Absorption Kinetics of Subcutaneously Administered Ceftazidime in Hypoperfused Guinea Pigs

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ABSTRACT

Background: Pneumonia is the most common cause of death in patients with severe motor and intellectual disabilities (SMID), and intravenous ceftazidime (CAZ) is a widely used treatment for such infections. However, intravenous administration in patients with SMID may be difficult because of insufficient vascular development.

Objectives: The aim of our study was to determine the feasibility of subcutaneous drug administration by mentholated warm compresses (WMCs) as an alternative delivery method for ceftazidime in patients with SMID.

Methods: CAZ was subcutaneously administered to the abdominal region of naphazoline-treated hypoperfused guinea pigs, which were used as a hemodynamic model of patients with SMID. WMCs or warm compresses (WCs) were applied to the injection site to increase blood flow. We calculated the cumulative CAZ absorption over time by using the deconvolution method.

Results: Application of WMCs or WCs increased blood flow at the administration site and increased CAZ plasma levels. Application of WMCs or WCs after subcutaneous CAZ injection led to higher CAZ plasma levels than the mutant prevention concentration for a longer period than was observed for CAZ administration without the application of WMCs or WCs.

Conclusions: The application of WMCs or WCs enhanced subcutaneous CAZ absorption by increasing blood flow. WMCs and WCs are considered to be safe and routine methods to induce defecation after surgery on the digestive system; thus, the combination of these methods and subcutaneous CAZ administration is a potential method for treating pneumonia in patients with SMID.

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People with SMID are often bedridden for long periods of time during which their movements are limited. In these people, vascular development may be insufficient, and blood vessels of sufficient thickness may be difficult to target for intravenous administration of CAZ for pneumonia treatment. It is necessary to consider alternative administration routes that can be used simply and reliably, and thus we focused on the subcutaneous route of administration for the treatment of pneumonia with CAZ in patients with hypoperfusion. This route of administration is simpler than the intravenous route, and reduces the incidence of peripheral phlebitis and systemic infections.

Patients with SMID often experience symptoms caused by disturbances in the autonomic nervous system. In particular, skin blood flow may be reduced by constriction of the skin blood vessels due to elevated sympathetic nervous system tone. Subcutaneous blood flow may be slower in patients with severe disabilities compared with healthy individuals; therefore, low drug absorption rates due to limited blood flow, which lead to sub-MIC or sub-MPC blood levels, must be considered when treating such patients.

We have previously reported that reduced cutaneous blood flow decreased skin permeation of nicardipine hydrochloride, and that the addition of a strong permeation enhancer that had been screened in vitro to the formulation most likely caused the observed decrease in cutaneous blood flow. Thus, it is important that methods that promote absorption increase blood flow at the site of drug administration.

Hot compresses applied locally to the site of drug administration have been shown to increase local subcutaneous blood flow through vasodilation. Mentholated warm compresses (MWCs) and warm compresses (WCs) are used widely in clinical settings to induce defecation after gastrointestinal tract surgery, and their safety is well established.

In our study we investigated the use of MWC and WC methods to enhance the absorption of subcutaneously administered drugs with the goal of reaching blood levels that exceed the MIC and MPC in patients with hypoperfusion. The vasoconstrictor naphazoline was used to produce hypoperfusion in guinea pigs as a model of reduced blood flow in patients with SMID.

### Methods

#### Materials

Naphazoline nitrate was purchased from Wako Pure Chemical Industries (Osaka, Japan). Ceftazidime was obtained from GlaxoSmithKline Co Ltd (Tokyo, Japan). Cephalexin hydrate (CEX) was purchased from Sigma Chemical Co Ltd (St Louis, Missouri). Mentha oil was acquired from Yoshida Pharmaceutical Co Ltd (Saitama, Japan). All other chemicals and solvents were of reagent grade or HPLC grade and used without further purification.

#### Animals

Male Hartley guinea pigs (Japan SLC, Shizuoka, Japan) of identical age (7 weeks, 450–500 g; n = 42) were reared under constant temperature (22 C ± 2 C) and humidity (55% ± 5%), with a 12-hour light cycle from 7 AM to 7 PM. Feed and water were supplied ad libitum. All experiments using animals were approved by the Institutional Animal Care and Use Committee of Josai University (approval No. H22043-2010/05/14).

#### Blood flow measurement using laser Doppler flowmetry

Guinea pigs were anesthetized with urethane (1.5 g/kg IP), and blood flow in the abdominal region was measured using a laser Doppler flow meter (Peri Flux PF3; Perimed KB Co, Lund, Sweden). Animals were divided into 3 groups (saline group, n = 8; naphazoline group, n = 4 each). A 0.15 or 1.5 mg/kg dose of naphazoline was administered to guinea pigs to induce hypoperfusion. Control animals received a physiologic saline solution injection into the femoral muscle. The laser Doppler probe (6 mm diameter; Perimed KB Co) was attached to the shaved abdominal skin, and blood flow was measured for 6 hours. Blood flow was measured every 5 seconds for 3 minutes, and the mean of these measurements was used for each blood flow value. The data are represented as changes in blood flow (ie, percent of initial flow).

![Blood flow changes](image)

* Figure 1. Blood flow changes in hypoperfused guinea pigs. Guinea pigs received an intramuscular injection of saline and either 0.15 mg/kg naphazoline or 1.5 mg/kg naphazoline as a vasoconstrictor, 150 minutes after anesthesia. Blood flow was measured every 10 minutes for 6 hours. Each point represents the mean (SD) (saline group, n = 8; 0.15 mg/kg naphazoline group, n = 4; 1.5 mg/kg naphazoline group, n = 4). P < 0.05 (0.15 mg/kg vs. saline). *P < 0.05 (1.5 mg/kg vs saline).
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CAZ (17 mg/kg) and CEX (7 mg/kg) were injected into the jugular vein.

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immediately centrifuged (4

10, 15, 20, 40, 60, 90, 120, 240, and 360 minutes) for 6 hours, and
taken from the contralateral jugular vein at different time intervals (5,

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Animals were divided into 2 groups (saline group, n = 3; 1.5 mg/kg naphazoline group, n = 5). Immediately after the intramuscular administration of naphazoline (15 mg/kg) or saline (as a control), CAZ (17 mg/kg) and CEX (7 mg/kg) were injected into the jugular vein. The dose of 17 mg/kg represents the converted clinical dose (1 g/administration for a 60-kg adult). Blood samples (0.25 mL) were taken from the contralateral jugular vein at different time intervals (5, 10, 15, 20, 40, 60, 90, 120, 240, and 360 minutes) for 6 hours, and immediately centrifuged (4 C; 3000 g; 10 minutes) to collect plasma, which was stored at –45 C until the analysis. Heparin sodium (5 U/mL) was used as an anticoagulant for plasma sampling. The plasma concentration-time profile of CAZ and CEX were analyzed by

2-compartment model, where the plasma concentrations after intravenous administration are described as follows:

\[ C(t) = \frac{D(\alpha - K_{21})}{V_1(\alpha - \beta)} e^{-\alpha t} + \frac{D(K_{21} - \beta)}{V_1(\alpha - \beta)} e^{-\beta t} \] (1)

where \( \alpha \) and \( \beta \) are constants, \( V_1 \) is the volume of drug within the central compartment, \( V_2 \) is the volume of drug within the peripheral compartment, \( K_{21} \) is the migration rate constant for migration from the peripheral to central compartment, \( K_{12} \) is the migration rate constant for migration from the central to peripheral compartment, \( K_{10} \) is the elimination rate constant for elimination from the central compartment, and \( CL_{tot} \) is the total body clearance.

The value of \( \alpha, \beta, V_1, \) and \( K_{21} \) were estimated by fitting the above equation 1 to the concentration-time curve after intravenous administration using MULTI to the nonlinear least-squares method (algorithm; Gauss-Newton method). The values obtained for \( \alpha, \beta, V_1, \) and \( K_{21} \) were used in equations 2 to 7 to calculate the other parameters.

\[ t_{1/2a} = \frac{0.693}{\alpha} \] (2)

\[ t_{1/2b} = \frac{0.693}{\beta} \] (3)

\[ V_2 = \frac{K_{12} \cdot V_1}{K_{21}} \] (4)

\[ K_{12} = \alpha + \beta - K_{21} - K_{10} \] (5)

\[ K_{10} = \frac{\alpha \beta}{K_{21}} \] (6)

\[ CL_{tot} = \frac{D \alpha}{A \beta + B \alpha} \] (7)

Subcutaneous administration of CAZ to hypoperfused guinea pigs

Animals were divided into 4 groups (n = 3 each) according to the dose level, and injection volume. CEX, which has a high urinary excretion rate, was coadministered with CAZ to correct a decrease in the elimination rate of CAZ in naphazoline-treated animals. Guinea pigs were anesthetized by intraperitoneal administration of urethane (1.5 g/kg). Naphazoline saline solution (1.5 mg/kg) was injected into the femoral muscle to reduce blood ow 2.5 hours after anesthesia administration. Intravenous administration of a CEX saline solution (7 mg/kg) in the jugular vein immediately followed. A CAZ saline solution (17 mg/320 mL [1D1V], 17 mg/640 mL [1D2V], 34 mg/320 mL [2D1V], or 34 mg/640 mL [2D2V]) was subcutaneously administered over a 30-minute period at 2 sites in the abdominal region. After CAZ administration, blood samples (0.25 mL) were taken from the contralateral jugular vein at predetermined times (0, 5, 10, 15, 20, 40, 60, 90, 120, 180, 240, 300, and 360 minutes) for 6 hours and immediately centrifuged to collect plasma. The supernatant was 1 stored at –45 C until analysis, in which plasma concentrations of CAZ and CEX were determined using an HPLC system. In addition, the blood ow at the administration sites was measured using a laser Doppler ow meter.

Effect of the mentholated warm compress on CAZ subcutaneous absorption

Animals were divided into 3 groups (n = 3 each). To prepare an MWC, mentha oil (0.5 mL) was dripped into warm tap water (65 C; 500 mL) to prepare saturated mentha water. The MWC consisted of a towel (11 33 cm) that was soaked in saturated mentha water until its temperature reached 65 C. The administration site was covered with the MWCs immediately after CAZ administration, and
the MWCs were entirely covered with plastic cling wrap to prevent mentha water evaporation. WCS that did not contain mentha oil were similarly applied to the administration site. In addition, the abdomen was wrapped with a towel. The MWCs or WCS were replaced every 30 minutes, and they were replaced a total of 13 times during the duration of the experiment. To ensure that the mentha water saturation of the administration site was maintained, mentha oil was added 5 minutes before the MWCs were changed.

HPLC separation and quantification of CAZ and CEX

A 7.0% (vol) perchloric acid solution (100 μL) was added to each plasma sample (100 μL), and this mixture was centrifuged for 15 minutes (4 C; 13,000 g). The supernatant (20 μL) was injected into the HPLC system to determine CAZ and CEX plasma concentrations.

The HPLC system consisted of a pump (LC-10ATvp; Shimadzu Corp), a column (TSK-gel ODS-80TM, 5 μm, 4.6 × 250 mm; TOSOH Corp, Tokyo, Japan), and an ultraviolet detector (SPD-6A; Shimadzu Corp). A mobile phase consisting of 9:91 (v/v) acetonitrile:10 mM phosphate buffer (pH 3.0) was used for elution. The flow rate was 1.0 mL/min, the column temperature was 50°C, and the detector operated at a wavelength of 258 nm.

Under the HPLC condition for the analysis of CAZ, the peaks of CAZ and CEX appeared on the chromatogram of the ultraviolet detector at 8.8 and 15.8 minutes, respectively. From the standard deviation of the response and the slope of the calibration line, the detection limit for CAZ was calculated to be 0.02 μg/mL, whereas the detection limit for CEX was 0.05 μg/mL. Similarly, the lower limit of quantification for CAZ was calculated to be 0.06 μg/mL, whereas that of CEX was 0.13 μg/mL. A calibration line was produced every time the HPLC pump was stopped.

Estimation of CAZ absorption rate by the deconvolution method

The deconvolution method was used to estimate subcutaneous CAZ absorption rates (Research Institute of TTS Technology, Saitama, Japan). The time course of the subcutaneous absorption rate, I(t), was estimated from the time course of the blood concentration after subcutaneous (C[t]) and intravenous (W[t]) administrations. C(t) is the response function, W(t) is the weight function, and I(t) is the input function. C(t) is expressed as follows:

\[ C(t) = \int_0^t W(t-u) \cdot I(u) \cdot du \]  

The volume of distribution of CAZ and its elimination rate constant were decreased by naphazoline-induced hypoperfusion; thus, correction of the weighting function was necessary to estimate the absorption rate of CAZ. Ratios comparing the pharmacokinetic parameters of CEX in hypoperfused guinea pigs and normal guinea pigs were determined. The pharmacokinetic parameters of CAZ in normal guinea pigs were multiplied by these ratios to estimate the pharmacokinetic parameters of CAZ in hypoperfused guinea pigs. Using these parameters as weighting functions, and the CAZ plasma level-time curve after subcutaneous administration in hypoperfused guinea pigs as a response function, the time-course of cumulative CAZ absorption was estimated using the deconvolution method. The average absorption rate was used to compare the absorption rates between administration methods. The average absorption rate is the slope of the cumulative absorption profile.

Statistical analysis

All data are presented as mean (SD). Differences in blood flow and plasma concentrations of CAZ among the groups were analyzed using ANOVA. When an ANOVA showed a significant effect (P < 0.05), Tukey’s honest significant difference test was used as a post-hoc test to compare means, and differences were considered to be significant if the value of P was < 0.05. All analyses were conducted using software from the R Project for Statistical Computing (R version 2.15.2, R Foundation for Statistical Computing, Vienna, Austria).

Results

Blood flow measurement using laser Doppler flowmetry in hypoperfused guinea pigs

After administration of 0.15 and 1.5 mg/kg naphazoline, blood flow immediately decreased by 30% and 50%, respectively, compared with baseline. The reduction of blood flow caused by 1.5 mg/kg naphazoline administration persisted until the end of the measurement period (Figure 1).
Effects of naphazoline on the disposition of CAZ after intravenous administration

The CAZ and CEX plasma concentration-time curve after intravenous CAZ and CEX administration following intramuscular administration of naphazoline or saline is shown in Figure 2, and the corresponding pharmacokinetic parameters are presented in Table 1.

The elimination of CAZ, determined using a 2-compartment model, was significantly altered, most likely due to a blood flow reduction after naphazoline administration, and had to be corrected to estimate the subcutaneous absorption rate of CAZ. Thus, intravenous coadministration of CEX, which has a high urinary excretion rate, was performed at the same time as subcutaneous CAZ administration. The elimination parameters of CAZ were then determined based on the changes observed in the elimination parameters of CEX.

CAZ subcutaneous absorption profile in hypoperfused guinea pigs

We hypothesized that CAZ blood levels would be approximately equivalent after intravenous administration of identical doses per time unit (eg, 17 mg for 1D1V and 1D2V or 34 mg for 2D1V and 2D2V). However, doubling the volume of vehicle (2V) resulted in significantly increased CAZ blood levels compared with the original vehicle volume (1V) (Figure 3A). CAZ blood levels exceeded its MIC90 (16 μg/mL) under all conditions of administration. However, in the 1D1V condition, CAZ blood levels did not exceed the MPC (32 μg/mL), which was exceeded in the other conditions.

Blood flow changes occurring during CAZ subcutaneous administration are shown in Figure 3B. Blood flow rapidly decreased after naphazoline administration in all conditions, and then reached a constant rate. No difference was observed among groups. Figure 3C shows the CAZ cumulative absorption profile estimated using the deconvolution method. The subcutaneous absorption of CAZ was not complete 6 hours after CAZ administration in the 1D1V and 2D1V groups, whereas almost all of the administered CAZ was absorbed in the 1D2V and 2D2V groups.

Effect of WMCs on CAZ subcutaneous absorption in hypoperfused guinea pigs

MWCs and WCs were applied to the 1D1V group. CAZ plasma levels were markedly increased in both the MWC and WC groups compared with the control group, and were approximately 4 and 6 times higher, respectively, than in the control group 6 hours after administration (Figure 4A). Naphazoline administration resulted in a blood flow decrease, which was partially inhibited by the application of an MWC or a WC (Figure 4B). Figure 4C shows the cumulative CAZ absorption as estimated using the deconvolution method. CAZ absorption after subcutaneous administration was about 84.0% complete in the MWC group (141 mg absorbed) after 6 hours, whereas 60.0% of the administered CAZ was absorbed in the WC group. The average absorption rate throughout the period of experimentation was increased compared with the 1D1V group by the application of WCs or MWCs (1D1V, 0.013 mg/min; with WC, 0.027 mg/min; with MWC, 0.039 mg/min). The increased absorption that was produced by application of an MWC or WC reached levels equivalent to those of the 1D2V and 2D1V groups (Figure 3C).

Discussion

Abdominal subcutaneous blood flow was reduced by intramuscular administration of naphazoline. In the 0.15 mg/kg
A subcutaneous injection of CAZ was administered at a dose of 17 mg (1D) over a 30-minute time period (1D1V). We expected plasma levels of CAZ to be equivalent within these pairs of treatment groups. However, doubling the volume of vehicle resulted in elevated CAZ levels. It has been previously reported that subcutaneously administered drugs spread horizontally in the subcutaneous tissue at the site of administration.

Conclusions

The subcutaneous administration of CAZ in combination with MWCs or WCs is a potential alternative to intravenous CAZ.
administration, and should be investigated clinically for the treatment of pneumonia in patients with hypoperfusion associated with SMID.

Acknowledgments

Tsuyoshi Ebihara and Shinji Oshima contributed the data analysis, data interpretation and writing. Kousuke Ohara, Akio Negishi and Shigeru Ohshima contributed the literature search and figure creation. Mitsuyoshi Okita and Sayumi Shiina contributed the data collection. Hiroyuki Iwasaki, Akira Yoneyama and Eiji Kitazumi contributed the study design for clinical practical application. Daisuke Kobayashi contributed the study design, the overall responsibility of the article and the writing.

Conflicts of Interest

The authors have indicated that they have no conflicts of interest regarding the content of this article.

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