

Distribution of metronidazole in muscle tissue of patients with septic shock and its efficacy against *Bacteroides fragilis* *in vitro*

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Objectives: Studies investigating the target site concentration of antibiotics, such as β -lactams and fluoroquinolones, have demonstrated differences between the drug concentrations in healthy volunteers and septic patients. The aims of this study were (i) to evaluate the muscle tissue concentration of metronidazole in patients with septic shock and (ii) to test the efficacy of metronidazole in an *in vitro* pharmacodynamic model at different single doses.

Materials and methods: Six patients admitted to the ICU of Tartu University Clinics with a diagnosis of septic shock were studied. Patients receiving metronidazole treatment within 48 h before the study or with a BMI > 35 were excluded. Metronidazole muscle tissue concentration was assessed by a microdialysis technique. Based on the microdialysis data, similar kinetics were simulated in *in vitro* experiments using *Bacillus fragilis* strains with MIC_{90s} of 0.125 mg/L (BF125) and 1.0 mg/L (BF1).

Results: Metronidazole concentrations in plasma achieved a mean (s.d.) value of 11.4 ± 2.0 mg/L at 30 min after administration of a single 500 mg intravenous dose, while in the muscle tissue, maximum concentrations of 8.2 ± 4.5 mg/L were achieved at 140 ± 92.3 min after the dose. When this metronidazole time course was simulated *in vitro*, the time to 99.9% kill ranged from 1.0 to 1.4 h for BF125 and from 1.8 to 3.5 h for BF1, while the eradication time ranged from 1.7 to 2.5 h and from 3.4 to 6.5 h, respectively. No regrowth was detected.

Conclusion: Pharmacokinetic–pharmacodynamic simulation of metronidazole interstitial concentrations shows a high efficacy of the drug in septic patients.

Keywords: microdialysis, pharmacokinetics, pharmacodynamics

Introduction

Sepsis and septic shock are major causes of mortality in intensive care units (ICUs) and aggressive fluid resuscitation, adequate antimicrobial therapy and local surgical treatment are the cornerstones of sepsis management.^{1–3} In patients with severe sepsis, the interstitial space often becomes the major site for bacterial infection, and, consequently, the target site for antimicrobial drugs. The target site concentration of protein-free antibiotics in patients with sepsis or septic shock has been evaluated in several studies. These studies have shown that interstitial concentrations of both β -lactams and fluoroquinolones differ between healthy volunteers and septic patients.^{4–6}

Pharmacokinetic–pharmacodynamic (PK–PD) approaches to the study of antimicrobial action are gaining in popularity and

possibly could become a new standard for drug development,^{7,8} as they reflect drug–microorganism relations in a time-dependent manner. Protein-free drug concentrations in tissues can be measured using a microdialysis technique³ and based on these data, the pharmacokinetics of the drug can be evaluated and simulated *in vitro* in the presence of microorganisms for pharmacodynamic study.^{6,9}

Metronidazole is a well established antimicrobial agent, which is widely used for the prophylaxis and treatment of anaerobic infections.¹⁰ The pharmacokinetic studies that led to the current dosage of metronidazole had several disadvantages, as plasma or total tissue concentrations were measured instead of target-tissue concentration^{11,12} and were also conducted on healthy volunteers.^{11–13} Metronidazole has a clinical efficacy profile in the surgical population. However, in patients with microcirculatory impairment, such as severe sepsis or septic shock,

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the pharmacokinetics and, consequently, the target-site concentration of the drug might be severely altered.

The aims of this study were (i) to describe pharmacokinetics of metronidazole using a muscle tissue microdialysis technique in patients with septic shock and (ii) to test the activity of metronidazole in an *in vitro* pharmacodynamic model at different single doses.

Materials and methods

The ethics committee of the University of Tartu approved the study. Because the patients were unconscious, their relatives received a detailed description of the study protocol and written informed consent was obtained. The study was carried out in accordance with the Declaration of Helsinki.

Patients

Six male patients admitted to the General ICU of Tartu University Clinics with a diagnosis of septic shock according to the American College of Chest Physicians and the Society of Critical Care Medicine consensus conference criteria were included.¹⁴ Overweight patients with a body mass index (BMI) over 35 and patients that had received metronidazole treatment within 48 h before the study were excluded. Mean age of the patients was 58.2 years (range 32.0–69.0 years), mean weight 77.5 kg (range 70.0–95.0 kg) and mean BMI 25.8 (range 22.9–31.0). Causes of septic shock were two cases of bronchopneumonia, two cases of peritonitis from the purulent cholecystitis and colonic perforation, one case of urosepsis and one case of phlegmon of the shoulder and arm. Metronidazole was not included in the treatment regimen and was administered once as a dose of 500 mg intravenously (iv).

Microdialysis

Microdialysis catheters (CMA 60 catheters with 30 mm long polyamide membrane with cut-off of 20 kDa) were placed into the *m. vastus lateralis* of the right thigh just above the knee. Microdialysis was carried out using a CMA 107 microdialysis pump and CMA T1 perfusion fluid containing Na⁺ 147 mmol/L, K⁺ 4.0 mmol/L, Ca²⁺ 2.3 mmol/L and Cl⁻ 156 mmol/L, which was perfused at a rate of 2 µL/min. Baseline sampling (approximately 60 µL) was carried out during the first 30 min after insertion of the probe. During the next 30 min, another sample of approximately 60 µL was collected during retrodialysis with a metronidazole 5 mg/L solution to assess *in vivo* recovery of the drug.¹⁵ A 15 min washout period was then allowed. Thereafter, 500 mg of metronidazole (Metronidazol Nycomed; Nycomed Austria GmbH, Linz, Austria) was given intravenously by infusion over 10 min. Individual microdialysate samples were collected between 0 and 0.5 h, 0.5 and 1.0 h, 1.0 and 1.5 h, 1.5 and 2.0 h, 2.0 and 2.5 h, 2.5 and 3.0 h, 3.0 and 4.0 h, 4.0 and 5.0 h, 5.0 and 6.0 h, 6.0 and 7.0 h, 7.0 and 8.0 h, 8.0 and 9.0 h, and 9.0 and 10.0 h. Samples were immediately frozen and stored at -20°C until further analysis.

Plasma samples

Blood samples (6 mL) were taken at the end of each microdialysis collection from the arterial line located either in the radial or the brachial artery. The blood was drawn into the Vacutainer test tubes with lithium heparin. The samples were centrifuged immediately at 2.8g for 10 min, and plasma was separated and stored in Eppendorf tubes at -20°C until further analysis.

Drug assay

The metronidazole assay was described in detail in our previous paper.¹³ Briefly, the metronidazole concentration was analysed by an HPLC method. Samples were prepared by treatment with acetonitrile. The chromatographic system consisted of a Lichrosorb RP-18 pre-column, Lichrosorb RP-18, 5 µm, 250×3.2 mm column, and an ultraviolet detector measuring at 318 nm. The mobile phase consisted of acetonitrile/0.01 M aqueous phosphate solution (NaH₂PO₄), 15:85 (v/v), with a flow rate of 0.7 mL/min and a column temperature of 23–25°C (room temperature).

Pharmacokinetic data

Individually obtained values were used for the calculation of *in vivo* recovery of metronidazole and the concentration of the drug in muscle tissue according to the following equations:

$$\text{Recovery (\%)} = 100 - \left(\frac{\text{concentration}_{\text{dialysate}}}{\text{concentration}_{\text{perfusate}}} \right) \times 100$$

$$\text{Tissue concentration (mg/L)} = \left(\frac{\text{concentration}_{\text{dialysate}} \times 100}{\text{in vivo recovery value}} \right)$$

where the perfusate is a solution which was used for perfusion of microdialysis probe and the dialysate is a solution which was obtained from the microdialysis vial.

Pharmacokinetic profile: V_{ss} , $t_{1/2}$, CL and AUC_{0–10} values were obtained from a two-compartmental model, calculated using Kinetics 2000 (version 3.0 demo; InnaPhase Corporation, USA). $t_{1/2}$ in muscle tissue was calculated using non-compartmental analysis. The AUC_{0–10} muscle/AUC_{0–10} plasma ratio was used as a measure of metronidazole penetration into the muscle tissue.

Pharmacodynamic model

Microorganisms. MIC₉₀ and time–kill studies were carried out with two clinical strains of *Bacteroides fragilis* BF1 and BF125. Two replicates of each strain were used and the arithmetical mean is presented in the results.

Media. Growth and time–kill assays were carried out in pre-reduced and cation-adjusted Wilkins-Chalgren broth (Oxoid, Basingstoke, UK), containing vitamin K and haemin. Wilkins-Chalgren agar (Oxoid) enriched with 5% sheep blood was used for plating experimental samples for colony number determination. All media were pre-reduced in an anaerobic chamber before inoculation.

Susceptibility testing. The MIC₉₀ of metronidazole for both isolates used was determined by Etest (AB Biodisk, Solna, Sweden) as proposed by the manufacturer. Pre-reduced blood agar plates were used for susceptibility testing. Inoculated plates were incubated at 35°C inside an anaerobic chamber (Bactron, Sheldon Manufacturing, Portland, OR, USA) at 5% CO₂, 5% H₂ and 90% N₂ for 48 h. Both *B. fragilis* strains were fully susceptible to metronidazole according to published breakpoints. The MICs of metronidazole for *B. fragilis* BF1 and BF125 were 1.0 and 0.125 mg/L, respectively.

Inoculum. Both organisms were placed on Wilkins-Chalgren agar plates and incubated overnight at 35°C in an anaerobic chamber. The microorganisms were diluted with pre-reduced sterile saline until the turbidity of the suspension matched that of a 0.5 McFarland standard (~1×10⁸ cfu/mL). The suspension (0.5 mL) was then used to inoculate into test flasks with Wilkins-Chalgren broth. This yielded a starting inoculum of approximately 1–5×10⁶ cfu/mL. The actual size of each inoculum was also determined via colony counts.

Metronidazole in septic shock

Time-kill assay. Based on the microdialysis-derived mean time-concentration curve from the muscle tissue after 500 mg of iv metronidazole administration, we simulated a similar curve in an *in vitro* model. We also carried out similar experiments for tissue concentration-time profiles following 250 mg and 1000 mg doses of metronidazole by multiplying the 500 mg profile by factors of 0.5 and 2, respectively, as a linear relationship exists between dose and plasma concentration for doses of 200–2000 mg.¹⁶ The bacterial suspension was added to a test flask with 50 mL of Wilkins-Chalgren broth. Thereafter, metronidazole was added to achieve a step by step increase in the drug concentration up to the level equivalent to the peak level in the interstitial space fluid. Commercially available metronidazole solution, (Metronidazol Nycomed; Nycomed Austria GmbH), with a concentration of 5 mg/mL was used in *in vitro* experiments. Adding an appropriate amount of broth medium to the inoculated broth at 30 min intervals simulated decreasing metronidazole concentration. Altogether, six flasks containing no antimicrobial agent were used as a growth control and for the construction of the growth curve without metronidazole. After homogenization, all flasks were incubated at 35°C in an anaerobic chamber. At predetermined time points during each experimental run (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9 and 10 h), samples (50 µL) were removed from the inoculated flask, serially diluted in sterile saline and plated (50 µL) on Wilkins-Chalgren agar palates. Samples were also taken after 24 h to check for possible regrowth. Dilution were utilized to increase the accuracy of viable counts and to minimize antibiotic

carryover. The lowest limit of detection was 1 cfu per 50 µL of Wilkins-Chalgren broth or 1.3 log cfu. Inoculated plates were incubated anaerobically at 35°C for 48 h and colony counts were carried out visually. A resazurin indicator was used to ensure that conditions remained anaerobic throughout the experiments. Results were assessed by plotting log₁₀ cfu against time. The killing rate over time was determined as bactericidal if a reduction of 3 log₁₀ cfu/mL (≥ 99.9% reduction cfu/mL) could be achieved. No corrections were made for dilutional effects and the metronidazole concentration/time profile was not confirmed by assay.

Statistical analysis. The change in colony counts over time was determined by linear regression analysis of the time-kill plots. The time to ≥ 99.9% reduction in cfu/mL, the time to total eradication, and the rate of reduction of cfu/mL were determined by linear regression. The rate of killing was defined as the slope of the killing curve from the start of the experiment to the time of the detection limit. The reduction in viable counts and figures were calculated using Graph Pad Prism (San Diego, CA, USA) software.

The data are presented as arithmetical means ± standard deviation (s.d.), if not otherwise stated.

Results

Patient demographic data are shown in Table 1.

Table 1. Patients' data

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 |
|---|---------------|-----------|-----------|-----------|-----------|-----------|
| Age | 66 | 32 | 63 | 65 | 69 | 54 |
| APACHE II (at admission) | 16 | 5 | 15 | 22 | 44 | 24 |
| Vasopressors during the study | Dopa/Nor/Dobu | Dopa/Nor | Dopa/Nor | Dopa/Nor | Dopa/Nor | Dopa/Nor |
| Volume replacement during 12 h, mL | 1625 | 2475 | 2095 | 2170 | 3500 | 2280 |
| Urine output during 12 h, mL | 540 | CVVHD | 1950 | 1350 | 900 | 1350 |
| Leucocytes count, at baseline, mm ⁻³ | 15.5 | 5.53 | 17.3 | 15.7 | 18.2 | 29.5 |
| CRV, mmol/L | 178 | 255 | 251 | 101 | 292 | 289 |
| Haemodynamic profile at baseline | | | | | | |
| heart rate, min | 142 | 86 | 102 | 78 | 112 | 84 |
| MAP, mmHg | 99 | 86 | 110 | 103 | 84 | 89 |
| mean PAP, mmHg | 46 | 33 | 43 | 30 | 29 | 40 |
| PAOP, mmHg | 36 | 28 | 29 | 15 | 12 | 21 |
| CI, L/min/m ² | 4.54 | 3.12 | 3.28 | 3.69 | 2.67 | 3.75 |
| SVRI, dynes·s·cm ⁻⁵ /m ² | 1444 | 1894 | 2144 | 1974 | 2159 | 2049 |
| PVRI, dynes·s·cm ⁻⁵ /m ² | 176 | 128 | 341 | 325 | 510 | 406 |
| Oxygen profile at baseline | | | | | | |
| D _{O2} , mL/min/m ² | 557 | 442 | 471 | 490 | 559 | 624 |
| V _{O2} , mL/min/m ² | 152 | 193 | 138 | 164 | 180 | 132 |
| Q _s /Q _t , % | 22.6 | 13.2 | 9.9 | 6.8 | 20.6 | 13.6 |
| F _i O ₂ , % | 50.0 | 40.0 | 45.0 | 45.0 | 50.0 | 40.0 |
| P _a O ₂ , mmHg | 86.0 | 80.0 | 176 | 155 | 180 | 114 |
| S _v O ₂ , % | 71.0 | 55.0 | 72.0 | 68.0 | 65.0 | 79.0 |
| pH | 7.37 | 7.37 | 7.44 | 7.42 | 7.29 | 7.43 |
| BE, mmol/L | -0.2 | -3.3 | 2.5 | -5.4 | -9.0 | 3.4 |
| lactate, mmol/L | 3.8 | 6.0 | 1.4 | 2.8 | 5.1 | 1.2 |
| Hgb, g/L | 108 | 107 | 104 | 124 | 162 | 123 |

^aAPACHE II, Acute Physiology And Chronic Health Evaluation; Dopa, dopamine; Nor, noradrenaline; Dobu, dobutamine; MAP, mean arterial pressure; PAP, pulmonary artery pressure; PAOP, pulmonary artery occlusion pressure; CI, cardiac index; SVRI, systemic vascular resistance index; PVRI, pulmonary vascular resistance index; D_{O2}, oxygen delivery; V_{O2}, oxygen consumption; Q_s/Q_t, shunt fraction; F_iO₂, fraction of oxygen; P_aO₂, oxygen partial pressure; S_vO₂, mixed venous blood saturation; BE, base excess; Hgb, concentration of haemoglobin; CRV, C-reactive protein concentration.

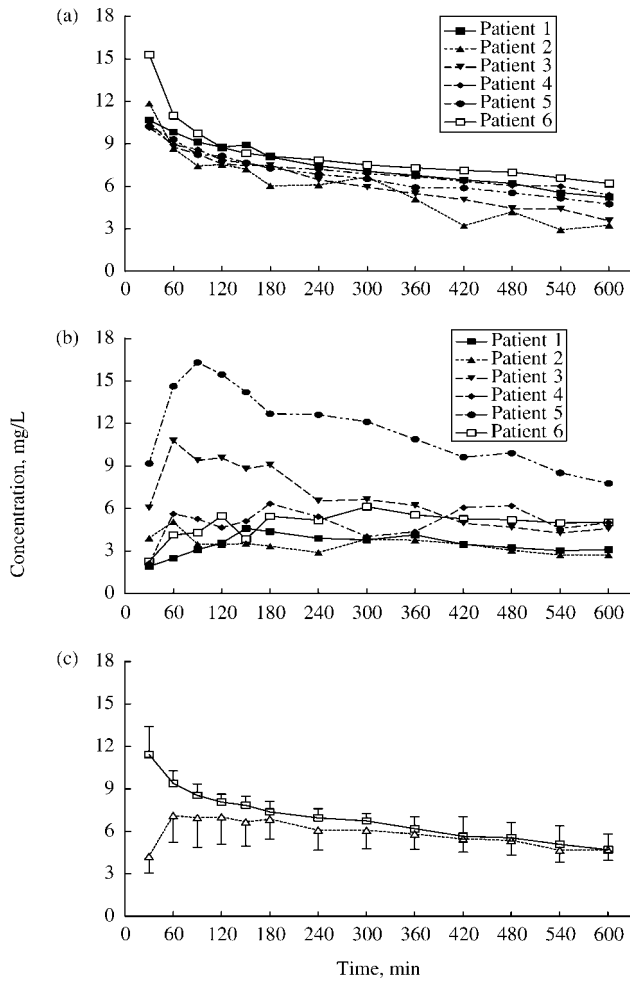


Figure 1. Time course of metronidazole concentrations in plasma and muscle tissue in septic shock patients after a single iv dose of 500mg. The individual time course of metronidazole in plasma (a) and in muscle tissue (b); patients are represented by individual lines: filled squares and solid line for patient one; filled triangles and dotted line for patient two; filled inverted triangles and dashed line for patient three; filled diamonds and dashed-dotted line for patient four; filled circles and dashed double-dotted line for patient five and open squares and solid line for patient six. (c) Mean time course in plasma, open squares and solid line; and mean time course in muscle tissue, open triangles and dotted line; vertical solid lines represent standard deviations (S.D.).

Pharmacokinetics

Recovery of metronidazole from the muscle tissue during retrodialysis ranged from 24.7% to 82.0% (mean value 54.9 ± 20.0%).

The individual and mean concentration versus time curves of metronidazole in plasma and muscle tissue after a single dose of 500mg are shown in Figure 1. The AUC_{0-10} muscle/ AUC_{0-10} plasma ratio of 0.88 ± 0.47 indicates a good penetration of metronidazole into the muscle tissue in patients with septic shock. Other pharmacokinetic parameters, calculated from plasma and muscle tissue concentrations of the drug, are shown in Table 2.

Pharmacodynamic model

The pharmacodynamics of metronidazole against two strains of *B. fragilis* (BF1 and BF125) are shown in Figure 2 and Table 3. All three simulated metronidazole doses were rapidly bactericidal against both strains of *B. fragilis*, with viable counts falling 5–6 logs to undetectable levels and remaining below this limit for the following 10h. The control regimens (with no drug) exhibited approximately a 3 log increase in cfu/mL by 10h test time. However, no regrowth on the control plates was detected after 24 h.

Discussion

The pharmacokinetics of metronidazole have been assessed in several studies, using plasma concentrations,^{10,12} biopsies¹¹ or skin blisters.¹⁷ However, few studies have been carried out on critically ill patients, where disturbances of macro- and microcirculation could significantly alter the pharmacokinetics and pharmacodynamics of the drug.¹²

In this study, protein-free interstitial concentrations of metronidazole have been measured for the first time in patients with septic shock. Using a microdialysis technique, we have shown that the maximum concentration of the drug in muscle tissue was 8.2 ± 4.5 mg/L in these patients. The important advantage of the microdialysis technique is that it allows us to assess the protein-free concentrations of the drug in tissue, i.e. to study the fraction of the drug which exerts an effect. We have used a similar technique in healthy gynaecological patients and found the mean maximal concentration of the drug in muscle tissue was comparable to this study.¹³ Metronidazole penetration into the muscle tissue, measured as the AUC_{0-10} muscle/ AUC_{0-10} plasma ratio, therefore appears to be similar between septic patients and healthy volunteers, 0.88 ± 0.47 and 0.91 ± 0.19 ,¹³ respectively. The C_{max} in plasma appears to be slightly lower in septic patients than in healthy volunteers, 11.4 ± 2.0 and 16.5 ± 4.6 mg/L,¹³ respectively. Increased V_{ss} due to capillary leakage and interstitial oedema as well as differences in haemodynamic profile and use of haemodialysis could most likely explain these findings. These two factors (capillary leakage and

Table 2. Main pharmacokinetic parameters calculated for the study population

| | AUC_{0-10} (mg/L × h) | $t_{1/2}$ (h) | C_{max} (mg/L) | T_{max} (min) | CL (mL/min) | V_{ss} (L) |
|--------|----------------------------|-------------------|------------------|-----------------|-----------------|----------------|
| Plasma | 66 ± 8.3 | 13.2 ± 5.3 | 11.4 ± 2.0 | 30 ^a | 56.2 ± 26.9 | 53.5 ± 4.0 |
| Muscle | 57.9 ± 29.9 | 27.3 ± 23.4^b | 8.2 ± 4.5 | 140 ± 92.3 | | |

Results presented as arithmetical means ± S.D.

^aTime of the first plasma sample.

^bCalculated using non-compartmental analysis.

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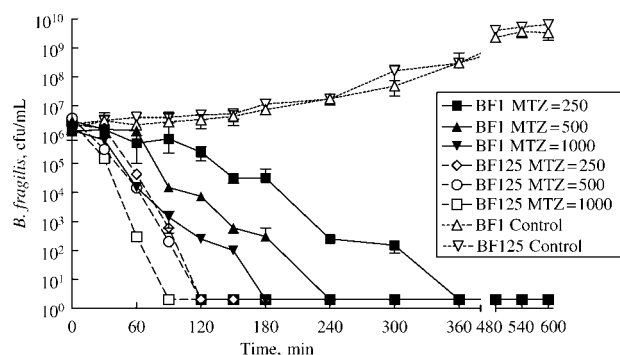


Figure 2 Time–killing curves of *B. fragilis* after *in vitro* simulation of concentration versus time profile of metronidazole in the muscle tissue of patients with septic shock. Time–killing curves are represented as follows: solid line and filled squares for BF1 and metronidazole (MTZ) dose of 250 mg; solid line and filled triangles for BF1 and metronidazole dose of 500 mg; solid line and filled inverted triangles for BF1 and metronidazole dose of 1000 mg; dashed line and open diamonds for BF125 and metronidazole dose of 250 mg; dashed line and open circles for BF125 and metronidazole dose of 500 mg; dashed line and open squares for BF125 and metronidazole dose of 1000 mg. Dotted line and open triangles for BF1 control growth without metronidazole and dotted line and open inverted triangles for BF125 control growth.

interstitial oedema) might also explain the wide inter-subject variation of data seen in this study where the maximum concentration of metronidazole in the muscle tissue of septic patients differs over a factor of two-fold between the individuals (Figure 1b).

Metronidazole penetration into tissues has also been studied by Bielecka-Grzela & Klimowicz,¹⁸ who used cutaneous microdialysis in healthy volunteers. They assessed the AUC_{0–8} skin/AUC_{0–8} plasma ratio as a measure of tissue penetration, finding a value of 0.672 ± 0.196 . Thus, our data indicate that the penetration of metronidazole into the peripheral tissues of septic patients is at least as good as in healthy patients.

The results of C_{max} , V_{ss} , CL and AUC, calculated from the plasma measurements of metronidazole in this study, were quite similar to our previous observations in a relatively healthy population,¹³ and as described in the review by Lamp *et al.*¹⁰ The results of the study are in accordance with low metronidazole protein binding (<20%).¹⁰ As can be seen from Figure 1(c), the difference between the total plasma concentration and the protein-free muscle tissue concentration after equilibration is small.

On the basis of our findings, it could be concluded that septic shock has a minimal influence on the distribution of metronidazole in plasma and muscle tissue.

The pharmacokinetics of iv metronidazole in critically ill patients has been studied by Plaisance *et al.*¹² They found that in patients with renal or liver disease there is a prolongation of the clearance and half-life of the drug (half-life ranged from 7.98 to 42.4 h). Our results did not address these findings, as none of our patients had liver function impairment.

The half-life calculated by fitting the plasma concentrations to a two-compartment model was longer than in the healthy volunteers described by Lamp *et al.*,¹⁰ 13.2 ± 5.3 versus 6–10 h, respectively. However, in our previous study, we found the half-life of metronidazole was 12.9 ± 4.9 h in relatively healthy female patients.¹³ Therefore, it is difficult to conclude whether the half-life of metronidazole is prolonged or not in patients with septic shock.

It has been suggested that metronidazole exhibits a concentration-dependent killing effect against anaerobic pathogens.¹⁰ As the MIC₉₀ of metronidazole for the *B. fragilis* group ranges from <0.25 to 8 mg/L,¹⁹ there is a possibility that a sufficient target concentration of the drug may not be achieved. Therefore we carried out a PK–PD analysis with two different *B. fragilis* strains, isolated in the microbiology laboratory of our hospital. The model was based on the interstitial concentrations of metronidazole, measured in the muscle tissue of the septic patients, and this confirmed the high activity of the drug against *B. fragilis* strains. Eradication time for the *B. fragilis* with the highest MIC (1 mg/L) was only 6.49 ± 0.05 h for the lowest dose studied and no regrowth was detected within the next 24 h.

An important limitation of the present study is that the metronidazole concentrations in the test flasks were not controlled during the *in vitro* experiments. However, to the best of our knowledge, metronidazole is chemically stable and not metabolized by *B. fragilis* in amounts which could affect the final concentration of the drug in the test flasks. Another factor which theoretically could influence the bacterial killing rate, is the dilution effect caused by adding extra broth in the *in vitro* experiments. At 3 h, the volume of broth in the test flask had increased by 15%, whereas the count of bacteria had decreased at least by 10 times (Figure 2); at 6 h, the respective numbers were 30% and 10^6 times. Based on these data, we believe that the effect of dilution on bacterial killing rate is minimal. The third limitation concerns sampling. Plasma samples were taken

Table 3. Pharmacodynamic data obtained from the *in vitro* experiment

| Strain, metronidazole dose | C_{max}/MIC_{90} | AUC _{0–10} /MIC ₉₀ | Time to kill 99.9%, ^a h | Time to eradication, ^b h | Slope of the kill curve (kill rate), h ⁻¹ | r^2 (kill curve) |
|----------------------------------|--------------------|--|------------------------------------|-------------------------------------|--|--------------------|
| <i>B. fragilis</i> 0.125–250 mg | 28.4 | 231 | 1.41 ± 0.01 | 2.53 ± 0.01 | -2.39 ± 0.30 | 0.942 |
| <i>B. fragilis</i> 0.125–500 mg | 56.8 | 463 | 1.29 ± 0.08 | 2.27 ± 0.08 | -2.75 ± 0.15 | 0.991 |
| <i>B. fragilis</i> 0.125–1000 mg | 113 | 926 | 0.95 ± 0.03 | 1.69 ± 0.02 | -3.63 ± 0.42 | 0.974 |
| <i>B. fragilis</i> 1.0–250 mg | 3.55 | 29.0 | 3.47 ± 0.05 | 6.49 ± 0.05 | -0.90 ± 0.08 | 0.945 |
| <i>B. fragilis</i> 1.0–500 mg | 7.10 | 57.9 | 2.43 ± 0.12 | 4.33 ± 0.08 | -1.42 ± 0.13 | 0.955 |
| <i>B. fragilis</i> 1.0–1000 mg | 14.2 | 115 | 1.84 ± 0.00 | 3.42 ± 0.00 | -1.71 ± 0.14 | 0.966 |

Results presented as means \pm s.d.

^aTime required to achieve a 99.9% kill of the inoculum.

^bTime required to decrease viable counts below the limit of detection.

at the end of microdialysate collection and plasma time points do not exactly match microdialysis time points, which produces a difficulty in interpretation of the AUC muscle/AUC plasma ratio. But, as the decline in plasma and microdialysate concentrations was not steep, such inaccuracy has minimal influence on the AUC muscle/AUC plasma ratio.

Taken together, our clinical and experimental data indicate that metronidazole could be administered twice or even once daily in patients with septic shock. However, such a change in clinical practice needs studies with multiple dosing regimes, like those carried out by Lewis *et al.*²⁰ They used a PK–PD model of oral administration of metronidazole to determine whether the newer extended release oral preparation, twice or once daily, had the same activity compared with immediate release oral preparations given three times a day. Similar to our findings, the authors observed rapid bactericidal activity ($\geq 99.9\%$ reduction) by 12 h with both regimens and no regrowth during the next 48 h.²⁰

In conclusion, the present data demonstrate that the distribution of metronidazole is not affected in patients with septic shock. After a single iv dose of 500 mg metronidazole, effective concentrations against *B. fragilis* are achieved in muscle tissue of these patients. Further studies are needed to clarify whether once or twice daily administration of metronidazole is appropriate for clinical use of the drug.

Acknowledgements

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