Imipenem Antagonism of the In Vitro Activity of Piperacillin Against 

*Pseudomonas aeruginosa*

MIRIAM A. BERTRAM* AND Lowell s. YOUNG

Clinical Microbiology Laboratory and Division of Infectious Disease, University of California, Los Angeles Medical Center, Los Angeles, California 90024

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The MICs of imipenem and piperacillin, alone and in combination, against *Pseudomonas aeruginosa* were determined in a checkerboard titration microdilution assay. A dramatic, one-way antagonism of imipenem for piperacillin was demonstrated in 28 of the 35 strains examined; antagonism was associated with the induction of a β-lactamase.

Imipenem (IMI) is a β-lactamase-stable carbapenem with a broad antibacterial spectrum (2, 8, 10, 13). Piperacillin (PIP) is a piperazinopenicillin with remarkable broad-spectrum activity and minimal toxicity (3, 4, 7, 19). However, the use of piperacillin as a single agent has been associated, in some studies, with the emergence of resistant organisms (5, 18). The combined use of two β-lactams should provide antimicrobial coverage of a very broad spectrum with low toxicity. To investigate this possibility for IMI and PIP, we examined the two drugs, alone and in combination, for their in vitro activities against *Pseudomonas aeruginosa*.

Aminoglycoside-resistant and aminoglycoside-susceptible P. aeruginosa were included in this study. Susceptibility to aminoglycosides was determined by microdilution in Mueller-Hinton broth supplemented with calcium and magnesium (MH-CS) (12). All aminoglycoside-resistant strains (n = 25) were resistant to gentamicin (MICs, >6 μg/ml); in addition, some strains were resistant to amikacin (MICs, >16 μg/ml) or to tobramycin (MICs, >6 μg/ml) or both. These strains were collected over several years and stored at −70°C. All aminoglycoside-susceptible strains (n = 10) were recent clinical isolates from the University of California, Los Angeles Medical Center and were susceptible to the three aminoglycosides examined. IMI was obtained from Merck & Co., Inc., Rahway, N.J., and PIP was obtained from Lederle Laboratories, Pearl River, N.Y.

The interaction of IMI and PIP was examined by microdilution in MH-CS (12). Wells of standard microtiter plates containing 0.1 ml of antimicrobial solution were inoculated with a diluted stationary-phase culture to give a final concentration of 5 × 10^5 CFU/ml. Trays were incubated at 35°C for 18 h, and the MICs of the antimicrobial agents alone and in combination were determined. The antimicrobial agents were arranged in a checkerboard titration pattern with serial twofold dilutions (9); IMI was tested at concentrations of 0.12 to 8 μg/ml and PIP was tested at concentrations of 2 to 128 μg/ml. The sum of the fractional inhibitory concentration index (ΣFIC) was calculated to determine the nature of the interaction of the two antibiotics (6) by the formula ΣFIC = (MIC(combination)/MICaloneB) + (MIC(combination)/MICaloneA), where A and B are two different antibiotics. Antagonism was defined as a ΣFIC of >4.

The effect of combining IMI and PIP on the interactive index is given in Table 1; of the 35 strains examined, 28 demonstrated antagonism. The distributions of the ΣFICs between aminoglycoside-resistant and aminoglycoside-susceptible *P. aeruginosa* strains were similar; antagonism was demonstrated for 9 of the 10 aminoglycoside-susceptible and for 19 of the 25 aminoglycoside-resistant strains.

Individual components contributing to the antagonistic interactions were examined. The MIC of IMI for any of the strains was not affected by the addition of 2 to 128 μg of PIP per ml; however, the MIC of PIP was increased by the addition of IMI. The MIC of IMI for 50% of the strains (3 μg/ml) was not changed by the addition of PIP, whereas the MIC of PIP for 50% of the isolates increased from 8 to 128 μg/ml upon addition of IMI. In all cases, this antagonism was observed at the lowest concentration of added IMI (0.12 μg/ml) and was observed over the range of IMI concentrations (0.12 to 8 μg/ml) that were less than the MIC of IMI. However, when the concentration of added IMI approached the MIC of IMI, the antagonism was less pronounced, since the antimicrobial activity of IMI overrode the decreased antimicrobial activity of PIP. This effect was generally observed at an IMI concentration which was one-half the MIC of IMI.

Since β-lactamase activity could account for this antagonism (17) and induction of β-lactamase by IMI has been demonstrated for *Serratia marcescens* (11), we tested for constitutive and induced β-lactamase activity in these *P. aeruginosa* strains.

Strains were suspended in broth to obtain a turbidity equivalent to a 0.5 McFarland Standard (15). The culture medium was MH-CS with or without added IMI (0.1 μg/ml) or PIP (1 μg/ml). The culture was incubated at 35°C for 18 h, and then 50-μl samples of the culture suspensions were tested for β-lactamase activity. β-Lactamase activity was detected by addition of 50 μl of the chromogenic cephalosporin nitrocefin (500 μg/ml) (14) and examination for a color change from yellow to red-brown.

Under these assay conditions, both constitutive and induced β-lactamase activities were detected. Induction was observed after overnight incubation in the presence of IMI but not PIP.

β-Lactamase was induced in 21 of the 28 strains in which antagonism had been demonstrated. In five strains, β-lactamase activity was constitutive, and in two strains, enzyme was not detected. Constitutive β-lactamase activity was observed in five of the seven strains which did not display antagonism; in two strains, no activity was observed. Incu-
bation in the presence of 10 or 100 μg of PIP per ml or for 6 h instead of overnight did not give different results. The limitations of the test procedure did not allow for reliable observation or quantitation of an increase in activity (from induction) over constitutive β-lactamase activity.

The correlation of β-lactamase induction and inactivation of PIP was examined in four strains. Each strain was suspended in MHCS with or without IMI (0.1 μg/ml) or PIP (1 or 10 μg/ml) to a final turbidity equivalent to a 0.5 McFarland Standard. After 18 h of incubation at 35°C, the cells were removed by filtration (0.45-μm pore size), and PIP was added in a 20-μl volume to a final concentration of 200 μg/ml. The mixture was incubated at 35°C for 6 h and then frozen at −70°C. An inoculation control was frozen at −70°C at the beginning of the experiment; a PIP stability control, 200 μg of PIP in 1 ml of MH-CS, was included. PIP activity was measured by a standard agar diffusion bioassay with Bacillus subtilis var. globigii as the seed organism (1).

Piperacillin was stable during the 6-h incubation in MH-CS at 35°C. A small loss of PIP activity, ca. 20%, was observed in the control tube containing filtrate from organisms grown without antibiotics. No further reduction of activity from this culture control was observed in the filtrate from organisms grown in the presence of 1 or 10 μg of PIP per ml. However, an additional 60 to 90% reduction in activity was observed in the presence of filtrate from IMI-induced cells which produced β-lactamase. Thus, induction of β-lactamase was found to correlate with PIP destruction.

The cause of IMI antagonism of PIP remains theoretical. One possible explanation may be the induction of a β-lactamase which is capable of destroying PIP but not IMI. However, other factors, such as competition for binding sites, cannot be excluded from consideration. This could be especially true for the two strains in which antagonism occurred and β-lactamase activity was either below the limits of detection or not induced.

The combination of IMI and PIP theoretically augments the antipseudomonal activity of each antimicrobial agent and provides antistaphylococcal activity and greater anti-Klebsiella activity. However, this study demonstrates that low concentrations of IMI actually antagonize the in vitro activity of PIP against P. aeruginosa. The antagonism occurs irrespective of the susceptibility of the organism to aminoglycosides.

Our findings extend an earlier observation of IMI antagonism of second- and third-generation cephalosporins in Aeromonas hydrophila and Serratia marcescens (11); Miller et al. were able to demonstrate IMI induction of β-lactamase in S. marcescens but not in A. hydrophila. Thus, we provide further evidence that the interactions of β-lactam antibiotics and β-lactamase are more complex than originally expected (16, 17). The use of an agent such as IMI combined with a penicillin such as PIP in clinical settings should be approached with caution.

LITERATURE CITED