



A survey of subcutaneous blood flow in patients with SMID and subcutaneous ceftazidime administration using mentholated warm compresses in healthy subjects

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Abstract

Objectives: To investigate subcutaneous blood flow rate (SBFR) in healthy volunteers and patients with severe motor and intellectual disabilities (SMID), and evaluate the effect of mentholated warm compresses (MWCs) on SBFR and subcutaneous ceftazidime absorption in healthy volunteers.

Methods: SBFR at the forearm, chest and abdomen were evaluated in Japanese healthy volunteers and in adults with SMID. The effects of MWCs on blood flow rate and ceftazidime pharmacokinetics were evaluated in healthy volunteers.

Results: SBFR was significantly lower in the forearms of female patients with SMID ($n = 11$) than in the forearms of healthy females ($n = 6$); it was not significantly lower in the abdomen or chest. There were no significant differences between male patients ($n = 18$) or controls ($n = 12$) in SBFR at any site. MWC application increased SBFR 1.3- to 2.0-fold compared with baseline in healthy controls ($n = 6$). MWC application increased ceftazidime maximum blood concentration, SBFR and time above mutant prevention concentration in a single healthy subject.

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Conclusions: Abdominal SBFR in patients with SMID did not differ from that of healthy subjects. MWC application increases SBFR and subcutaneous drug absorption rate in healthy humans.

Keywords

Severe motor and intellectual disabilities, mentholated warm compress, subcutaneous administration, blood flow rate, mutant prevention concentration

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Introduction

Patients with severe motor and intellectual disabilities (SMID) may have complications including cerebral palsy, scoliosis, epilepsy, abnormal muscle tone, dysphagia, respiratory problems and various digestive disorders.¹ They may also experience elevated heart rate, impaired blood pressure regulation or respiratory function, and reduced circulating blood volume. In addition, long periods of motor disruption may cause patients to become bedridden, and this often leads to disuse syndrome.

Pneumonia, particularly aspiration pneumonia, is the leading cause of death in patients with SMID.² Maintaining antibiotic concentrations within the therapeutic range facilitates recovery and prevents drug resistance, but this is a significant challenge. Patients with SMID have a hyperactive sympathetic nervous system, causing vasoconstriction and reduced blood flow; this makes it difficult to locate veins for intravenous administration of drugs, including antibiotics.³ Venepuncture may take excessive time or may not even be possible due to narrowing of the vascular diameter.

We have previously reported the use of subcutaneous antibiotic injection as an alternative to intravenous administration for treatment of pneumonia in patients with SMID.⁴ Subcutaneous administration is more accessible and less painful than intravenous administration; it also reduces the risk of tissue damage, which is

particularly important given the reduced muscle volume associated with long-term bed rest in patients with SMID.⁵ The plasma concentration of ceftazidime, a third-generation cephalosporin widely used in the treatment of aspiration pneumonia, exceeded the minimum concentration required for inhibiting 90% of bacterial growth (MIC90), while remaining below the mutant prevention concentration (MPC; the minimum concentration required to prevent the development of bacterial resistance) in guinea pigs injected with naphazoline nitrate to simulate SMID haemodynamics.⁴ Doubling the administration rate or concentration of ceftazidime increased the plasma concentration above MIC90 and MPC,⁴ but these results could not be applied clinically because doubling the administration rate causes pain at the injection site.^{6,7}

The application of mentholated warm compresses (MWCs) to the ceftazidime administration site ameliorated the effect of naphazoline nitrate on blood flow in our animal model, resulting in plasma ceftazidime concentrations exceeding MIC90 and MPC without increasing the drug administration rate.⁴ It is not known whether this finding would be observed in humans, however.

The aim of this present study was to investigate whether the findings of our animal study are replicated in humans. Subcutaneous blood flow rate in healthy volunteers and patients with SMID was

investigated and the effect of MWCs on blood flow rate in healthy volunteers was evaluated. In addition, change in plasma drug concentration over time was analysed after application of MWCs in a healthy volunteer, in order to evaluate the association between subcutaneous absorption rate and blood flow rate.

Patients and methods

Study population

The study enrolled adult patients with SMID, resident since childhood at the National Rehabilitation Centre for Children with Disabilities, Tokyo, Japan. The study was conducted between 24 April 2012 and 29 May 2012. Patients who were considered suitable for inclusion and those for whom consent was provided were enrolled. Healthy adult volunteers were recruited from the staff at the National Rehabilitation Centre for Children with Disabilities, and also from Josai University in Saitama, Japan. Control subjects were required to not be attending any clinic or taking any regular medication.

The study protocol was approved by the Ethics Review Committee of the National Rehabilitation Centre for Children with Disabilities (approval date: 25 October 2010). All healthy participants and families of participants with SMID provided written informed consent.

Subcutaneous blood flow rate measurement

All participants were placed at rest in the supine position for at least 15 min, then blood flow rate was measured using laser Doppler blood flowmetry (moorVMS-LDF1, Moor-Instruments Ltd, Devon, UK). Measuring probes (VP1T) were adhered with 5-cm double-sided tape to the measurement sites (forearm, 10 cm from the medial wrist joint to the elbow; abdomen,

3 cm lateral from the umbilical region; chest, from the clavicular and manubrium junction to immediately below), to measure blood flow rate for 3 min. The laser characteristics were as follows: 780 nm wavelength; 20–22 kHz bandwidth; 0.1 s time constant; 40 Hz sampling frequency. Data were transferred to a data analysis system (moorVMS-PC, Ver3.0, Moor-Instruments, Ltd), and a 180-s mean value representing the steady blood flow rate was computed. Each participant's blood flow was measured under quiet conditions, adjusted to 22–26°C.

Subcutaneous blood flow enhancement

Mentha oil (0.5 ml; Yoshida Pharmaceutical Co., Ltd, Tokyo, Japan) was added to hot water (500 ml, 65°C) to make a 0.1% menthol emulsion. To prepare the MWCs, towels (19 cm × 16 cm, with a 6-cm diameter hole in the centre for attaching the blood flow-measuring probe) were immersed in the menthol emulsion until they reached 65°C in temperature.

A subset of six healthy adult subjects were placed at rest in the supine position for at least 15 min. Blood flow was then measured for 10 min at the MWC abdominal application site (6 cm left lateral of the umbilical region), and the mean skin blood flow rate at rest (baseline) was calculated. Next, the MWC was applied to the abdomen and the entire towel was wrapped with plastic wrap to prevent evaporation of the menthol emulsion. The MWC was replaced eight times (once every 10 min) for a total application time of 90 min. To maintain the towels' temperature at 65°C, each MWC was prepared just before adhesion. Blood flow rate and skin surface temperature were recorded every 0.5 s over 120 min (for the 90 min adhesion period and 30 min after removal). Mean blood flow rate and mean skin surface temperature were calculated every minute and recorded as the blood flow rate and skin surface temperature,

respectively. To evaluate the effect of MWCs on blood flow rate, changes in blood flow rate were calculated as a percentage of blood flow rate value compared with baseline.

Ceftazidime pharmacokinetics

The abdominal blood flow rate of a healthy male subject was measured for 10 min at rest, after which an MWC was applied for 90 min. Continuous ceftazidime subcutaneous administration (Modacin[®]; Glaxo SmithKline K.K., Tokyo, Japan) began at the left abdominal site 60 min after the start of MWC application (administration rate: 0.5 g/10 ml per 30 min × 2 sites). Using ethylenediaminetetra-acetic acid disodium as an anticoagulant, 8 ml blood was collected before ceftazidime administration and at 10, 20, 30, 60, 90, 120, 180 and 240 min after the start of administration. Collected blood was centrifuged (4°C, 3000g, 10 min) for plasma isolation and stored at -45°C until ceftazidime concentration was determined.

Plasma (0.75 ml) was mixed with an equal volume of 7% perchloric acid and centrifuged (4°C, 13,000g, 15 min). The resulting supernatant (20 µl) was diluted with methanol as needed. Plasma ceftazidime concentration was determined via high performance liquid chromatography (HPLC), as previously described.⁴ The calibration curve was created from 0.02, 0.05, 0.1, 0.2, 0.5, 1, 2, 5 and 10 µg/ml ceftazidime, and linearity was confirmed within this concentration range.

Each parameter of the ceftazidime absorption profile was calculated with the use of a one-compartment model using MULTI (algorithm; Simplex method), a program using the nonlinear least squares method.⁸ The cumulative absorption rate was estimated through deconvolution of pharmacokinetic parameters and plasma concentration data collected after intravenous administration in healthy volunteers.⁹⁻¹¹

Statistical analyses

Data were presented as mean ± SEM. Biometric data were compared using Student's *t*-test. Comparisons of blood flow rate in patients with SMID and healthy volunteers were made using two-way analysis of variance (ANOVA) with paired comparison for one factor (factor A: healthy volunteers or patients with SMID; factor B: forearm, chest or abdomen). When ANOVA showed a significant effect ($P < 0.05$), means were compared with Tukey's honest significant difference post-hoc test. P -values < 0.05 were considered statistically significant. All analyses were performed using R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria) for Windows[®].¹²

Results

The study enrolled 41 healthy adults (16 male/25 female; mean age 45.7 ± 1.30 years; age range 21–58 years) and 48 patients with SMID (23 male/25 female; mean age 45.9 ± 1.28 years; age range 22–70 years). Blood flow rate analysis was performed using data obtained from 40- to 54-year-old male and 45- to 49-year-old female patients (control group: 12 male/six female; SMID group: 18 male/11 female), who formed the largest subpopulation in the SMID group. Biometric data for this subpopulation are shown in Table 1. There were significant between-group differences in height, weight and body mass index (BMI) for both sexes ($P < 0.001$ for each comparison). There was no significant between-group difference in age for both sexes.

A sex-based comparison of blood flow rates at each site is shown in Table 2. In both healthy male and female subjects, blood flow rate was significantly higher in the forearm than in both the chest and abdomen ($P < 0.01$ for each comparison). There were

Table 1. Biometric characteristics of males aged 40–54 years and females aged 45–49 years included in a Japanese study investigating subcutaneous blood flow rate in healthy volunteers and patients with severe motor and intellectual disabilities (SMID), stratified according to sex.

| Characteristic | Male participants | | Female participants | |
|------------------------|-------------------------|----------------------|------------------------|----------------------|
| | Healthy group n = 12 | SMID group n = 18 | Healthy group n = 6 | SMID group n = 11 |
| Age, years | 48.8 ± 0.97 | 46.3 ± 1.01 | 46.8 ± 0.70 | 47.2 ± 0.54 |
| Height, cm | 171.2 ± 1.43 | 152.4 ± 2.14*** | 157.8 ± 2.01 | 142.2 ± 3.03*** |
| Weight, kg | 69.1 ± 2.17 | 35.5 ± 1.84*** | 52.7 ± 2.59 | 32.5 ± 1.81*** |
| BMI, kg/m ² | 23.6 ± 0.74 | 15.1 ± 0.56*** | 21.2 ± 1.10 | 15.9 ± 0.60*** |

Data presented as mean ± SEM.

BMI, body mass index.

***P < 0.001 versus same-sex healthy group; Student's t-test.

Table 2. Subcutaneous blood flow rates in healthy volunteers and patients with severe motor and intellectual disabilities (SMID) included in a Japanese study investigating subcutaneous blood flow rate in healthy volunteers and patients with SMID, stratified according to sex.

| Injection site | Male subjects ^a | | Female subjects ^b | |
|----------------|----------------------------|----------------------|------------------------------|--------------------------|
| | Healthy group n = 12 | SMID group n = 18 | Healthy group n = 6 | SMID group n = 11 |
| Forearm | 36.9 ± 7.67 ^{c,d} | 21.6 ± 5.46 | 50.0 ± 9.32 ^{e,f} | 17.1 ± 1.60 ^g |
| Chest | 19.7 ± 2.38 | 16.7 ± 0.89 | 16.8 ± 1.28 | 17.4 ± 1.39 |
| Abdomen | 12.5 ± 1.62 | 15.8 ± 1.28 | 11.6 ± 0.57 | 18.1 ± 2.06 |

Data presented as mean ± SEM.

^aAged 40–54 years; ^baged 45–49 years; ^cP < 0.01 vs chest in same group; ^dP < 0.01 vs abdomen in same group; ^eP < 0.001 vs chest in same group; ^fP < 0.001 vs abdomen in same group; ^gP < 0.001 vs same site in healthy female subjects; Tukey's honest significant difference post-hoc test.

no significant between-site differences in blood flow rate in male or female patients with SMID.

Blood flow rate in the forearm was significantly lower in female patients with SMID than it was in healthy female volunteers ($P < 0.001$). There was no significant between-group difference in blood flow rate at any site in males, or at the chest and abdomen in females.

Data regarding changes in blood flow rate and skin surface temperature following MWC application in a subset of six healthy volunteers are shown in Figure 1. Skin

surface temperature increased by 15–20% following MWC application in each subject, and then gradually decreased until MWCs were replaced. This change was observed repeatedly until completion of MWC treatment, at which time skin surface temperature slowly decreased. Blood flow rate was increased relative to baseline following MWC application in each subject, although changes varied among subjects. In subjects 1–4, blood-flow rate increased immediately after MWC application. In comparison, subjects 5 and 6 began showing moderate elevation after 20 min.

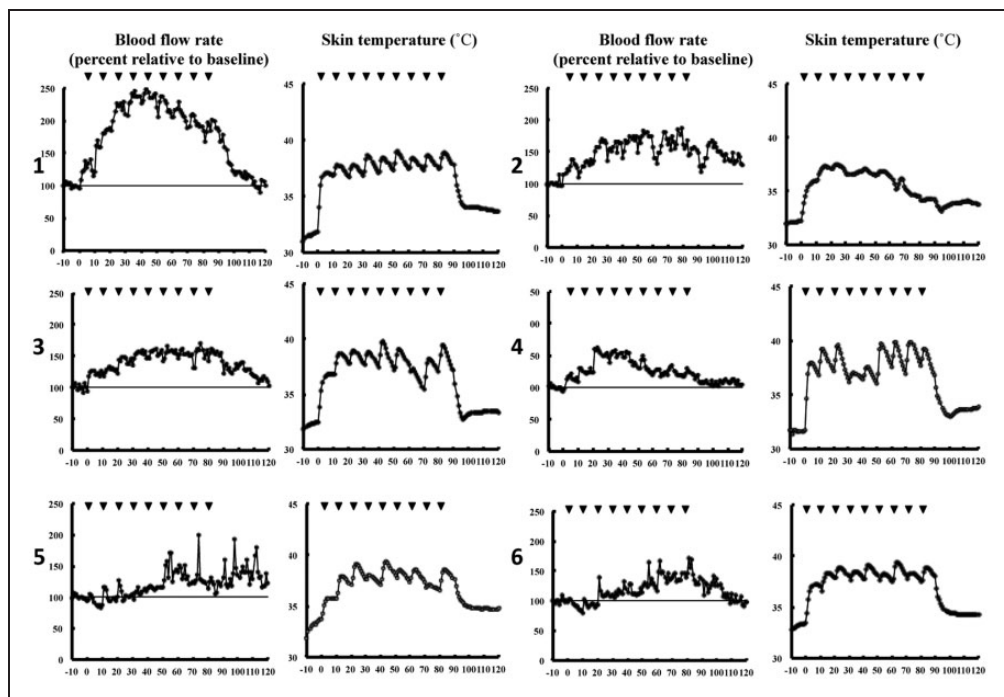


Figure 1. Effect of mentholated warm compresses (MWCs) on subcutaneous blood flow rate in Japanese healthy volunteers ($n = 6$) in a study investigating subcutaneous blood flow rate in healthy volunteers and patients with severe motor and intellectual disabilities, stratified according to sex. In each graph, the X-axis indicates minutes and the Y-axis represents either the blood flow relative to baseline or skin surface temperature ($^{\circ}\text{C}$). ▼, application of MWC.

Blood flow rate increased 1.3- to 2.0-fold in all subjects after MWC application (Figure 2).

The effects of MWCs on ceftazidime pharmacokinetics in a single healthy subject are shown in Figure 3. Ceftazidime plasma concentration rapidly increased and reached the maximum (C_{\max}) within ~ 90 min of subcutaneous administration, regardless of MWC application (Figure 3a). The C_{\max} was $44.8 \mu\text{g/ml}$ without MWC application, and $57.4 \mu\text{g/ml}$ with MWCs. The duration that MPC ($32 \mu\text{g/ml}$) was exceeded was 182.2 min with MWCs and 130.7 min without; this is a difference of 51.5 min (Figure 3a). The time to complete absorption was 120 min with MWCs and 205 min without (Figure 3b). The effect of MWC

application on blood flow rate in this individual is shown in Figure 3c. Flow rate rapidly elevated when MWCs were applied, peaking at 185.9% of the baseline value immediately before subcutaneous ceftazidime administration (55 min after application). Although the flow rate moderately declined after cessation of MWC treatment, it remained above the baseline value until blood collection was completed.

Discussion

Intravenous drug administration is the preferred method when there is a need for rapid achievement of therapeutic blood concentrations. Narrow vascular diameters in some patients with SMID may complicate

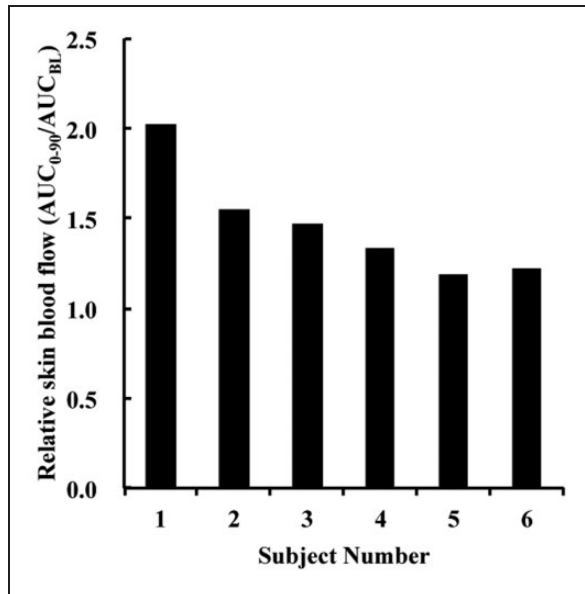


Figure 2. Change in subcutaneous blood flow rate relative to baseline in six healthy Japanese volunteers following application of mentholated warm compresses (in a study investigating subcutaneous blood flow rate in healthy volunteers and patients with severe motor and intellectual disabilities, stratified according to sex). AUC₀₋₉₀, area under the blood flow rate-time curve from 0 to 90 min; AUC_{BL}, area under the baseline blood flow rate-time curve from 0 to 90 min.

intravenous injection, resulting in missed venepuncture and undue pain.

Studies investigating absorption into systemic circulation via subcutaneous (rather than intravenous) administration have indicated that absorption rate may depend on subcutaneous blood flow.^{13,14} The small arteries and veins in the skin, prevalence of arteriovenous anastomotic branches, high pressure on the smaller artery walls and distensibility of the venous walls allow the thermoregulatory function to significantly alter blood flow to the skin. Changes in cutaneous blood flow through constriction and distention of blood vessels in the skin are controlled by neural factors (e.g. the skin's sympathetic nerves) and environmental factors (e.g. the local temperature).¹⁵ Since the sympathetic nervous system in patients with SMID is reportedly enhanced

compared with that of healthy subjects,^{16,17} cutaneous blood vessels may be constricted, resulting in reduced blood flow.

The present study evaluated subcutaneous blood flow rates in the forearm, abdomen and chest, as candidate sites for subcutaneous ceftazidime administration. Blood flow rate was significantly lower in the forearms of women with SMID than in the forearms of healthy female volunteers; this was possibly due to vasoconstriction. This finding enforces that it is difficult to distinguish veins for venepuncture in patients with SMID. There were no significant between-group differences in blood flow rate in the abdomen or chest, however, demonstrating that subcutaneous blood flow rate in patients with SMID does not always differ from that in healthy volunteers; it depends on the site being evaluated.

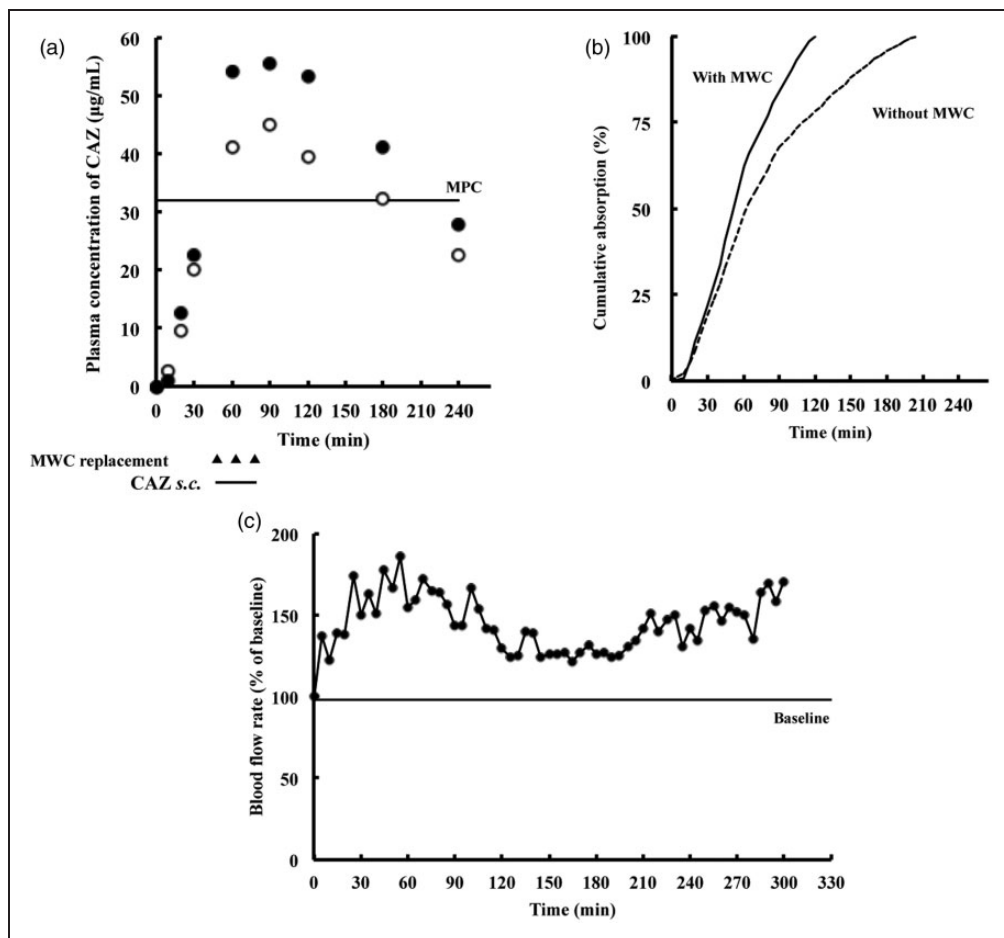


Figure 3. Effect of mentholated warm compresses (MWCs) on subcutaneous absorption of 1.0g/30 min ceftazidime (CAZ) in a Japanese healthy volunteer ($n = 1$) taking part in a study investigating subcutaneous blood flow rate in healthy volunteers and patients with severe motor and intellectual disabilities, stratified according to sex. (a) Ceftazidime plasma concentration-time curves. ●, with MWCs; ○ without MWCs. (b) Cumulative ceftazidime absorption, determined by deconvolution method. (c) Changes in subcutaneous blood flow rate. MPC, mutant prevention concentration (minimum concentration required to prevent the development of bacterial resistance).

The present finding that the application of MWCs increased blood flow rate in healthy subjects confirms the results of our animal study.⁴ It has been reported that blood flow rate in the right forearm rises rapidly to approximately 2.5 times the baseline value following application of 2% menthol in white petrolatum.¹⁸ Similarly, application of 3% menthol

increased blood flow, possibly via direct action of menthol against Transient Receptor Potential M8 (TRPM8; present on blood vessel walls).¹⁹ If menthol does increase blood flow via TRPM8, individual response may vary depending on the levels of this protein.

In pharmacotherapy, the plasma drug concentration of antibiotics should be

maintained above the MPC to prevent emergence of resistant bacteria. Subcutaneous administration of ceftazidime combined with MWC application sustained plasma concentrations above the MPC for a longer period than without MWCs. Notably, the subjects' skin surface temperatures never exceeded 45°C during MWC application, which would have caused burn injuries. Thus, MWCs may be considered a safe enhancer of absorption for subcutaneously administered drugs.²⁰ There were significant differences between patients and controls in physical characteristics (including body weight and BMI) in the present study, making it difficult to extrapolate our findings regarding the effect of MWCs on blood flow in healthy subjects to patients with SMID.

In conclusion, this study demonstrates that MWC application increases the subcutaneous blood flow rate and subcutaneous drug absorption rate in healthy humans. It was expected that blood flow rate would be lower in patients with SMID than in healthy subjects, regardless of measurement position, but this was not the case. Subcutaneous blood flow rate at the abdomen (a candidate site for subcutaneous administration) was not significantly different in patients with SMID and healthy subjects. Further studies are required to determine whether the application of MWCs increases the blood flow rate and absorption rate of subcutaneous ceftazidime in patients with SMID.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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