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Short Communication

In Vitro Combination Effects of Aztreonam and Aminoglycoside against Multidrug-Resistant *Pseudomonas aeruginosa* in Japan

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SUMMARY: The aim of this study was to evaluate the in vitro combination effects of aztreonam (AZT) and aminoglycosides against multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains in Japan. We investigated 47 MDR *P. aeruginosa* strains collected from 8 facilities. We selected the aminoglycosides amikacin (AMK), gentamicin (GM), and arbekacin (ABK) to examine their effects when combined with AZT using the checkerboard method. Of the 47 MDR *P. aeruginosa* strains, 41 tested positive for metallo-β-lactamase (MBL). In all combinations, aminoglycosides decreased the minimum inhibitory concentrations of AZT in a dose-dependent manner, and there was no apparent antagonism. The combination effects were scored on a scale of 0 to 4, and statistical analysis was performed using the Wilcoxon signed-rank test. In all 47 strains, AZT + ABK (mean score, 2.02) had the highest score, followed by AZT + AMK (1.68) and AZT + GM (1.38) (ABK versus GM, $P < 0.0001$). In 41 MBL-positive strains, AZT + ABK (mean score, 2.05) had the highest score, followed by AZT + AMK (1.56) and AZT + GM (1.37) (ABK versus AMK, $P = 0.02$, and ABK versus GM, $P < 0.0001$). AZT + ABK was the most promising combination regimen against MDR *P. aeruginosa* strains; the other promising combinations were AZT + AMK and AZT + GM.

Pseudomonas aeruginosa is a clinically significant Gram-negative rod and an important cause of hospital-acquired infection, particularly in immunocompromised patients. Multidrug-resistant (MDR) *P. aeruginosa* is emerging as a serious problem in clinical settings worldwide. Since intravenous colistin is not available in Japan, combination therapy is required. Tateda et al. have suggested the usefulness of a ''Break-point Checkerboard Plate'' to screen appropriate antibiotic combinations against MDR *P. aeruginosa* (1). A ''Break-point Checkerboard Plate'' is used to evaluate the effect of combination therapy with reference to the breakpoint concentration, which correlates with clinical efficacy. It allows for simultaneous evaluation of the effect of combination antimicrobial therapy using 8 clinically important agents (ceftazidime, piperacillin, imipenem [IMP], aztreonam [AZT], gentamicin [GM], ciprofloxacin, polymyxin B, and rifampicin) on a single plate. In Japan, a commercially available ''Break-point Checkerboard Plate'' is the BC plate EIKEN (Eiken Chemical, Tokyo, Japan), which includes amikacin (AMK), meropenem, and colistin instead of GM, IMP, and polymyxin B. IMP-type metallo-β-lactamase (MBL) production is frequently observed in highly resistant *P. aeruginosa* strains isolated in Japan (2). MBL-producing *P. aeruginosa* strains often remain susceptible to monobactams (3,4). Our previous study also demonstrated that the combination of AZT and aminoglycoside was often effective against *P. aeruginosa*, both in vitro and in vivo (5). In this study, we investigated the in vitro effects of a combination of AZT and aminoglyco-

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side against MDR *P. aeruginosa* strains collected from multiple centers.

MDR *P. aeruginosa* was defined as *P. aeruginosa* resistant to aminoglycosides (minimum inhibitory concentration [MIC] of AMK $\geq 32\,\mu g/mL$), carbapenems (MIC of IMP $\geq 16\,\mu g/mL$), and fluoroquinolones (MIC of ciprofloxacin $\geq 4\,\mu g/mL$).

Between 2003 and 2006, 47 MDR *P. aeruginosa* strains were collected by the ABX Combination Therapy Study Group (ACTs) from unrelated patients under treatment at 8 facilities throughout Japan. The MDR *P. aeruginosa* strains were obtained from the urinary tract in 24 patients, blood in 6, respiratory tract in 6, and other sites in 4. The survey did not indicate the source of the remaining 7 strains. AMK, GM, and arbekacin (ABK) were selected as aminoglycosides to be combined with AZT using the checkerboard method. The Clinical and Laboratory Standards Institute recommended standards of antibiotic susceptibility testing were applied in this study (6,7). MBL was detected using the sodium mercaptoacetate (SMA) disc method, employing SMA, ceftazidime, and IMP disks.

The clinically therapeutic MIC for AZT was 16 $\mu g/mL$ (intermediate). The combination of AZT and each aminoglycoside was defined as effective at the following concentrations: 16 $\mu g/mL$ AZT (intermediate) + 32 $\mu g/mL$ AMK (intermediate), 16 $\mu g/mL$ AZT (intermediate) + 8 $\mu g/mL$ GM (intermediate), and 16 $\mu g/mL$ AZT (intermediate) + 8 $\mu g/mL$ ABK (defined as intermediate). Since the breakpoint of ABK against *P. aeruginosa* was not defined, the GM criterion was used as an alternative (7). Combination effects were scored and evaluated. In this study, scoring was performed using a 0–4 scale to evaluate the combination effects with reference to the breakpoint concentration established by the ''Break-point Checkerboard Plate'' correlated with clinical efficacy. A score of 4 (inhibited bacterial growth in the following combination: AZT [susceptible] + aminoglycoside [susceptible]) indicated the most promising combined effect, while a score of 0 (bacterial growth not inhibited even by the following combination: AZT [intermediate] + aminoglycoside [intermediate]) indicated that there was no combination effect (Fig. 1). Statistical analysis was performed using the Wilcoxon signed-rank test. A P value < 0.05 was considered significant. All analyses were conducted using SPSS (version 11.0 for Windows; SPSS Inc., Chicago, Ill., USA).

Of the 47 MDR *P. aeruginosa* strains, 41 were MBL positive and 6 were negative. The MICs of AZT as a single agent were as follows: 8 $\mu g/mL$ (2 strains), 32 $\mu g/mL$ (18 strains), 64 $\mu g/mL$ (18 strains), and > 128 $\mu g/mL$ (9 strains). The clinically therapeutic MIC of AZT was 16 $\mu g/mL$ (intermediate). AZT as a single agent achieved an effect that is likely to be clinically significant in only 4% of strains (2 of 47 strains). In all combinations, aminoglycoside decreased the MICs of AZT in a dose-dependent manner, and there was no apparent antagonism. The combinations of AZT and aminoglycosides required to achieve these effects were as follows: 16 $\mu g/mL$ AZT + 32 $\mu g/mL$ AMK (77%, 36 of 47 strains), 16 $\mu g/mL$ AZT + 8 $\mu g/mL$ GM (43%, 20 of 47 strains), and 16 $\mu g/mL$ AZT + 8 $\mu g/mL$ ABK (79%, 37 of 47 strains).

In addition, the combination effects were scored and evaluated as described above. For the combination effect score, AZT + ABK (mean score, 2.02) was the highest, followed by those of AZT + AMK (mean score, 1.68) and AZT + GM (mean score, 1.38) in all 47 strains. The combined effect of AZT + ABK was significantly higher than that of AZT + GM ($P < 0.0001$) (Fig. 2). The combination effects on 41 MBL-positive strains were highest for AZT + ABK (mean score, 2.05), followed by AZT + AMK (mean score, 1.56) and

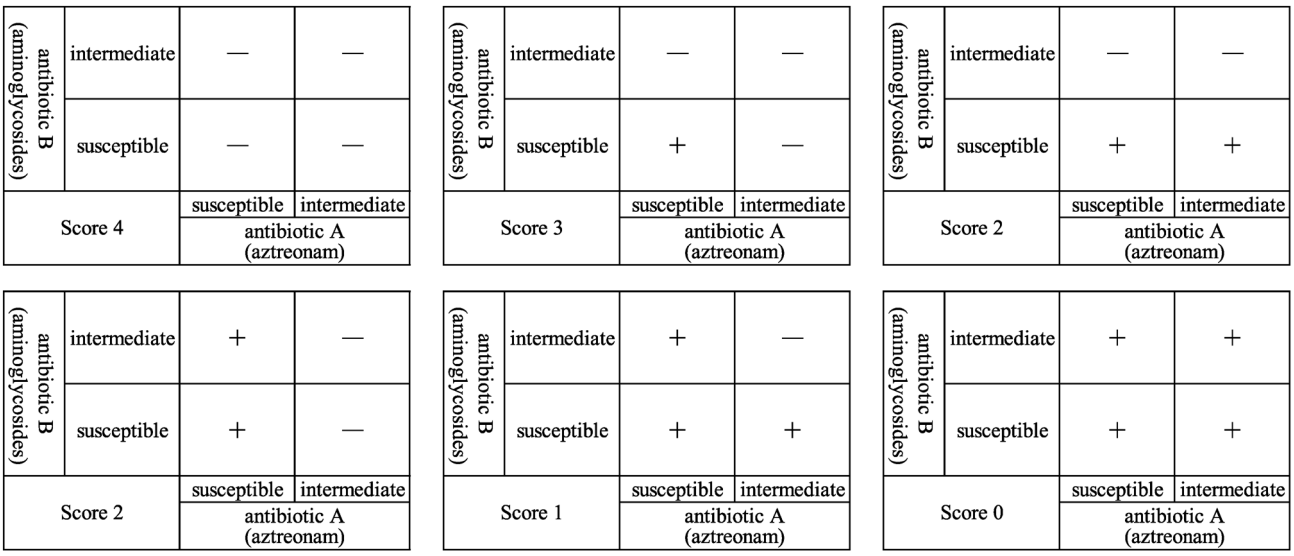

Fig. 1. Combination effects were scored and evaluated. Minus signs indicate each drug combination concentration that inhibited bacterial growth. Scoring was performed using a scale from 0 to 4 in this study. Score 4 (inhibited bacterial growth in the following combination, aztreonam [AZT]: susceptible + aminoglycoside: susceptible) indicated the most promising combined effect, while score 0 (bacterial growth not inhibited even by the following combination, AZT: intermediate + aminoglycoside: intermediate) indicated that there was no combination effect.

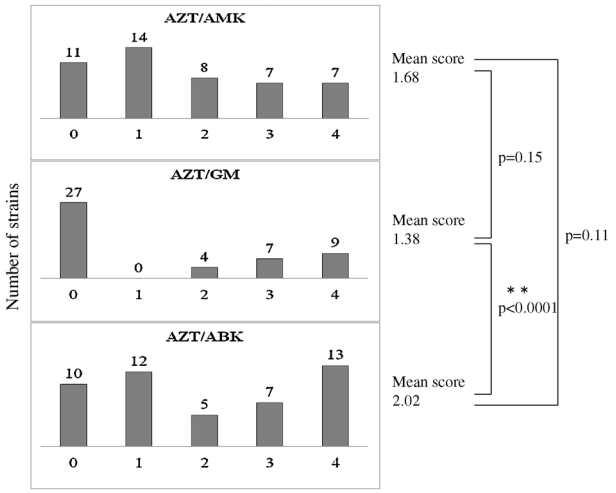

Fig. 2. Scoring of combination effects for each drug combination against all 47 multidrug-resistant (MDR) *Pseudomonas aeruginosa* strains. AZT, aztreonam; AMK, amikacin; GM, gentamicin; ABK, arbekacin.

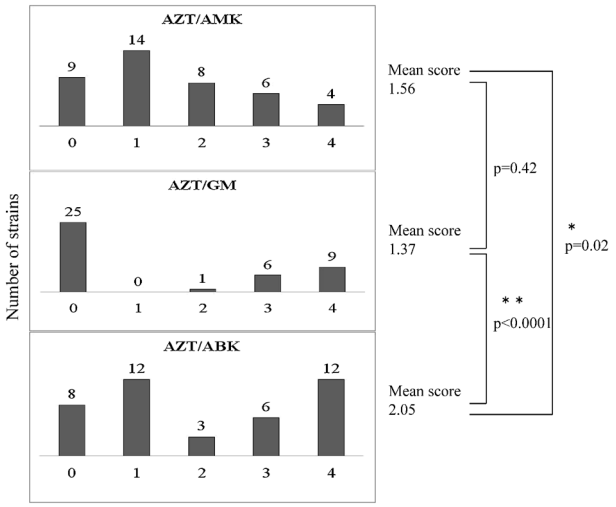

Fig. 3. Scoring of combination effects for each drug combination against 41 metallo-β-lactamase (MBL)-positive MDR *P. aeruginosa* strains.

AZT + GM (mean score, 1.37). The combined effect of AZT + ABK was significantly higher than that of AZT + GM ($P < 0.0001$) and AZT + AMK ($P = 0.02$) (Fig. 3).

In Japan, the production of MBL is often implicated in the high-level resistance of *P. aeruginosa*. It has been reported that IMP is encoded by the bla_{IMP} gene on an integron (2). In this study, out of the 47 MDR *P. aeruginosa* strains, 41 were MBL positive (18 strains for IMP-1, 10 for IMP-7, 12 for IMP-10, and 1 for VIM-2); 6 strains were MBL negative, and most MBL-producing MDR *P. aeruginosa* strains were the IMP type, as previously reported (2). PCR detection of various MBLs was performed by Hiroshi Kataoka, and this information was received as a personal communication.

Strains producing MBLs often remain susceptible to monobactams. However, AZT as a single agent achieved a clinical effect in only 4% of the strains (2 of 47 strains). AZT and aminoglycosides in combination were associated with a high probability of achieving a clinical effect. In addition, there was no antagonism. Therefore, the combination of AZT and aminoglycosides seems to be promising. This regimen may provide an effective second line of therapy for patients in whom intravenous colistin cannot be used. Our study was conducted to evaluate which aminoglycoside was appropriate to be used in combination with AZT. With respect to the combination effects, AZT + ABK showed the highest scores, followed by AZT + AMK and AZT + GM. Statistical analysis indicated that the combined effect of AZT + ABK was significantly higher than that of AZT + GM in all 47 strains.

In Japan, studies have shown that the mechanism most frequently underlying resistance to aminoglycosides was inactivation of the antibacterial agent by aminoglycoside-modifying enzymes (8,9). Other known mechanisms include the methylation of 16S rRNA (10) and increased expression of drug-efflux pumps (11). ABK, the semisynthetic aminoglycoside used in clinical settings in Japan, is effective against Gram-negative bacilli, including *P. aeruginosa*, as well as methicillin-resistant *Staphylococcus aureus* (MRSA) (12). ABK is stable against most aminoglycoside-modifying enzymes, and only the bifunctional enzyme AAC(6′)/APH(2″) is known to have low or moderate resistance to ABK (13,14). Therefore, ABK, similar to AMK, is regarded a strong candidate for combination use with AZT. Further studies are needed to analyze MDR *P. aeruginosa* strains from the perspective of aminoglycoside-modifying enzymes.

In conclusion, our study showed that AZT + ABK was the most promising combination regimen against MDR *P. aeruginosa* strains; the other promising regimens were AZT + AMK and AZT + GM.

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Conflict of interest None to declare.

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