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Note

Clinical pharmacokinetics of oral azithromycin in epididymal tissue☆

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ABSTRACT

Objectives: *Chlamydia trachomatis* is one of the major pathogens causing acute epididymitis. Azithromycin (AZM) has a good efficacy against *C. trachomatis*; however, the ability of AZM to penetrate into human epididymal tissue has not yet been fully elucidated. Here, we examined the appropriate dosage of oral AZM for human epididymal tissue by site-specific pharmacokinetic/pharmacodynamic (PK/PD) analysis.

Methods: Patients with prostate cancer who underwent orchiectomy were included in this study. All patients received a 1-g dose of AZM before orchiectomy. Both epididymal tissue and blood samples were collected during surgery, and the drug concentrations were measured by high-performance liquid chromatography. All concentration-time data were analyzed with a three-compartment model with first-order absorption and elimination processes to simulate AZM concentrations in serum and epididymal tissue.

Results: A total of 10 patients were enrolled in the current study. For the observed values, the ratio of the epididymal concentration to the serum concentration was 5.13 ± 3.71 (mean ± standard deviation). For the simulated values, the maximum concentrations were 0.64 μg/mL at 2.42 h in serum and 1.96 μg/g at 4.10 h in epididymal tissue. The 24-h concentrations were 0.239 μg/mL in serum and 0.795 μg/g in epididymal tissue.

Conclusions: The penetration of oral AZM into human epididymal tissue was examined to assess the potential application of AZM for the treatment of acute epididymitis. Based on the previous reports mentioning drug-susceptibility of *C. trachomatis*, multiple doses of oral AZM 1 g would be recommended for epididymitis based on the site-specific PK/PD.

Acute epididymitis due to sexually transmitted organisms, particularly *Chlamydia trachomatis*, has become a major public health concern. Inappropriate treatment is associated with recurrent epididymitis, infertility, chronic pelvic pain, and testicular infarction [1]. Recently, the incidence of antimicrobial-resistant *C. trachomatis* isolates has reported [2]. Thus, appropriate dosing regimens determined using a pharmacokinetic/pharmacodynamic (PK/PD)-based approach for epididymitis due to *C. trachomatis* is urgently needed. Azithromycin (AZM), a macrolide antibiotic containing a 15-member azalactone ring, has good antimicrobial activity against *C. trachomatis* [3] and is one of the recommended antimicrobials for urethritis due to *C. trachomatis* by Japanese guidelines [4]. However, the use of AZM for acute epididymitis is not specifically recommended since tissue distribution characteristics of AZM and its PK/PD profiles in human epididymis have not been reported. In this study, we aimed to analyze the distribution of oral AZM in human epididymal tissue and to investigate whether penetration into the epididymis exceeded enough concentrations for previously reported minimum inhibitory concentration (MIC) for *C. trachomatis*.

Patients with prostate cancer who underwent orchiectomy for hormonal therapy at Okayama University Hospital and its related institutions in Japan between July 2012 and June 2014 were

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included in this study. All patients provided informed consent prior to enrollment. Patients with active urogenital infection, a history of treatment with antimicrobials within 1 week, or liver disfunction were excluded. Participants were administered a 1-g dose of AZM (ZITHROMAC® tablet; Pfizer Japan Inc., Japan) orally around 1 h before entering operation room for orchiectomy. Next, 0.5 g epididymal tissue was excised, and the surface was immediately cleaned lightly by physiological saline after surgery followed by wiping up and storing at −60 °C until analysis. Blood samples (3 mL) were collected immediately after right orchiectomy, immediately after left orchiectomy, and 1 h after the end of surgery. Serum (1.5 mL) was then collected and stored at −60 °C until measurement. Concentrations of AZM in serum and epididymal tissues were measured by high-performance liquid chromatography (HPLC), as previously reported [5,6], with modifications. Epididymal tissue samples (0.5 g) were homogenized with an overhead mixer in four volumes (w/v) of 50 mM phosphate buffer (pH 7.0) and centrifuged [7]. The tissue homogenate supernatants or serum samples (1 mL each) were extracted with diethyl ether (5 mL). After vortex-mixing and centrifugation, the upper aqueous layer was aspirated with a micro vacuum pump. The organic layer (4 mL) was directly transferred to a clean test tube without pipetting, and the organic solvent was evaporated to dryness. Next, the residue was reconstituted with 160 μL of 9-fluorenylmethyloxycarbonyl chloride (2.5 mg/mL in acetonitrile) and 40 μL of 100 mM borate buffer (pH 7.4). After vortex-mixing, the samples were kept at 50 °C for derivatization reaction (40 min) and stopped by adding 10 μL of 100 mM glycine solution. After 1 min, the sample mixture (40 μL) was then injected into the HPLC system. The HPLC system employed a phenyl column at 62 °C and detected AZM with a fluorescence detector at 260 nm for excitation and 315 nm for emission. The mobile phase consisted of a mixture of 50 mM phosphate buffer containing 0.2% triethylamine (pH 5.9) and methanol (29:71 [v/v]) at a flow rate of 1 mL/min. The quantification limits for AZM were 0.1 μg/mL and 0.2 μg/g in serum and epididymal tissue, respectively, and both calibration curves were linear up to 5 μg/mL and 10 μg/g, respectively. The interday and intraday accuracy and precision were within 10%. PK model analysis was performed with the MULTI program [8]. As a naive pooled data method, all concentration-time data for serum and epididymal tissue were fitted simultaneously to a three-compartment model with first-order absorption and elimination processes. The PK parameters were volumes of distribution for the central compartment (V_1, L), peripheral compartment (V_2, L), and epididymal compartment (V_3, L); clearance (Cl, L/h); bioavailability (F); absorption rate constant (Ka, h^{-1}); and transfer rate constants between the central and peripheral compartments (K_{12}, K_{21}; h^{-1}) and between the central and epididymal compartments (K_{13}, K_{31}; h^{-1}).

In total, 10 patients were enrolled in this study. None of the patients had active urogenital infection, a history of treatment with antimicrobials within 1 week, or liver dysfunction before surgery. The patients' background characteristics are shown in Table 1. Neither treatment-related adverse events nor postoperative urogenital infections were observed. Measurements of the mean AZM concentrations in serum and epididymal tissue were 0.47 ± 0.33 μg/mL and 1.68 ± 0.95 μg/g, respectively. The mean ratio of the epididymal concentration to serum concentration (E/S ratio) was 5.13 ± 3.71. The PK parameters of oral AZM were estimated as $V_1/F = 1228.0$ L, $V_2/F = 454.4$ L, $V_3/F = 90.8$ L, $Cl/F = 66.6$ L/h, $Ka = 3.73$ h^{-1}, $K_{12} = 0.0256$ h^{-1}, $K_{21} = 0.0693$ h^{-1}, $K_{13} = 0.274$ h^{-1}, and $K_{31} = 1.22$ h^{-1}. The simulated concentrations using these PK parameters fit the corresponding observed concentrations (Fig. 1). The simulated values were as follows: the maximum concentrations were 0.64 μg/mL at 2.42 h in serum and 1.96 μg/g at 4.10 h in epididymal tissue. The 24-h concentrations were 0.239 μg/mL in serum and 0.795 μg/g in epididymal tissue. The areas under the concentration-time curve for 24 h were 10.27 μg·h/mL in serum and 50.05 μg·h/g in epididymal tissue.

Table 1
Patient characteristics.

Patients	Age (yr)	AST (U/L)	ALT (U/L)	γ-GTP (U/L)	T-Bil (mg/dL)
1	84	17	12	30	0.8
2	64	25	21	34	0.7
3	68	20	13	7	0.3
4	88	22	19	22	0.8
5	84	26	18	47	0.7
6	88	28	20	23	0.3
7	88	21	14	14	0.7
8	88	28	7	47	0.8
9	77	21	15	21	0.7
10	84	26	26	23	0.3
Mean ± SD	81.3 ± 8.8	23.4 ± 3.7	16.5 ± 5.4	26.8 ± 12.9	0.61 ± 0.22

Abbreviations: AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; T-Bil, Total bilirubin; SD, Standard deviation.

In the current study, we determined the PK/PD characteristics of oral AZM in the human epididymal tissue by evaluating their concentrations in serum and epididymal tissues from patients with prostate cancer who received an oral dose of AZM 1 g at 1 h before orchiectomy. In addition, this study evaluated the therapeutic appropriateness of oral AZM 1 g for epididymitis caused by *C. trachomatis* by determining site-specific PK/PD characteristics.

The wide intersubjective variations in the concentrations of AZM observed in serum in the current study were consistent with those of other previous studies [9]. A previous study suggested that penetration into rat epididymal tissue was dependent on the vascular permeability associated with inflammation and that the concentration of the antibiotic amdinocillin was significantly higher at the site of inflammation [10]. Namely, AZM concentrations in epididymal tissue should depend on the presence and extent of inflammation while patients in this study were uninfected. This suggested the possibility that the PK/PD characteristics identified in this study may underestimate the epididymal concentrations and possibly the therapeutic effects of AZM regimens for acute epididymitis. However, it is clinically difficult to evaluate epididymal tissue from patients with acute epididymitis. Therefore, the PK/PD characteristics of oral AZM identified in this study seem to be significant.

It is important to investigate antimicrobial concentrations owing to their site-specific PK/PD characteristics. Unfortunately, there are no reports on the penetration of AZM into human epididymal tissue. Although the current study included a limited number of cases, we found that the mean ratio of epididymal AZM concentration to serum concentration was 5.13. Recently, we reported that the mean epididymal concentration to serum concentration ratios of levofloxacin 500 mg and sitafloxacin 100 mg in humans were 1.60 and 1.36, respectively [7]. In a rat model of epididymitis, trimethoprim and DOX showed the greatest degree of epididymal penetration compared with serum concentrations (2.56 for trimethoprim and 2.57 for DOX) [11]. Our current findings indicated that the penetration of oral AZM into human epididymal tissue was better than that of other antimicrobials.

In the nationwide surveillance of the antimicrobial susceptibility of *C. trachomatis* from male urethritis, no macrolide-resistant strains have been identified in Japan [12]. The MICs of AZM in chlamydial strains isolated from the urethra range from 0.125 to 0.50 μg/mL [3,12]. Additionally, the MIC_{90} of AZM was reported to range from 0.25 to 0.50 μg/mL [3,12]. Nevertheless, recently, a macrolide-resistant *C. trachomatis* isolate was identified in North

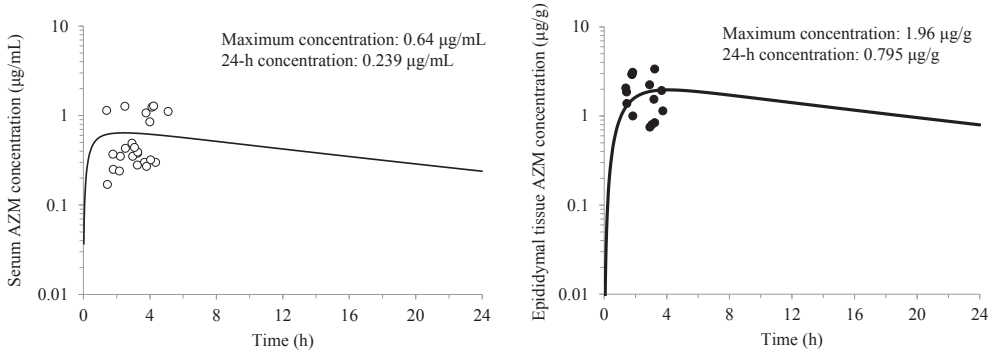

Fig. 1. Observed concentrations of oral azithromycin 1 g (23 serum and 17 epididymal tissue samples from 10 patients) and simulated curves drawn using the estimated pharmacokinetic parameters ($V_1/F = 1228.0$ L, $V_2/F = 454.4$ L, $V_3/F = 90.8$ L, Cl/F = 66.6 L/h, Ka = 3.73 h^{-1}, $K_{12} = 0.0256$ h^{-1}, $K_{21} = 0.0693$ h^{-1}, $K_{13} = 0.274$ h^{-1}, $K_{31} = 1.22$ h^{-1}).

America [13]. Thus, it is important for physicians to use macrolides appropriately to avoid the emergence of such resistant strains even in Japan. Regarding PK/PD parameters for analysis of the efficacy of AZM, the AUC and MIC of AZM against *C. trachomatis* have not been defined, even in the Clinical and Laboratory Standards Institute guidelines. It is presumed that a dose of AZM exceeding the MIC_{90} must be administered for 5–10 days for effective treatment of *C. trachomatis* [14]. For oral AZM 1 g, the maximum and 24-h concentrations were 1.96 and 0.795 μg/g, respectively. These results showed that a therapeutic regimen of single-dose AZM 1 g for acute epididymitis due to *C. trachomatis* was inadequate. Thus, additional administration of oral AZM are recommended for acute epididymitis to exceed the MIC previously reported for 5–10 days.

There are some limitations to the current study. First, only 10 patients were enrolled in this study, and young patients or patients with liver disfunction were not enrolled. Second, it may be more appropriate to collect blood samples at the elimination phase (3–6 h after the start of the operation) for more accurate evaluation of PK parameters. Third, epididymal tissues were uninfected and noninflammatory in our study. However, a previous study reported that penetration into rat epididymal tissue was dependent on vascular permeability associated with inflammation and that the concentration of antibiotic amdinocillin was significantly higher at the site of inflammation [10]. Further studies are needed to evaluate epididymal tissues from patients with acute epididymitis. Fourth, although single-dose of AZM 2 g and multiple doses of AZM 0.5 g are confirmed for chlamydial urethritis and acute bronchitis, respectively, by Japanese insurance, other doses of AZM than 1 g were not simulated in this study.

In conclusion, the penetration of AZM into human epididymal tissue was better than that of other antimicrobials reported previously. In addition, the dose of AZM 1 g for epididymitis could exceed the MICs of *C. trachomatis* previously reported. However, a single-dose of oral AZM 1 g was not sufficient for the treatment of acute epididymitis caused by *C. trachomatis* in terms of PK/PD analysis. Thus, multiple-dose regimens should be considered for the treatment of acute epididymitis. These results are helpful for developing rational optimized dosing regimens for acute epididymitis based on acceptable tissue PD characteristics related to the MIC of *C. trachomatis*.

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Conflicts of interest

None to declare.

Ethical approval

This clinical study was approved by the Okayama University Institutional Review Board prior to study initiation (registration no. 1409).

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