with cephaloridine.
[d] Bold face figures indicates the highest enzyme production using each drugs.

Antibacterial activity and inducer activity of various β-lactam antibiotics to *P. vulgaris* GN76 are also shown in Table 2.

*P. vulgaris* GN76 was also resistant to many β-lactam antibiotics including cefuroxime, except for the newly-introduced drugs. The CSase from *P. vulgaris* had a broad substrate specificity, easily hydrolyzed most of the substrate tested including cefuroxime and was very similar to the enzymes from *Pseudomonas cepacia*[12] and *Bacteroides fragilis*[13] in activities suggesting it belonged to a cefuroxime-hydrolyzing β-lactamase group[7].

Determination of the inducibility in *Proteus vulgaris* was similar to that of *P. rettgeri* GN4430. Under these conditions, most of the drugs showed little or no effect on the growth of *P. vulgaris* GN76. As shown in Table 2, the drugs assigned to group (A) are similar to those for *P. rettgeri* GN4430 except for clavulanic acid. Cefotaxime, ceftizoxime, latamoxef and carbenicillin belonged to group (B). Cefoperazone, piperacillin, apalcillin and clavulanic acid belonged to group (C).

Table 2. Antibacterial activity and inducer activity of various β-lactam antibiotics against *P. vulgaris* GN76.

| Drug | MIC[a] (μg/ml) | Enzyme induction[b] with MICs | | | | Relative[c] rate of hydrolysis (%) | Group |
|---|---|---|---|---|---|---|---|
| | | 1/100 | 1/10 | 1 | 10 | | |
| Cephaloridine | >800 | 0.091 | **0.177**[d] | 0.114 | — | 100 | |
| Cephalothin | >800 | 0.063 | **0.116** | 0.083 | — | 173 | |
| Cephalexin | >800 | 0.074 | **0.155** | 0.149 | — | 274 | |
| Cefazolin | >800 | 0.065 | **0.109** | 0.100 | — | 387 | |
| Cefamandole | >800 | 0.056 | **0.105** | 0.083 | — | 381 | |
| Cefotiam | >800 | 0.076 | **0.143** | 0.135 | — | 351 | |
| Cefsulodin | 800 | 0.037 | **0.188** | 0.160 | — | 9 | |
| Cefuroxime | >800 | 0.065 | **0.193** | 0.139 | — | 1140 | A |
| Cefoxitin | 25 | 0.129 | 0.138 | 0.093 | **0.205** | <1 | |
| Cefmetazole | 12.5 | 0.053 | **0.282** | 0.196 | 0.107 | <1 | |
| Cefotetan | 0.78 | **0.188** | 0.165 | 0.187 | 0.133 | <1 | |
| Benzylpenicillin | >800 | 0.035 | 0.163 | **0.420** | — | 20 | |
| Ampicillin | >800 | 0.070 | 0.172 | **0.230** | — | 15 | |
| Methicillin | >800 | 0.006 | 0.037 | **0.243** | — | <1 | |
| Cloxacillin | 800 | 0.005 | 0.018 | **0.300** | — | <1 | |
| Latamoxef | 0.39 | 0.012 | 0.018 | 0.066 | **0.127** | <1 | |
| Cefotaxime | 0.78 | 0.016 | 0.017 | 0.040 | **0.088** | 80 | B |
| Ceftizoxime | 0.2 | 0.014 | 0.015 | 0.048 | **0.095** | 5 | |
| Carbenicillin | 6.25 | 0.007 | 0.003 | 0.048 | **0.130** | <1 | |
| Cefoperazone | 3.13 | 0.004 | 0.006 | **0.008** | 0.007 | 15 | |
| Piperacillin | 6.25 | 0.006 | 0.007 | 0.005 | **0.014** | 8 | C |
| Apalcillin | 1.56 | 0.004 | 0.003 | 0.007 | **0.008** | 1 | |
| Clavulanic acid | 50 | 0.005 | 0.005 | **0.039** | 0.006 | — | |

[a] MIC was determined by agar dilution method.
[b] β-Lactamase production is expressed as the specific activity of the induced enzyme (units/mg of protein) using cephaloridine as a substrate.
[c] Relative rate of hydrolysis of various β-lactam antibiotics by the enzyme is expressed in percent of the activity with cephaloridine.
[d] Bold face figures indicates the highest enzyme production using each drugs.

The inducer activities of 23 β-lactam antibiotics for β-lactamases of *P. rettgeri* GN4430 and of *P. vulgaris* GN76 were similar to that for *E. cloacae* GN5797. Cephaloridine, cephalothin, benzylpenicillin, *etc.*, which are easily hydrolyzed by the enzyme and the cephamycin derivatives which are resistant to hydrolysis by the enzyme, showed high inducer activity. Methicillin and cloxacillin, which are good inducers in Gram-positive bacteria[10], also showed high inducer activity. Cefotaxime, ceftizoxime, latamoxef, carbenicillin and apalcillin showed intermediate or low inducer activity. However cefoperazone and piperacillin showed low inducer activity in 3 strains of *P. rettgeri, P. vulgaris* and *E. cloacae*.

β-Lactamase has been considered to play a significant role in bacterial resistance against the β-lactam antibiotics, and attention should therefore be paid to the stability of the drug to β-lactamases. Unfortunately, there are no clear relationships between inducer activity and chemical structure at present.

Acknowledgements

This work was supported in part by the grant (No. 211912 and 311201) from the Ministry of Education, Science and Culture in Japan.

## References

1) MINAMI, S.; A. YOTSUJI, M. INOUE & S. MITSUHASHI: Induction of β-lactamase by various β-lactam antibiotics in *Enterobacter cloacae*. Antimicrob. Agents Chemother. 18: 382~385, 1980
2) CHRISTINE, C. S. & W. E. SANDERS, Jr.: Emergence of resistance of cefamandole: Possible role of cefoxitin-inducible beta-lactamases. Antimicrob. Agents Chemother. 15: 792~797, 1979
3) HENNESSEY, T. D.: Inducible β-lactamase in *Enterobacter*. J. Gen. Microbiol. 49: 277~285, 1967
4) JOHN, L. O.; J. R. TURNER & D. F. MAHONEY: Lack of correlation between β-lactamase production and susceptibility to cefamandole or cefoxitin among spontaneous mutants of *Enterobacteriaceae*. Antimicrob. Agents Chemother. 15: 14~19, 1979
5) NORDSTRÖM, K. & R. B. SYKES: Effect of sublethal concentrations of benzylpenicillin on *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 6: 741~746, 1974
6) NORDSTRÖM, K. & R. B. SYKES: Induction kinetics of β-lactamase biosynthesis in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 6: 734~740, 1974
7) MATSUBARA, N.; A. YOTSUJI, K. KUMANO, M. INOUE & S. MITSUHASHI: Purification and some properties of a cephalosporinase from *Proteus vulgaris*. Antimicrob. Agents Chemother. 19: 185~187, 1981
8) MATSUURA, M.; H. NAKAZAWA, M. INOUE & S. MITSUHASHI: Purification and biochemical properties of β-lactamase produced by *Proteus rettgeri*. Antimicrob. Agents Chemother. 18: 687~690, 1980
9) MITSUHASHI, S.; S. YAMAGISHI, T. SAWAI & H. KAWABE: Biochemical mechanism of plasmid-mediated resistance. *In* R Factor-drug resistance plasmid. *Ed.* S. MITSUHASHI, pp. 195~254. University of Tokyo Press, Tokyo. 1977
10) NOVICK, R. P.: Staphylococcal penicillinase and the new penicillins. Biochem. J. 83: 229~235, 1962
11) NEU, H. C. & K. P. FU: Clavulanic acid, a novel inhibitor of β-lactamases. Antimicrob. Agents Chemother. 14: 375~377, 1978
12) HIRAI, K.; S. IYOBE, M. INOUE & S. MITSUHASHI: Purification and properties of a new β-lactamase from *Pseudomonas cepacia*. Antimicrob. Agents Chemother. 17: 355~358, 1980
13) SATO, K.; M. INOUE & S. MITSUHASHI: Activity of β-lactamase produced by *Bacteroides fragilis* against newly introduced cephalosporins. Antimicrob. Agents Chemother. 17: 736~737, 1980