



# Evaluation of the Microbiological Efficacy of a Single 2-Gram Dose of Extended-Release Azithromycin by Population Pharmacokinetics and Simulation in Japanese Patients with Gonococcal Urethritis

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**ABSTRACT** The objective of this study was to analyze the relationship between the pharmacokinetic (PK)/pharmacodynamic (PD) parameters of a single 2-g dose of extended-release formulation of azithromycin (AZM-SR) and its microbiological efficacy against gonococcal urethritis. Fifty male patients with gonococcal urethritis were enrolled in this study. In 36 patients, the plasma AZM concentrations were measured using liquid chromatography-tandem mass spectrometry, the AZM MIC values for the *Neisseria gonorrhoeae* isolates were determined, and the microbiological outcomes were assessed. AZM-SR monotherapy eradicated *N. gonorrhoeae* in 30 (83%) of the 36 patients. AZM MICs ranged from 0.03 to 2 mg/liter. The mean value of the area under the concentration-time curve (AUC), estimated by population PK analysis using a two-compartment model, was 20.8 mg · h/liter. Logistic regression analysis showed that the PK/PD target value required to predict an *N. gonorrhoeae* eradication rate of  $\geq 95\%$  was a calculated AUC/MIC of  $\geq 59.5$ . The AUC/MIC value was significantly higher in patients who achieved microbiological cure than in patients who achieved microbiological failure. Monte Carlo simulation using this MIC distribution revealed that the probability that AZM-SR monotherapy would produce an AUC/MIC exceeding the AUC/MIC target of 59.5 was 47%. Furthermore, the MIC distribution for strains isolated in this study was mostly consistent with that for strains currently circulating in Japan. In conclusion, in Japan, AZM-SR monotherapy may not be effective against gonococcal urethritis. Therefore, use of a single 2-g dose of AZM-SR either with or without other antibiotics could be an option to treat gonococcal urethritis if patients are allergic to ceftriaxone and spectinomycin or are diagnosed to be infected with an AZM-sensitive strain.

**KEYWORDS** azithromycin, gonococcal urethritis, PK/PD, *Neisseria gonorrhoeae*, Monte Carlo simulation

Gonorrhea is one of the most common sexually transmitted infections. Although several antimicrobial agents have been recommended for the treatment of gonorrhea, the introduction of new agents has repeatedly led to the development of resistance to these agents in clinical strains of *Neisseria gonorrhoeae* (1). Fluoroquinolones and oral third-generation cephalosporins were recommended as first-line agents for the treatment of gonorrhea after the emergence and spread of penicillin-resistant

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*N. gonorrhoeae* (2). However, these agents are no longer used to treat gonorrhea because of increases in the numbers of clinical strains exhibiting resistance to them (3). Although ceftriaxone monotherapy has been recommended as another first-line treatment (4), some clinical strains of *N. gonorrhoeae* exhibiting decreased susceptibility to ceftriaxone have already emerged (5, 6). Currently, we cannot expect the rapid development of promising new agents to treat gonorrhea caused by antimicrobial-resistant *N. gonorrhoeae* strains; therefore, it is necessary to manipulate existing agents to enhance their efficacy toward gonorrhea and prevent the emergence and spread of antimicrobial-resistant *N. gonorrhoeae* strains.

Azithromycin (AZM), a macrolide antibiotic containing a 15-member azalactone ring, exhibits antimicrobial activity against *N. gonorrhoeae*. Some studies report a good efficacy of a single 1-g dose of conventional AZM against gonorrhea (7, 8). However, since the early 1990s, clinical strains exhibiting decreased susceptibility to AZM have been observed in several countries worldwide (9–11). Although a single 2-g dose of an immediate-release formulation of AZM (conventional AZM) remains effective against uncomplicated gonorrhea (12), this monotherapy could cause an unacceptable rate of adverse events, particularly gastrointestinal upset. Recently, a single 2-g dose of a microsphere-based extended-release formulation of AZM (AZM-SR) was developed and was found to be effective against *N. gonorrhoeae* (13–15), and the frequency of adverse events also decreased compared with that achieved with a single 2-g dose of conventional AZM (12, 15–18). Therefore, a single 2-g dose of AZM-SR is considered useful for the treatment of gonorrhea. Generally, the efficacy of antimicrobial agents is known to be correlated with their pharmacokinetic (PK)/pharmacodynamic (PD) properties. With regard to AZM, previous studies have reported that the probability of success according to the clinical and bacteriological responses of respiratory tract infections is positively associated with the area under the concentration-time curve (AUC)/MIC ratio (19). However, the PK/PD properties of AZM-SR in Japanese male patients with gonococcal urethritis have been unclear. To find the target value of the PK/PD parameters positively associated with the efficacy of AZM-SR, we analyzed the relationship between the PK/PD properties and the efficacy of a single 2-g dose of AZM-SR for the treatment of gonococcal urethritis. Additionally, we explored the prospects of the regimen as an alternative treatment for gonorrhea caused by currently circulating clinical strains of *N. gonorrhoeae* using Monte Carlo simulation.

## RESULTS

**Efficacy of AZM-SR.** Overall, 13 patients who did not revisit the clinic for further examination and 1 additional patient were excluded because of a lack of laboratory data. Of the remaining 36 patients, 30 (83%) were judged to be microbiologically cured after treatment with AZM-SR. There was no significant difference in the background characteristics between the patients who achieved a microbiological cure and those who achieved a microbiological failure (Table 1).

**MIC.** The AZM MICs of the strains collected from the 36 patients ranged from 0.03 to 2 mg/liter. The distribution of the AZM MICs against the *N. gonorrhoeae* strains is shown in Table 2. All cases of microbiological failure were caused by strains with an AZM MIC of  $\geq 0.5$  mg/liter.

**Determination of plasma AZM concentrations.** Plasma AZM concentrations were determined using our established method without interference from matrix products (Fig. 1A). The calibration curve was linear from 10 to 300 ng/ml, with correlation coefficients being  $>0.99$ . All validations, including intraday and interday accuracies and stabilities, met the criteria (within  $\pm 15\%$ ). The rates of AZM recovery ranged from 83.6% to 99.9%. The limits of quantification and detection were 2.3 and 0.7 ng/ml, respectively (signal-to-noise ratios, 10 and 3, respectively).

**PK modeling and model development.** The population PK model was based on that described in a previous report (19), and a two-compartment model with age and body weight as covariates was chosen as the final model. Goodness-of-fit plots for observed versus population predicted concentrations (PRED), observed versus individ-

**TABLE 1** Background characteristics of subjects treated for gonococcal urethritis with a single 2-g dose of AZM-SR<sup>c</sup>

Characteristic	Value(s) for subjects who achieved microbiological:				
	Cure ( <i>n</i> = 30)		Failure ( <i>n</i> = 6)		<i>P</i> value <sup>b</sup>
	Mean ± SD	Range	Mean ± SD	Range	
Age (yr)	31.5 ± 8.2	20–54	30.7 ± 9.4	24–45	0.83
Wt (kg)	68.5 ± 11.3	55–100	67.3 ± 13.8	47–80	0.82
WBC count (no./μl)	6,677 ± 2,457	3,800–16,500	7,083 ± 1,938	4,700–8,800	0.71
AST concn (IU/liter)	19.8 ± 10.7	12–72	20.3 ± 6.9	12–31	0.91
ALT concn (IU/liter)	24.0 ± 28.5	9–168	21.5 ± 10.7	8–39	0.84
ALP concn (U/liter)	234.0 ± 65.5	159–462	246.8 ± 58.8	139–315	0.66
T-bil concn (mg/dl)	0.63 ± 0.23	0.1–1.1	0.67 ± 0.25	0.3–0.9	0.70
Cr concn (mg/dl)	0.83 ± 0.09	0.65–0.98	0.86 ± 0.10	0.71–0.94	0.38
CL <sub>CR</sub> <sup>a</sup> (ml/min)	125.6 ± 22.7	95.1–187.1	117.8 ± 17.9	99.0–143.2	0.44

<sup>a</sup>CL<sub>CR</sub> was calculated using the Cockcroft-Gault equation.

<sup>b</sup>Determined by Student's *t* test.

<sup>c</sup>The data are for 36 subjects. WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; T-bil, total bilirubin; Cr, creatinine; CL<sub>CR</sub>, creatinine clearance.

ual predicted concentrations (IPRED), and conditional weighted residuals (CWRES) versus population predicted concentrations are presented in Fig. 1B to D. The plots of the observed versus the predicted concentrations suggested a slight overestimation at higher concentrations and indicated that the model described the data well with no systematic bias.

**PK/PD analysis.** AUC values were calculated using population PK parameters and ranged from 5.7 to 58.1 mg · h/liter, with the mean value being 20.8 mg · h/liter. AUC values did not significantly differ between the patients with microbiological cure and those with microbiological failure (Fig. 2A). The mean MIC was 0.5 mg/liter (range, 0.03 to 2.0 mg/liter). In the treatment failure group, all MICs were >0.5 mg/liter. As shown in Fig. 2B, the AZM MICs in the microbiologically cured patients were significantly lower than those in patients exhibiting treatment failure. The mean AUC/MIC ratio was 68.9 (range, 5.9 to 324.9), and the AUC/MIC ratios for patients who failed treatment were significantly higher than those for patients who were cured (Fig. 2C).

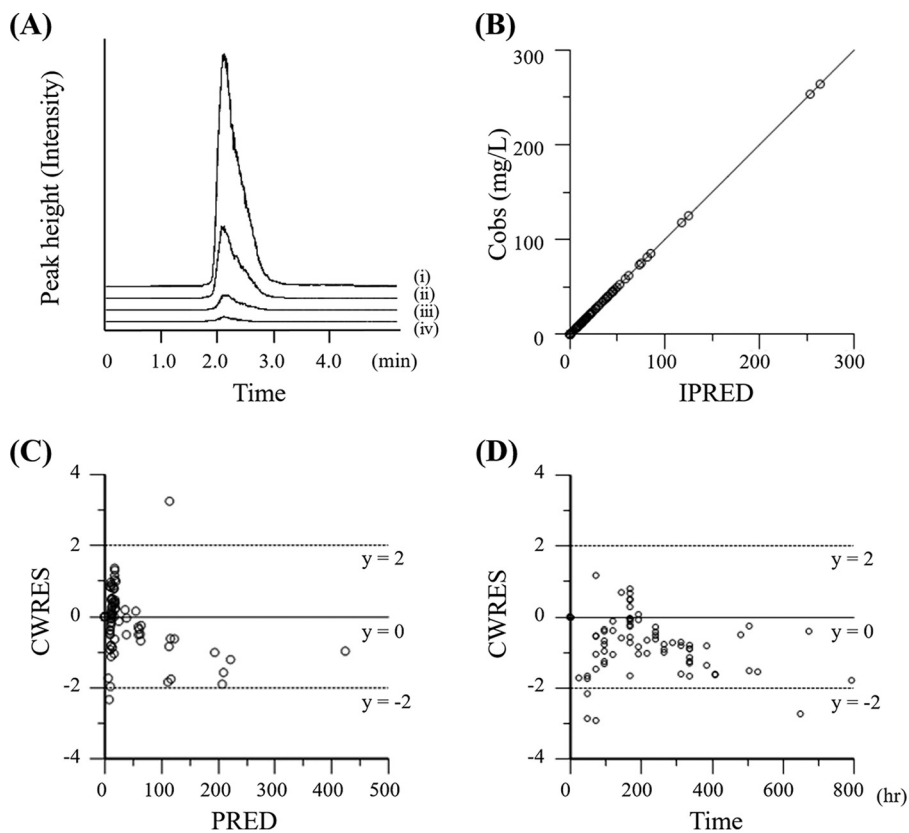
The probability of the correct classification as treatment cures or failures was calculated to be 83% for AUC and 86% for AUC/MIC. The probability of microbiological cure (POC) from the AUC/MIC was computed using a logistic function, where  $POC = \{1/[1 + e^{(0.798 - 0.063 \times AUC/MIC)}]\}$ , and then the target value of the AUC/MIC required to predict a POC of ≥95% was determined to be 59.5 (Fig. 3).

Although AZM-SR provided high a probability of target attainment (PTA; >90%) at an MIC of ≤0.125 mg/liter, the PTA for MICs of 0.25 and 0.5 mg/liter decreased to 66% and 22%, respectively (Fig. 4A). In our patient population, the PTA obtained with 2 g AZM was almost similar to that obtained with AZM-SR; however, a satisfactory PTA was

**TABLE 2** Microbiological efficacy of a single 2-g dose of AZM-SR for gonococcal urethritis treatment

MIC (mg/liter) of AZM	No. of isolates from subjects who achieved microbiological <sup>a</sup> :	
	Cure	Failure
0.03	1	0
0.06	1	0
0.125	0	0
0.25	15	0
0.5	11	3
1	2	1
2	0	2
Total	30	6

<sup>a</sup>The judgments were performed microbiologically.

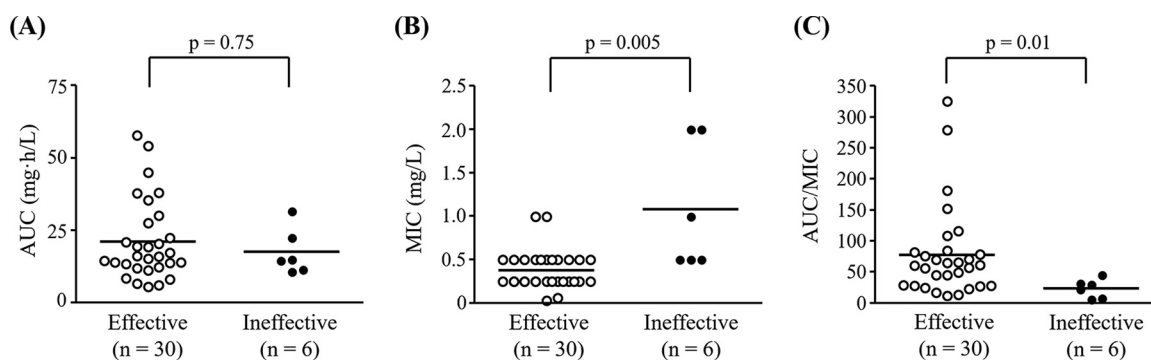


**FIG 1** Typical AZM LC-MS/MS chromatograms and goodness-of-fit plots for the final PK model. (A) Chromatograms showing the results for plasma AZM concentrations of 300 (i), 100 (ii), 30 (iii), and 10 (iv) ng/ml; (B) scatter plots of observed plasma AZM concentrations (Cobs) versus individual model predictions (IPRED); (C) conditional weighted residuals (CWRES) versus population predictions (PRED); (D) CWRES versus time after dose.

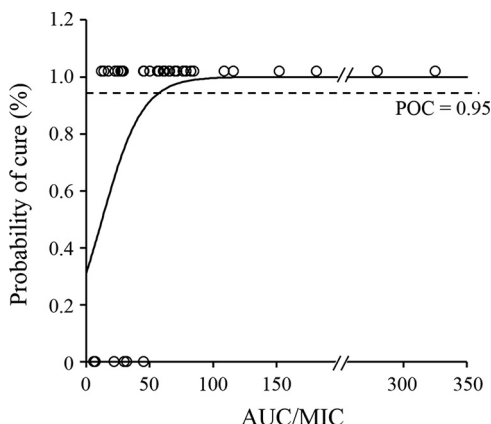
not obtained with 1 g AZM, even at an MIC of  $>0.125$  mg/liter (PTA  $< 55\%$ ). Furthermore, as a result of the Monte Carlo simulation according to the MIC distribution in the subjects in the present study, the cumulative fractions of responses (CFRs) of three AZM monotherapies at the target AUC/MIC value of 59.5 were calculated to be 47% for AZM-SR, 41% for 2 g AZM, and 10% for 1 g AZM (Fig. 4B).

## DISCUSSION

In the present study with Japanese male patients with gonococcal urethritis, we established the way to measure plasma AZM concentrations, with good validation.



**FIG 2** Comparison of AZM PK parameters (AUC, MIC, and AUC/MIC) in 36 patients with gonococcal urethritis. (A) AUC; (B) MIC; (C) AUC/MIC. Each line represents the median. *P* values were calculated by the Mann-Whitney U test.

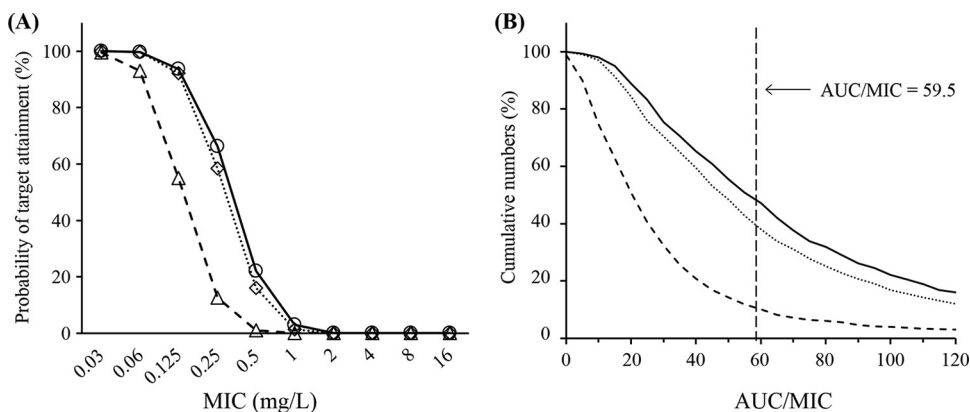


**FIG 3** Probability of cure following AZM-SR administration. The solid line shows the logistic regression curve, and open circles represent clinical data.

Moreover, we calculated the PK parameters by the use of population PK and found that AUC/MIC ratios were related to microbiological efficacy. To our knowledge, this is the first study showing the PK parameters associated with microbiological efficacy and the target values of the PK parameters associated with microbiological efficacy.

A simple liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the measurement of AZM was established. In this method, solid-phase extraction (SPE) instead of liquid-liquid extraction was used, unlike in previous studies (20, 21). Plasma AZM concentrations were measured within 5 min, with good validation. Moreover, the measurement range was sufficient to measure the trough levels; therefore, this method was considered applicable to the PK analysis of AZM.

Estimation of the values of PK parameters optimally requires large numbers of blood samples; however, it is difficult and clinically invasive to collect multiple samples with various concentrations from patients. Moreover, iterative analysis and the development of new population models with every addition of patient data are discouraged. Therefore, the present analyses of the PK parameters were performed using the two-compartment model developed by Muto et al. (19). This model includes age and body weight as covariates, and our data showed a sufficient goodness of fit, indicating its wide applicability to the analysis of PK parameters for AZM, regardless of the disorder. Our estimated AUC value was slightly higher than that reported from a previous study of patients with respiratory tract infections (19), potentially reflecting the relatively small number of patients, with only two plasma samples from each



**FIG 4** Probability of target attainment achieved with 2 g AZM-SR, 2 g AZM, and 1 g AZM at each MIC and the cumulative probability of AUC/MIC distributions calculated by Monte Carlo simulation. (A) The plots show data for 2 g AZM-SR (open circles, solid lines), 1 g AZM (open triangles, dashed lines), and 2 g AZM (open diamonds, dotted lines). (B) Each line represents 2 g AZM-SR (solid lines), 1 g AZM (dashed lines), and 2 g AZM (dotted lines).

patient being tested. These limitations warrant future studies with larger numbers of samples and patients.

Here, we attempted to elucidate the relationship between PK parameters and microbiological efficacy in Japanese male patients with gonococcal urethritis treated with a single 2-g dose of AZM-SR. As a result of determination of the PK parameters reflecting the effectiveness of AZM, an AUC/MIC of 59.5 was calculated to be the target value for predicting the rate of *N. gonorrhoeae* eradication ( $\geq 95\%$ ) in males with gonococcal urethritis. However, a Monte Carlo simulation using the distribution of MICs for the *N. gonorrhoeae* strains recovered in the present study indicated that the CFR of AZM-SR monotherapy required to exceed an AUC/MIC of 59.5 is 47%. The recent distributions of MICs for *N. gonorrhoeae* strains in Japan were reported to have median values of 0.125 and 0.25 mg/liter in 2002 and 2014, respectively (22), whereas in this study (conducted with isolates collected from 2014 to 2016), the median MIC was 0.5 mg/liter. The distribution of MICs for the strains isolated in our study was mostly consistent with that previously reported (22), suggesting that the strains tested are typical of those circulating in Japan. Furthermore, the proportion of strains with MICs of  $\geq 1$  mg/liter (the breakpoint of the AZM MIC) significantly increased from 6.4% in 2014 to 24.1% in 2015 (22). In this study, the breakpoint AZM MIC associated with microbiological failure was 0.5 mg/liter. These findings indicate that the susceptibility of *N. gonorrhoeae* isolates to AZM is decreasing; therefore, there is concern that treatment failures will increase. Taken together with the findings described in previous reports (22, 23), our results suggest that adequate therapeutic effects cannot be obtained, even upon AZM-SR administration. Although descriptions of the effectiveness of AZM for the treatment of gonococcal urethritis have been added to the guidelines revised in 2016 (4), the decreasing susceptibility of *N. gonorrhoeae* to AZM has been reported in Japan (22, 24). In our current study, the microbiological efficacy of a single 2-g dose of AZM-SR was 83.3%, which was lower than that previously reported (93.8%) (14). Additionally, gonococcal strains exhibiting high levels of AZM resistance (MICs  $\geq 256$  mg/liter) have been reported in an increasing number of countries (10, 11, 25–27). The failure of treatment of gonorrhea with a single 2-g dose of AZM-SR (28) and conventional AZM (23) has also been reported; therefore, there is concern that microbiological failures will increase. These reports suggest that the use of AZM probably could induce AZM resistance. Therefore, AZM monotherapy cannot be recommended as a first-line treatment for gonorrhea; this is consistent with the results obtained by Yasuda et al. (22). Therefore, a single use of ASM-SR should be restricted to limited cases (e.g., patients allergic to ceftriaxone).

The Monte Carlo simulation performed in this study showed that the CFRs of single 1-g and 2-g doses of conventional AZM and a single 2-g dose of AZM-SR were 10%, 41%, and 47%, respectively. These results suggest that a single use of AZM would be ineffective for the treatment of gonorrhea. The guidelines of other countries (29–32) recommend the use of a combination of AZM with other antibiotics for gonorrhea treatment. Most countries, with the exception of Canada, recommend only intramuscular ceftriaxone (250 or 500 mg as a single dose) plus oral AZM (1 to 2 g as a single dose) as a first-line treatment (29–33). In Canada, oral cefixime (800 mg as a single dose) plus oral AZM (1 g as a single dose) is also recommended as a first-line therapy (33). A novel dual antimicrobial therapy has also been evaluated: intramuscular gentamicin (240 mg as a single dose) plus oral AZM (2 g as a single dose) and oral gemifloxacin (320 mg as a single dose) plus oral AZM (2 g as a single dose) (34). This treatment with agents with different mechanisms of action may hinder resistance development (35).

With regard to adverse events, particularly gastrointestinal symptoms, a single use of 1-g and 2-g doses of conventional AZM and a 2-g dose of AZM-SR caused adverse events in  $<10\%$  (8, 36), 24.4% to 35.3% (12, 18), and 17.9% to 21.5% (4, 15) of Caucasian patients, respectively. These results show that a higher dose of AZM would more frequently induce adverse events. In cases in which a single 2-g dose of AZM-SR was used, gastrointestinal symptoms were more likely to occur in Japanese patients (approximately 30%) (13, 14). However, the symptoms were mostly temporary and were

resolved within a day when the patients took AZM-SR (13, 14). Taken together, when a single 2-g dose of AZM-SR is administered with or without other antibiotics to Japanese patients, the patients should be carefully monitored for gastrointestinal adverse events, particularly within 1 day after AZM-SR administration.

In conclusion, assessment of the microbiological responses to AZM treatment of gonococcal urethritis can be performed using the AUC/MIC ratio. Our present results suggest that a single 2-g dose of AZM-SR may not be effective for the treatment of gonococcal urethritis in Japan. Therefore, AZM-SR monotherapy should not be used for the treatment of gonococcal urethritis. Currently, in Japan, the guideline recommends monotherapy with intramuscular ceftriaxone (1 g as a single dose) or intramuscular spectinomycin (2 g as a single dose) as a first-line treatment of gonococcal urethritis (4). So far, no reports have indicated the failure of treatment with these two drugs. Thus, if patients are allergic to ceftriaxone and spectinomycin and/or are diagnosed to be infected with AZM-sensitive *N. gonorrhoeae* isolates, either the combined use of a single 2-g dose of AZM-SR and other antibiotics or monotherapy using a higher dose of AZM-SR could be an option instead of monotherapy using a single 2-g dose of AZM-SR to treat gonococcal urethritis.

## MATERIALS AND METHODS

**Subjects.** We enrolled 50 Japanese male patients who visited iClinic between May 2014 and January 2016 and were diagnosed with gonococcal urethritis. All patients provided written informed consent, which was approved by the ethics committees of Gifu University (reference number 26-10). *N. gonorrhoeae* was isolated from these patients by culture of a swab specimen from the urethra and stored at  $-70^{\circ}\text{C}$ . Patients were administered a single 2-g dose of AZM-SR. Clinical data, including age, body weight, and laboratory values, such as white blood cell count, the levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin, and creatinine, and creatinine clearance, were also recorded.

**Microbiological outcomes.** The microbiological efficacy of AZM-SR against gonococcal urethritis was evaluated by use of the Aptima Combo 2 test for *N. gonorrhoeae* (Hologic, San Diego, CA, USA) and culture of a specimen collected from each patient by a clinician on every visit day between 5 and 41 days after AZM-SR administration, except for one patient who revisited 2 days after the first visit. If a positive judgment at the initial examination did not become a negative judgment by 14 days after administration, the treatment was defined to be ineffective.

**Antimicrobial susceptibility testing.** The AZM MICs for the pretreatment strains of *N. gonorrhoeae* which were isolated from the urethra of the patients and survived storage were determined by an agar dilution method authorized by the Clinical and Laboratory Standards Institute (37), as previously described (22).

**Determination of plasma AZM concentrations.** Plasma samples were collected twice, once between days 2 and 8 and then between days 7 and 33, from every patient and stored at  $-30^{\circ}\text{C}$  until use in AUC/MIC testing. The plasma AZM concentrations were measured using LC-MS/MS, as previously reported (38), with slight modifications. Briefly, an LC-MS/MS system (a Waters 2695 separations module and a Waters Quattro micro-mass spectrometer; Waters Corporation, Milford, MA, USA) with a Cadenza CD-C<sub>18</sub> column (2 mm by 100 mm; particle size, 3  $\mu\text{m}$ ; Imtakt Corporation, Kyoto, Japan) was set at  $40^{\circ}\text{C}$ . The mobile phase comprised 50 mM aqueous ammonium acetate and acetonitrile. The mass spectrometer was operated in the positive ionization mode to monitor transition ions  $m/z$  749.5  $\rightarrow$  157.9 for AZM and  $m/z$  752.6  $\rightarrow$  594.4 for the internal standard (IS; AZM-*d*3; Cosmo Bio Co., Ltd., Tokyo, Japan). The optimal parameters for MS were as follows: capillary voltage, 1.2 kV; cone voltage, 35 kV; source temperature,  $110^{\circ}\text{C}$ ; and desolvation temperature,  $350^{\circ}\text{C}$ . Nitrogen gas was used for desolvation and as a cone gas at flow rates of 600 liters/h and 50 liters/h, respectively. All data were processed using a MassLynx system (Waters Corporation).

An SPE cleanup procedure was used to extract AZM from plasma (39). Briefly, 50- $\mu\text{l}$  aliquots of distilled water were added to 200- $\mu\text{l}$  patient plasma samples or blank plasma (Cosmo Bio Co., Ltd.) that was spiked with an AZM reference standard (Sigma-Aldrich, St. Louis, MO, USA) at 10, 30, 100, and 300 ng/ml. Subsequently, 50- $\mu\text{l}$  aliquots of the IS (1,000 ng/ml) were vigorously mixed and loaded onto Oasis HLB columns (Waters Corporation) equilibrated with 1 ml of methanol and distilled water. After washing three times with 2% formic acid, the analytes were eluted with 1 ml of methanol and evaporated to dryness using a gentle stream of nitrogen. The residues were then dissolved in 100  $\mu\text{l}$  of the mobile phase and sonicated at an ultrasonic frequency of 42 kHz for 10 min. Subsequently, the diluents were passed through Millex-LG filters (pore size, 0.2  $\mu\text{m}$ ; 4 mm; Merck, Darmstadt, Germany), and 15- $\mu\text{l}$  aliquots were injected into the LC-MS/MS system.

The selectivity, calibration curve, accuracy, intraday and interday precisions, recovery, and stability were validated according to Food and Drug Administration guidelines (40).

**Population PK analysis.** Population PK analysis of AZM-SR was performed using the Phoenix NLME program (v1.3; Certara, Princeton, NJ, USA). On the basis of the findings of a previous study (19), the population PK model was generated and evaluated using the nonparametric bootstrap and visual predictive check options.

**PK/PD analysis and simulation.** AUC was calculated using the PK parameters obtained from the population PK analysis. To explore the relationship between the PK/PD parameters and antimicrobial effects, the predictive values of the microbiological efficacies were calculated for the PK parameters, such as AUC, the maximum concentration ( $C_{max}$ ), the AUC/MIC ratio, and  $C_{max}/MIC$ . The POC was computed using logistic regression, and the PK/PD target values were defined as the threshold for a 95% POC (41).

Simulation of the AUC for 1,000 cases was performed using the PK data from this study for AZM-SR and from previous studies for 1-g and 2-g doses of AZM (19, 42, 43) and Phoenix NLME software (Certara). AUC and MIC data were combined by Monte Carlo simulation (Crystal Ball; Oracle Corporation, Redwood Shores, CA, USA) on the basis of the MIC distribution from this study. The PTA was examined using the target AUC/MIC value. The cumulative fractions of responses (CFRs) of three monotherapies, 2 g AZM-SR, 2 g AZM, and 1 g AZM, were calculated on the basis of the AUC/MIC values.

**Statistical analysis.** Descriptive statistics were performed for all continuous data, and data are presented as the means  $\pm$  standard deviations, unless specified otherwise. Comparisons of continuous data between groups were performed using Student's *t* test after confirming the normal distribution of the data. PK parameters between groups were compared using the Mann-Whitney U test, and a *P* value of  $<0.05$  was considered statistically significant. Logistic regression analyses were performed to identify univariate predictors of treatment success (i.e., microbiological cure). All statistical analyses were performed using SPSS software (IBM Corp., Armonk, NY, USA).

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