DISSERTATIONES MEDICINAE UNIVERSITATIS TARTUENSIS
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THE PHARMACOKINETICS OF METRONIDAZOLE AND MEROPENEM IN SEPTIC SHOCK

A microdialysis study

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LIST OF ORIGINAL PUBLICATIONS

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I. **Karjagin, J**.; Pähkla, R.; Starkopf, J. (2004). Perioperative penetration of metronidazole into muscle tissue: a microdialysis study. European Journal of Clinical Pharmacology, 59(11), 809–813.

II. **Karjagin, J**.; Pähkla, R.; Starkopf, J. (2004). Metronidasooli kontsentratsioon septilises šokis olevate patsientide vereplasmas ja lihaskoes. Eesti Arst, 83(2), 87–92.

III. **Karjagin, J**.; Pähkla, R.; Karki, T.; Starkopf, J. (2005). Distribution of metronidazole in muscle tissue of patients with septic shock and its efficacy against *Bacteroides fragilis in vitro*. Journal of Antimicrobial Chemotherapy, 55(3), 341–346.

IV. **Karjagin, J**.; Lefeuvre, S.; Oselin, K.; Marchand, S.; Tikkerberi, A.; Starkopf, J.; Couet, W.; Sawchuk, RJ. Pharmacokinetics of meropenem determined by microdialysis in the peritoneal fluid of patients with severe peritonitis associated with septic shock. (accepted for publication in CPT and advanced online publication 8 August 2007).

ABBREVIATIONS

ACCP	American College of Chest Physicians
APACHE	Acute Physiology and Chronic Health Evaluation
AUC	area under the curve
AUC/MIC_{90}	the area under the concentration – time curve divided by the MIC_{90}
BMI	Body Mass Index
Cl	clearance
C_{max}	maximum concentration reached in the compartment of reference
C_{max}/MIC_{90}	maximum concentration level divided by MIC_{90}
C_{min}	minimum (trough) concentration
ECF	extracellular fluid
HPLC	high performance liquid chromatography
ICU	intensive care unit
LC-MS/MS	high-performance liquid chromatography coupled with tandem mass spectrometry
MIC_{90}	minimum inhibitory concentration
MPI	Mannheim Peritonitis Index
PD	pharmacodynamics
PF	peritoneal fluid
PK	pharmacokinetics
PK/PD	pharmacokinetic-pharmacodynamic
SCCM	Society for Critical Care Medicine
ss	at steady state
$T_{>MIC}$	time that the drug concentration exceeds the MIC_{90}
$T_{1/2}$	half-life
T_{max}	time to peak concentration
Vd	volume of distribution

1. INTRODUCTION

Sepsis – a systemic inflammatory response to infection – is one of the most frequent reasons for intensive care hospitalisation (Bone et al., 1992). Sepsis is often lethal, killing 20 to 50 percent of severely affected patients. It is the second leading cause of death among patients in noncoronary intensive care units (ICUs) and the tenth leading cause of death overall in the United States. Sepsis substantially reduces the quality of life of those who survive (Perl et al., 1995; Heyland et al., 2000).

Several recent studies suggest that the mortality of sepsis is not changed remarkably over the last 30 years. In United States, the incidence of sepsis is increased almost threefold from 1979 to 2000 (Martin et al., 2003). Although the mortality has declined from 27.8% to 17.9%, the increased incidence resulted in nearly tripling the number of in-hospital deaths related to this syndrome during that period of time. Vincent et al. studied the patients admitted to the intensive care departments all over the Europe from May the 1st till May the 15th in year 2002 and found that ICU mortality was almost doubled among patients with sepsis, 27% vs. 14%, respectively. ICU mortality of severe sepsis and septic shock was even higher, 32.2% and 54.1%, respectively (Vincent et al., 2006).

Overall mortality of secondary peritonitis is 22.6% (Barrie et al., 2004). Severe peritonitis, defined as Mannheim Peritonitis Index (MPI) from 21 to 29, has mortality of 22.5%; while MPI index above 29 is associated with mortality of 59.1% (Billing et al., 1994). Septic shock develops in 11% of patients suffering from peritonitis and this increases the relative risk for death by 13 times (Anaya and Nathens, 2003).

The cornerstones for therapy of sepsis are aggressive fluid resuscitation, source control, and antimicrobial treatment. The reduction of mortality from abdominal sepsis from 90% to 40% by surgical source control was already reported in early 20th century (Kirschner, 1926). Introduction of penicillin in 1942 opened a well-known new era in management of bacterial infections. However, soon it become clear that microorganisms are fought back and resistance develops to penicillin and every new antimicrobial drug. Today, despite of advanced medical technology, the mortality of sepsis has remained still unacceptably high. That is why we need to look for new treatment strategies and constantly improve the quality of the old ones. In present study we check the hypothesis that altered pharmacokinetics (PK) may lead to inadequately low target-tissue concentration of the metronidazole and meropenem in patients with septic shock.

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2. REVIEW OF LITERATURE

2.1. Pharmacokinetics in intensive care patients

Many drugs, including antimicrobial agents, are used in intensive care. The ultimate aim of whatever pharmacological treatment is to achieve safe and effective concentration of the drug in the target tissue (Fuhs et al., 1988).

Antimicrobials are consistently among the most important and commonly prescribed drugs in the ICUs (Paterson, 2003). Appropriate policies for their wise use should be accurately defined (Emmerson, 2000). Inappropriate use of antimicrobials may cause therapeutic failure or delayed clinical response in the individual patient; at the same time prolonged antimicrobial exposure contributes colonisation and spread of resistant pathogens (Eggiman and Pittet, 2001).

Selecting the appropriate antimicrobial in terms of spectrum of activity is the mainstay of antimicrobial therapy. Still, the consistent choice of correct dosage regimen (in terms of both dose and frequency of administration) has been repeatedly shown to be at least important for successful clinical cure and microbiological eradication (Hyatt et al., 1995; Hyatt and Schentag, 2000 and 2001; Schentag, 1999; Thomas et al., 1998; Craig, 1998).

PK changes can be a result of organ dysfunction, most notably the liver and kidneys, but can also be a consequence of the acute phase response, drug interactions and therapeutic interventions (Boucher et al., 2006). Even after brief period of hours significant changes of drug disposition, altered distribution or elimination processes, may be observed in individual critically ill patients (Pea et al., 2005).

Figure 1 schematically presents the possible mechanisms of altered PK in critically ill patients.

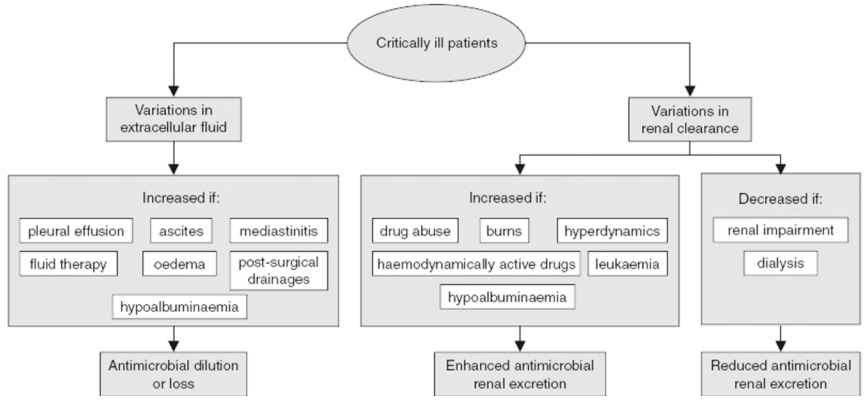

Figure 1. Pathophysiological or iatrogenic conditions affecting the distribution and elimination of antimicrobials (modified from Pea et al., 2005).

2.1.1. Increased volume of distribution

The oedematous state, commonly seen in critically ill, may alter the distribution of the drugs, especially of hydrophilic antimicrobials, which are limited to extracellular distribution. Sepsis and trauma are often associated with expanded extracellular fluid (ECF) (Pea et al., 2005). Both, the endothelial damage leading to increased capillary permeability (Bone, 1991; Glauser et al., 1991) and the conspicuous reduction of oncotic pressure due to severe hypoalbuminaemia (< 15 mg/L) (Vrhovac et al., 1995) may promote substantial fluid extravasation. The phenomenon is known as "third space" development or "capillary leak syndrome". In this situation aggressive fluid therapy is usually demanded for haemodynamic stabilisation, and this further aggravates the ECF expansion and increase in volume of distribution (Vd) (Pea et al., 2005).

Several studies have shown that oedematous state may cause clinical failure of antimicrobial therapy in sepsis and/or trauma. Dorman et al. assessed the initial peak plasma concentrations achievable after a 3 mg/kg loading dose of gentamicin and tobramycin in 52 critically ill surgical patients with life-threatening gram-negative infection. Based on data from healthy volunteers they expected values greater than 8.3 mg/L. In half of the cases this concentration was not achieved, while in 15.3% of the patients it remained below 5 mg/L (Dorman et al., 1998). Interstitial concentration of piperacillin has been assessed by microdialysis technique in patients undergoing open-heart surgery (Brunner et al., 2000) or suffering from septic shock (Joukhadar et al., 2001). In both conditions the peak concentrations were 5 to 10 times lower than in healthy volunteers. The authors speculate that this may lead to therapeutic failure against strains with high minimal inhibitory concentration (MIC_{90}). It also has been demonstrated that Vd of aztreonam (2g every 8 hours) and imipenem (500 mg every 6 hours) is higher in severe trauma patients than in historical controls (McKindley et al., 1996). This again suggests that initial standard dosages of these drugs may appear insufficient for critically ill patients.

Hypoalbuminemia is a frequent condition in critically ill patients being caused by increased albumin capillary escape rate through leaky endothelium, fluid overload or malnutrition. Hypoalbuminaemia may contribute to fluid extravasation and antimicrobial dilution. On the other hand, the increase of free fraction of the drug may increase Vd and clearance (Cl) (Pea et al., 2005). In ten critically ill patients with severe sepsis, the severe hypoalbuminaemia (22 ± 6.1 g/L) resulted in substantial increase of Vd for intravenous ceftriaxone, administrated 2g once daily. Furthermore, the drug Cl was almost doubled in patients with normal renal function when compared to healthy volunteers (41.3 ± 11.7 vs. 19.8 ± 2.5 mL/min). In overall, blood concentrations below the desired threshold (< 8 mg/L) were observed in five from ten patients with hypoalbuminaemia, which may cause suboptimal drug exposure (Joynt et al., 2001).

2.1.2. Changes in renal clearance

Early phase of sepsis is characterised by hyperdynamic cardiovascular response evidenced by increased cardiac output and enhanced renal blood flow. This, in turn, may lead to increased glomerular filtration rate and tubular secretion of renally eliminated drugs (Pinder et al., 2002). In critically ill trauma patients treated with ceftazidime 2g every 8 hours or 60 mg/kg continuous intravenous infusion, drug Cl was found to be significantly higher than in healthy volunteers (2.35 ± 0.89 vs 1.58 ± 0.23 mL/min/kg) (Hanes et al., 2000). The renal Cl of the levofloxacin (500mg every 12 hours) in critically ill is reported to be significantly higher than in healthy volunteers (3.40 vs. 2.42 mL/min/kg), most likely due to increase in both, glomerular filtration rate and tubular secretion (Pea et al., 2003). Mean ciprofloxacin Cl in critically ill patients who had severe sepsis and sustained renal function (creatinine Cl ≥30 mL/min) was similar to that observed in healthy volunteers (0.4 mL/min/kg). However, the coefficient of variation was as high as 50% in critically ill patients versus only 11% in historical controls, suggesting that very high interindividual pharmacokinetic variability may occur in this setting (Lipman et al., 1998). In summary, all these studies underline the necessity of defining higher than presently recommended dosages for most renally excreted antimicrobials in the treatment of septic patients with preserved renal function.

The vasoactive drugs may also influence on the PK of antimicrobials (Pea and Furlanut, 2001). In study of 18 intensive care patients, serum level of vancomycin was significantly decreased in 8 patients during co-treatment with dopamine, dobutamine and furosemide. The administrated dosage of vancomycin was 1.25- to 1.9-fold higher than recommended by Moellering's nomogram (Pea et al., 2000). Among other factors responsible for increased Cl of ceftriaxone in critically ill the use of inotropes was considered to contribute in three out of ten patients. The investigators therefore recommend a more aggressive dosage regimen for ceftriaxone in this setting, and suggest to decrease the dosage interval or to apply a continuous intravenous infusion (Joynt et al., 2001).

In critically ill patients, renal failure may occur because of underlying disease (i.e. trauma, multiple organ failure, extensive burns, cardiogenic or hypovolaemic shock) or it may be iatrogenically induced by nephrotoxic therapies. In these situations a reduction in the average daily dosage of most hydrophilic and moderately lipophilic antimicrobials may be required to avoid overexposure and toxic side-effects. In reality, the choice of appropriate dosage may be particularly difficult in cases when renal replacement therapies are applied (Pea et al., 2005).

Taking together, the dosage of antibacterial drugs in critically ill patients is not straightforward. Pathophysiological changes due to systemic inflammatory response may markedly alter pharmacokinetic and pharmacodynamic properties

of the drugs. For optimised drug dosing and improved patient outcome the knowledge of these alterations is essential (Nicolau, 1998). Today, we know very little about PK of antimicrobial drugs in conditions like severe sepsis or septic shock.

2.2. Microdialysis

The term microdialysis originates from the late 1950s, where it was used to describe a method of simultaneous dialysis and extraction of polar steroids from blood plasma with a volume of no more than 1mL (Kalant, 1958).

Microdialysis sampling, as an in vivo technique, makes possible the investigation of biochemical events in the ECF virtually of any tissue, organ or biological fluid. Development of the technique from a long-term dialysis sac implantation (Bito et al., 1966), through push-pull dialysis (Delgado et al., 1972), to the present continuous flow sampling method (Ungerstedt and Pycock, 1974; Zetterström et al., 1982) was accomplished largely by researchers in the neurosciences. In humans, microdialysis was first used for assessment of muscle tissue glucose concentration (Lönnroth et al., 1987). Later, the investigations of other human tissues such as blood, brain, heart, lung (Herkner et al., 2002), liver, kidney, peritoneal cavity, bile, bone (Thorsen et al., 1996), skin (Schnetz and Fartash, 2001), subcutaneous tissue (Stallknecht et al., 2004) and breast have been introduced.

The schematic representation of microdialysis probe is shown in Figure 2. The sampling is accomplished by implanting a probe consisting of a hollow fiber dialysis membrane into the organ or biological fluid of interest. The short length of dialysis fiber is affixed to pieces of narrow bore tubing, which serve as inlet and outlet tubes. A solution, termed as perfusate, is pumped slowly through the probe. The perfusate is an aqueous solution, which closely matches to the pH and ionic composition of the surrounding sample matrix, e.g. the ECF in case of *in vivo* tissue sampling (Davies, 1999; Plock and Cloft, 2005).

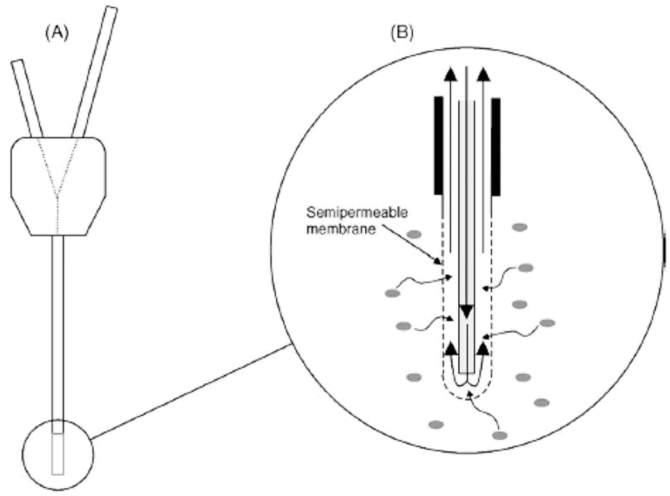

Figure 2. Concentric probe design: (A) rigid pin-style probe for brain microdialysis; (B) enlarged section of the same probe. Molecules pass the semipermeable membrane and are transported to the outlet with the perfusion fluid. Black arrows indicate the direction of the flow through the probe.

Microdialysis is a diffusion controlled process. Driving force for mass transport is the concentration gradient between the ECF and the fluid in the probe lumen. Low molecular mass compounds diffuse into (recovery) or out of (delivery) the probe lumen (Davies, 1999; Plock and Cloft, 2005). When the perfusate is correctly matched to the sample matrix, there should be no net exchange of ions or water across the membrane.

The method allows collecting a drug or an endogenous compound at the site of action. Most importantly, in difference from plasma or whole tissue concentration measurements, it becomes possible to collect protein-free samples. (Joukhadar et al., 2001; Plock and Cloft, 2005). The protein – unbound fraction of the drug is able to leave systemic circulation and penetrates into the tissues (Ryan, 1993). Also, it has been shown that only the freely available, unbound fraction of antibiotics exerts the anti-infective effect, both *in vitro* (Kunin et al., 1973) and *in vivo* (Merrikin et al., 1983).

The main challenge with microdialysis is to be sure that concentration measured in dialysate is somehow related to real tissue concentration. The concentration in the dialysate never exactly represents the concentration in the periprobe tissue and is a fraction of actual concentration, therefore $C_{tissue} > C_{dialysate}$ (Plock and Cloft, 2005). Accordingly, calibration of the probe is required for estimation of the absolute concentration of free analyte in the ECF (Davies, 1999).

The term relative recovery describes the ratio between the concentrations of a substance in the dialysate to that in the periprobe fluid. There is few probe

calibration methods described in the literature, like no-net flux method (Lönnroth et al., 1987, 1989), internal standards method (Alexander et al., 1988; Scheller and Kolb, 1991; Larsson, 1991), and slow perfusion method (Wages et al., 1986; Menacherry et al., 1992).

The most popular calibration technique in human PK studies is *in vivo* delivery or retrodialysis. The method assumes that diffusion through the membrane is equal in both directions (Ståhle, 1991). In retrodialysis, the analyte of interest is perfused through the microdialysis probe prior to the experiment. Probe recovery is calculated based on disappearance of analyte from perfusion solution by using the following equation (Plock and Cloft, 2005).

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

The retrodialysis is suitable for for *in vitro* recovery measurement for many different compounds (Zhao et al., 1995). Although absolute accurate determination of recovery is impossible, a close approximation is sufficient for clinical application as intraindividual coefficients of variation for interstitial concentration measurements by microdialysis were shown to range 10 to 20%, depending on analyte (Arner et al., 1988; Ståhle et al, 1991; Müller et al., 1995; Joukhadar et al., 2001).

Taking together, microdialysis is a sampling technique, allowing sampling a protein free fluid from tissue of our interest. Main challenge is a probe calibration, which has to be carefully performed for each experiment. Microdialysis was used for different applications as pharmacology, intensive care monitoring, cancer and brain studies (Klaus and Bahlmann, 2004)

2.3. Pharmacokinetic-pharmacodynamic (PK/PD) models

Currently, optimal dosing regimens are still poorly defined for antibiotics. The treatment of bacterial infections is frequently based on clinical experience rather than a rational scientific approach. To properly evaluate the clinical efficacy of antibiotics, not only the antibacterial activity of the drug but also the characteristics of the host and bacteria should be taken into account (CPMP, 1999).

Over the last decades, interest in the relationships between the PK and PD of antimicrobial agents has increased (Mouton et al., 2002). Pioneering studies on PK/PD modelling were performed soon after discovery of penicillin revealing that the effect of the penicillin is dependent on the time of its concentration above the MIC_{90} of the infecting pathogen (Eagle et al., 1950, 1953). The interest in this field was renewed only in 1980 and 1990s with studies demonstrating that PD of antibiotics is not important only for optimizing of their anti-bacterial effect but also for minimizing the risk of development of

drug resistant strains (Frimodt-Møller, 2002). The basic PK/PD indices determining the effect of the drug are illustrated by Figure 3. The PK/PD parameters commonly calculated form concentration/time relationship are (1) the ratio of the peak antibiotic concentration and MIC_{90} (C_{max}/MIC_{90}), (2) the area under the concentration – time curve and MIC_{90} ratio (AUC/MIC_{90}) and (3) the time the antibiotic concentration remains over MIC_{90} ($T_{>MIC}$) (Hyatt et al., 1995).

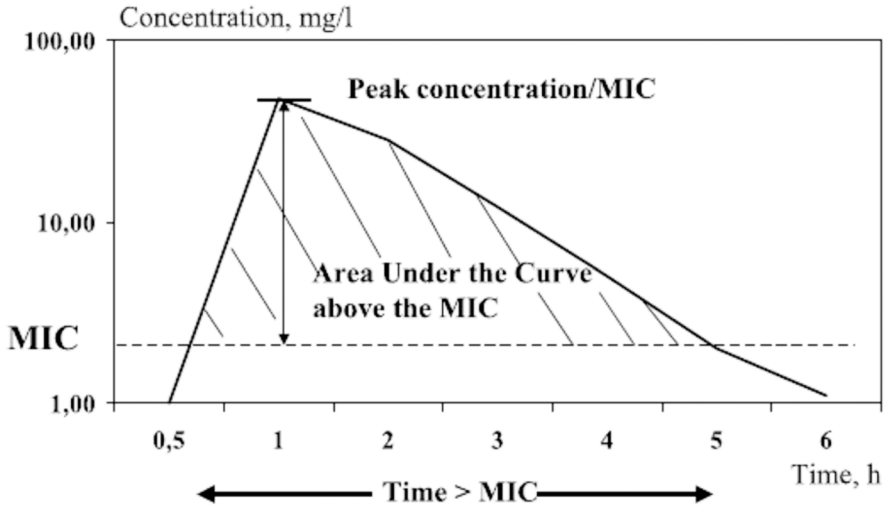

Figure 3. PK/PD parameters used for correlation with effect *in vivo*.

Currently, the most common PK/PD approach for anti-infective agents relies on plasma concentration as the PK input value and MIC_{90} as the PD input value (Liu et al., 2002). A PK/PD model is a mathematical concept that links the PK and PD of the drug, and describes the time course of pharmacological effect of a given dose, which is very helpful to determine appropriate dosing regimens (Liu et al., 2002).

The antimicrobial agents can be divided into two main groups: those that exhibit concentration-dependent killing and prolonged persistent effects and those that exhibit time-dependent killing and minimal-to-moderate persistent effects (Craig, 1998; Scaglione, 2002). Aminoglycosides, metronidazole and fluorochinolones belong to the former, while β-lactams, macrolides and glycopeptides are presenters of the latter group (Craig, 1998). Despite a general agreement about PK/PD indices governing efficacy of the antibiotics class by class, there is no agreement about the value of single index (MacGowan and Bowker, 2002). The $T_{>MIC}$ is an important indicator of efficacy of beta-lactams and carbapenems. Several studies suggest that it should last from 40 to 70% of the interval-time between the doses (Craig, 1998; Andes and Craig, 1998;

Mouton et al., 2000; Mouton and Punt, 2001). Both the C_{max}/MIC_{90} ratio and AUC/MIC_{90} ratio are considered to be useful indicators for aminoglycosides and fluoroquinolones (Andes and Craig, 1998; Drusano, 2000). For aminoglycosides, the C_{max}/MIC_{90} ratio higher than 8 should be the index of choice (Moore, 1987; Kashuba et al., 1999) because of toxicity considerations (toxicity is related to AUC and to trough concentrations). For fluoroquinolones some authors suggest that C_{max}/MIC_{90} ratio higher than 10 is the best parameter (Preston et al., 1998). Others believe that the AUC/MIC_{90} ratio is the best, and, that it depends on the type of bacteria: higher than 40 for Gram-positive and 100 – 125 for Gram-negative bacteria (Drusano, 2000).

In the last few decades, several techniques (e.g. saliva, skin blisters, tissue biopsy, and microdialysis) have been employed to monitor the free drug concentrations in the ECF (Liu et al., 2002). The advantages and disadvantages of these techniques are summarized in Table1. Biopsy samples do not allow the differentiation between intra- and extracellular drug fraction and continuous or repeated sampling is also impossible. Contradictory data exist about the use of salivary samples for PK studies (al-Obaidy et al., 1995; Lee et al., 1996; Brunner et al., 1998; Müller et al., 1998). This method, therefore, cannot be unrestrictedly recommended for determination of interstitial drug concentrations (Plock and Cloft, 2005). Another method applied for more than 30 years for interstitial drug concentration assessment is the skin blister method (Benfeldt et al., 1999). Both, suction and cantharides induced techniques have been compared to microdialysis sampling with controversial results. Resembling concentration–time profiles were obtained with penciclovir (Borg et al., 1999), paracetamol (Brunner et al., 1998) or acetylsalicylic acid (Benfeldt et al., 1999) while studies with fluconazole (Sasongko et al., 2003) or moxifloxacin (Müller et al., 1999) demonstrated the unsuitability of this method. Studies with cantharides skin blisters after the administration of theophylline showed that blister concentrations might be more similar to total plasma concentrations due to cumulation of proteins (Müller et al., 1998). Taking together, microdialysis is the only suitable of available techniques for measuring protein unbound concentration of the drug continuously in the tissue of interest.

Table 1. Comparison of different tissue sampling techniques (modified from Plock and Cloft, 2005).

	Microdialysis	Biopsy	Saliva	Skin blister
Collection at the site of action	+	+	–	–
No fluid loss	+	+/–	+/–	–
Protein-free samples	+	–	–	–
No or minimal invasiveness	+	–	+	–
Simple sampling technique	–	+	+	–
Continuous sampling at the same site	+	–	+	–
No size limitation of drug	–	+	+	+
No calibration necessary	–	+	+	+

Pharmacodynamic effect *in vivo* is the result of a dynamic exposure of the infective agent to unbound antimicrobial drug fraction at the relevant effect site (Joukhadar, 2001). Therefore dynamic simulation may provide a rational approach to describe and predict PDs at the relevant target site. Few recent publications describe microdialysis based *in vivo* PK / *in vitro* PD model for determination killing effect of antimicrobial agents (Delacher et al., 2000; Zeitlinger et al., 2003; Sauermann et al., 2003, 2005). The concentration of piperacillin in subcutaneous adipose and muscle tissue appeared up to ten times lower in septic patients than in healthy volunteers after 4 g intravenous bolus (Joukhadar et al., 2000). The PK profiles obtained in this study were further simulated *in vitro* with *S. aureus* and *P. aeruginosa* strains. Despite of low concentration of the drug in the tissue the antimicrobial killing effect of it was acceptable thanks to longer tissue Cl and thus longer $T_{>MIC}$ value (Sauermann et al., 2003). Another study used plasma and muscle tissue time – concentration profiles of levofloxacin obtained from patients with sepsis and simulated *in vitro* experiment with *S. aureus* and *P. aeruginosa* strains. The investigators observed large interindividual variability in PK in both plasma and muscle tissue. The killing of *S. aureus* was fast and there were no difference between simulated PK profiles. But, the picture was different with *P. aeruginosa*: plasma PK simulation produced fast and reliable killing, muscle tissue PK simulation gave significant interindividual differences in bacterial growth inhibition. (Zeitlinger et al., 2003). The distribution of cefpirome to subcutaneous adipose tissue in patients with sepsis with consecutive *in vitro* PD modelling is also studied. Effective antimicrobial killing against both *S. aureus* and *P. aeruginosa* in healthy and septic patients was similar despite of different PK (Sauermann et al., 2005).

2.4. Study drugs

2.4.1. Metronidazole

Metronidazole was introduced in early 60s of last century for the treatment of patients with *Trichomonas vaginalis*. Afterwards it became known as powerful antianaerobic agent (Kling and Burman, 1989; Lamp et al., 1999; Lares-Asseff et al., 1992).

Molecular weight of metronidazole is 171, Vd 0.5 to 1 L/kg, binding to plasma proteins 20%, $T_{1/2}$ about 8 hours, and PK express linear pattern over dosage ranges 0.5 to 2000 mg. Metronidazole is metabolised in liver, one of the five metabolites (hydroxyl-metronidazole) has significant antimicrobial activity (30–65% of parent drug). 7–12% of metronidazole excreted in urine unchanged. Metronidazole has also postantibiotic effect against anaerobes, which lasts for more than 3 hours (Lamp et al., 1999). The antimicrobial activity could be described as concentration dependent (Nix et al., 1995).

Bactericidal activity of metronidazole is dependent on the formation of a specific redox intermediate metabolite in the bacterium. Main effect of this metabolite is breakage of a DNA strand, inhibited repair, disrupted transcription and, ultimately, cell death (Edwards, 1979). Usually, the causative organisms for sepsis are aerobes, Gram-positive or Gram-negative. However, some infections, especially abdominal sepsis, are caused by mixed flora of aerobes and anaerobes, and require the treatment covering both of them. Abdominal sepsis deserves specific attention as it is the second leading cause of sepsis and septic shock (Friedman et al., 1998).

Postoperative wound infection is a recognised threat in elective surgery. The reported incidence of wound infection has been more than 20% without using prophylactic antibiotics. The patients who have had received antibiotic prophylaxis have had two to three-fold decrease in the frequency of wound infections. Metronidazole is widely used for prophylaxis and treatment of anaerobic infections in clinical practice (Mittendorf et al., 1993).

The PK of metronidazole has been investigated in number of studies. The vast majority of studies have used plasma, biopsy- or skin blister based methods (Lares-Asseff et al., 1992; Klimowicz et al., 1996; Kerin et al., 1992; Plaisance et al., 1988). Although metronidazole protein binding has reported to be less than 20% (Lamp et al., 1999), the inability of biopsy-based techniques to separate protein-bound and protein–unbound fractions of the drug could be a considerable disadvantage. In one study, metronidazole penetration into the tissues has been studied also by cutaneous microdialysis in healthy volunteers (Bielecka-Grzela and Klimowicz, 2003). No studies based on the microdialysis are available in septic shock patients.

2.4.2. Meropenem

Meropenem is relatively new member of family of carbapenems, being introduced more than 15 years ago. Meropenem is active against large spectrum of Gram-positive, Gram-negative and also anaerobic bacteria (Pfaller and Jones, 1997). Its bactericidal activity is based on inhibition of cell wall synthesis.

Molecular weight of meropenem is 383.5, apparent Vd of 0.2 to 0.3 L/kg, binding to plasma proteins 2%, $T_{1/2}$ is about 60 min. (Hurst and Lamb, 2000; Ververs et al., 2000; Robatel et al., 2003). Meropenem is primarily excreted by the kidney, with 54 to 79% of 1 g dose excreted unchanged in urine and further 19 to 27% excreted as microbiologically inactive opened β-lactam metabolite. Meropenem also exhibits postantibiotic effect against Gram-positive and -negative bacteria lasting up to three hours (Hurst and Lamb, 2000). PK/PD index describing the efficacy of β-lactams is $T_{>MIC}$, which for most of β-lactams has to be over 60% of interdosing time. However, significant postantibiotic effect leads to reducing the $T_{>MIC}$ to 40–50% for meropenem (Mouton and Punt, 2001). PK of meropenem is extensively studied using different techniques including plasma concentrations (Bedikian et al., 1994; Lovering et al., 1995; Kitzes et al., 2002), whole tissue biopsies (Condon et al. 1997; Gal et al., 1997) and microdialysis (Tomaselli et al., 2004). Some studies have been performed in critically ill patients exhibiting major disorders in circulation (Kitzes-Cohen et al., 2002; Novelli et al., 2005), but no studies are performed on patients with septic shock caused by severe secondary peritonitis. Beside surgery, the anti-microbial therapy is an essential part of management of peritonitis. Often the therapy should begin empirically. In Tartu University Hospital meropenem is a drug of choice, alongside with imipenem, for the empirical treatment of severe secondary peritonitis associated with septic shock.

Taking together, although there is a little doubt about clinical efficiency of metronidazole or meropenem in surgical population, and despite that PK of these drugs have been evaluated in numerous studies, we do not know whether the sufficient target site concentration of these drugs is achieved in patients with septic shock. PK studies based upon measurements plasma concentrations, have demonstrated remarkable differences between plasma, even free plasma concentration, and tissue concentration of these drugs. In patients with septic shock the PK may be altered due to microcirculatory impairment, and consequently, the target-site concentration might be even more different from plasma concentration. The insufficient target-site concentration of antimicro-bials in turn, may be causative for poor outcome of septic shock. In present study we were aimed to clarify PK of metronidazole and meropenem in septic shock patients. For these purposes target-site (muscle tissue, and peritoneal cavity) concentration of the drugs was evaluated by the aid of microdialysis sampling. The results of the study were expected to give us important additional information in assessment of antibacterial efficacy of these drugs

3. AIMS

The main purpose of the present work was to elucidate PK of metronidazole and meropenem in clinical conditions such as perioperative prophylaxis, septic shock and severe peritonitis. Particular preference was paid to target-site concentration of the drugs. The specific aims were the following:

1. To assess the muscle tissue concentration of metronidazole in setting of perioperative antimicrobial prophylaxis in elective surgical patients undergoing gynaecological surgery.
2. To evaluate the muscle tissue concentration of metronidazole in septic shock patients. To study whether muscle tissue concentration of metronidazole is sufficient for antimicrobial effect in these patients.
3. To investigate if metronidazole induces antimicrobial killing in *in vitro* PK/PD model using the muscle tissue time-concentration profile obtained from patients in septic shock.
4. To evaluate the differences in muscle tissue concentrations of metronidazole between elective surgical and septic shock patients.
5. To study whether administration of intravenous single standard-dose of meropenem to patients suffering from severe secondary peritonitis and septic shock results in peritoneal concentration of the drug sufficient for anti-microbial killing and compare it with plasma concentration.
6. According to obtained concentration-time profile after administration of single dose of 1000 mg meropenem establish compartmental model for calculating optimal dosing regimen for meropenem in the treatment of severe peritonitis.

6

4. MATERIALS AND METHODS

4.1. Patients

18 patients were studied in three different studies. The ethics committee of University of Tartu approved all three studies. Because in studies on septic shock the patients were unconscious their relatives received a detailed description of the study protocol and written informed consent was obtained. The studies were performed in accordance with the Declaration of Helsinki.

4.1.1. Metronidazole in perioperative prophylaxis (paper I and paper III)

From March 2002 to July 2002 six female patients (patients one to six) scheduled for elective gynaecologic surgery were studied. For inclusion, the patients had to be relatively healthy, with risk grade not above II according to the American Society of Anaesthesiology physical status classification. An active inflammatory process, confirmed by white blood cell count, and overweight – body mass index (BMI) more than 30.0, were exclusion criteria

Metronidazole was administered 15 min before start of surgery. During the sampling period the patients were in operating theatre and in the post-surgical care unit until the next morning.

The *Bacteroides fragilis* group was chosen as the most frequent anaerobic bacteria group isolated in our hospital. MIC_{90} data were obtained from database of microbiology laboratory of the hospital. MIC_{90} for the metronidazole was tested with E-test (AB Biodisk, Sweden) and presented as mean value of the MIC_{90} for the *B. fragilis* group, which were taken over the year 1999–2002 (Lõivukene and Naaber, 2002).

4.1.2. Metronidazole in septic shock (paper II and III)

From August 2002 to Aprill 2003 six male patients (patients seven to twelve) admitted to the General ICU of Tartu University Clinics were studied. The main inclusion criteria was diagnosis of septic shock according to the American College of Chest Physicians and the Society for the Critical Care Medicine (ACCP/SCCM) consensus conference criteria (Bone, 1992) (Table 2). Overweight patients with a BMI over 35.0 and patients that had received metronidazole treatment within 48 h prior to the study were excluded. Metronidazole was not included in the treatment regimen of the patients and was administrated once at a dose of 500 mg intravenously.

Table 2. ACCP/SCCM sepsis criteria

Sepsis: infection + two or more of the followings	- Core temperature, > 38°C or < 36°C - Heart rate, > 90 beats/min - Respiratory rate, > 20 breaths/min (or $PaCO_2$ < 32 mm Hg) - Leucocytes count, > 12,000 cells/μL or < 4,000 cells/μL (or > 10% band forms)
Severe sepsis – sepsis plus organ dysfunction	- Hypotension - Hypoperfusion abnormalities: lactic acidosis, oliguria or encephalopathy
Septic shock	- Sepsis-induced hypotension (systolic blood pressure < 90 mm Hg or a drop of > 40 mm Hg in the absence of other cause of hypotension) plus hypoperfusion abnormalities despite adequate fluid resuscitation

4.1.3. Meropenem in severe peritonitis (paper IV)

From November 2004 to November 2005 six patients (patients 13 to 18) admitted to General ICU of Tartu University Hospital due to severe secondary peritonitis associated with severe sepsis or septic shock were included. Septic shock was diagnosed according to ACCP/SCCM consensus conference criteria (Table 2). Secondary peritonitis was defined severe, when MPI, (Table 3) equals or becomes over 19 points. Patients who had received meropenem during the last 48 hours before the study were excluded.

The antimicrobial treatment should be started as soon as possible after the sepsis diagnosis is made, therefore we were able to study meropenem PK after second dose. 1000 mg of meropenem (AstraZeneca, Macclesfield**,** UK) was given intravenously by infusion over 20 min.

Table 3. Mannheim Peritonitis Index

	NO	YES
Age over 50 y.	0	5
Female gender	0	5
Organ insufficiency	0	7
Malignancy	0	4
Peritonitis over 24 h. preoperatively	0	4
Colorectal origin of peritonitis	4	0
Diffuse peritonitis	0	6
Exudation		
Clear	0	0
Purulent	0	6
Fecal	0	12

4.2. Microdialysis samples

Microdialysis catheters from CMA company (Solna, Sweden) approved for human use were used in all patients. Two different types of catheters were utilized. CMA 60 catheters for subcutaneous and intramuscular use employed in studies on metronidazole and CMA 62 gastrointestinal catheters, specially designed for intraperitoneal use in study of meropenem. Microdialysis was performed using CMA 107 microdialysis pump.

4.2.1. Metronidazole sampling from muscle tissue

Microdialysis catheters were placed on the right thigh just above the knee into the *m. vastus lateralis*. CMA T1 perfusion fluid containing Na^+ 147 mmol/L, K^+ 4.0 mmol/L, Ca^{2+} 2.3 mmol/L, and Cl^- 156 mmol/L, was perfused at a rate of 2 μL/min. Baseline sampling (approximately 60 μL) was performed during the first 30 min after insertion of the probe. During the next 30 min another sample of approximately 60 μL was collected from retrodialysis with metronidazole 5 mg/L solution to assess *in vivo* recovery of the drug (Ståhle et al., 1991). A 15 min long 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 –20ºC until further analysis.

4.2.2. Meropenem sampling from abdominal cavity

Microdialysis catheters CMA 62 were placed into the peritoneal cavity during the operation as close as possible to the source of peritonitis, eg perforation. Microdialysis was performed using perfusion fluid containing Na^+ 154 mmol/L and Cl^- 154 mmol/L, which was perfused with rate of 1 μL/min. Two baseline samplings (20 min and approximately 20 μL each) were performed before the second planned meropenem dose. Thereafter, 1000 mg of meropenem (AstraZeneca, Macclesfield, UK) was given intravenously by infusion over 20 min. During the next 7 hours microdialysis samples were drawn in as follows: approximately 20 min, 40 min, 1, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0 h. Samples were immediately frozen and stored at –80ºC until further analysis. The retrodialysis for calibration of microdialysis probe (Ståhle et al., 1991)

were started at the end of experiment with meropenem 50 mg/L solution; three collections 15 min long and approximately 15 μL each were performed.

4.3. Plasma samples

Blood samples (6mL) were taken at the end (metronidazole) or in the middle (meropenem) 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 Li-heparin. The samples were centrifuged immediately plasma was separated and stored in Eppendorf's tubes at –20ºC (metronidazole) or –80ºC (meropenem) until further analysis.

4.4. Drug Assays

4.4.1. Metronidazole

Metronidazole concentration was analysed by a high-performance liquid chromatographic method (HPLC). The same procedure was used for preparation of plasma and perfusate samples, for calibration curve construction and for quality control (QC) samples.

Samples were prepared for analyses by extraction with acetonitrile. The chromatographic system consisted of a Lichrosorb RP-18 pre-column, Lichrosorb RP-18, 5μ, 250×3.2 mm column, and a ultraviolet detector measuring at 318 nm. Mobile phase consisted of acetonitrile–0.01 M phosphate solution (NaH_2PO_4), 15:85 (v:v), flow rate was 0.7 mL/min, column temperature 23–25°C (room temperature).

Analytical method was validated before determination of study samples with regard to the following parameters: specificity, limit of detection and quantification, linearity, precision and accuracy, inter-day variability, intra-day variability, stability in the freezer and stability in the autosampler. The minimum quantifiable concentration (lowest concentration in the calibration curve) was 0.1 μg/mL. The method was linear over concentration range 0.1–30 μg/mL.

4.4.2. Meropenem

Quantitative analysis of meropenem in plasma was preformed by standard HPLC technique over the concentration range of 0.1–150 μg/mL (9 samples) in plasma. The HPLC system consisted of WatersTM 717 plus Autosampler

(Waters Millipore, USA), Alltech 426 HPLC pump (Alltech Associates Inc, USA) and Waters 486 Tunable absorbance detector (Waters Millipore, USA). Chromatography was preformed on an analytical column Inertsil® ODS-3 C18 (150 x 4.6 mm I.D., 5 µm) (GL Science Inc., Japan), protected by a Platinum C18 (7.5 x 4.6 mm I.D., 5 µm) precolumn (Alltech GmbH, USA). Chromatography software Kromex, ver 32S (Akrom-EX, Estonia) was used for data acquiring. For plasma samples the adsorbance of meropenem was detected at 302 nm.

Meropenem was quantified in PF by a high-performance liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method. Dialysate samples were injected directly using a temperature-controlled (4°C) autosampler. The HPLC column [Xterra® MS C18 (150 mm x 3.9 mm; 5µm),Waters, Saint Quentin en Yvelines, France] was connected to the MS/MS system [Quattro Micro Mass Spectrometer (Waters, Saint Quentin en Yvelines, France) equipped with an electrospray ionisation interface. The mobile phase was composed of a mixture of solvent A (water, formic acid (99.9/0.1, v/v)) and solvent B (acetonitrile, formic acid (99.9/0.1, v/v)). The flow was split ¼ before entry in the Mass Spectrometer. MS/MS detection in positive ion mode by multiple reaction monitoring, nitrogen as desolvation gas (300 $L.h^{-1}$), and as cone gas (100 $L.h^{-1}$); argon as collision gas; temperature at 120°C, the desolvation temperature at 300°C, the cone voltage 20V and the capillary voltage 3.5kV] allowed detection of meropenem (m/z 384.3 → m/z 340.1).

4.5. Pharmacokinetic calculations

Individually obtained values were used for calculation of *in vivo* recovery of the metronidazole according to the following equation:

$$\text{Recovery (\%)} = 100 - (\text{Cdialysate} / \text{Cperfusate}) \times 100$$

The concentration where calculated using equation:

$$\text{Concentration (mg/L)} = (\text{Cdialysate} \times 100) / \textit{in vivo}\ \text{recovery},$$

PK profile of metronidazole was calculated using PCNONLIN software (version 4.2, SCI Software, a ClinTrials company, Lexington, Kentucky, USA) and Kinetica 2000 (version 3.0 demo, InnaPhase Corporation, USA). AUC's were calculated according to trapezoidal rule. Cl, half-life($T_{1/2}$), Vd at steady state(Vd_{ss}) were obtained from two-compartmental model. AUC_{0-10} muscle/AUC_{0-10} plasma and $AUC_{0-\infty}$ muscle/$AUC_{0-\infty}$ plasma ratios were used as characteristics of metronidazole penetration into muscle tissue. C_{max}/MIC_{90} ratio

and $T_{>MIC}$ were calculated and used as surrogate parameters for antimicrobial efficiency.

Meropenem concentrations were determined in the perfusate and in dialysates to determine the *in vivo* relative recovery by loss, but we performed the calibration at the end of study, which means that tissues were not free of study drug. Thus, we corrected measured recovery using following equation:

$$\text{Recovery (\%)} = [(\text{Cperfusate} - \text{Cdialysate})/ \text{Cdialysate}] + \text{Cpf*}/\text{Cperfusate}$$

where Cpf* is the estimated concentration of meropenem in the dialysate at the midpoint of the corresponding retrodialysis period during the post study timeframe, before the next dose was administered. Values of Cpf* were determined by extrapolation of the log-linear decline of the concentrations measured in each patient.

The concentration where calculated using equation:

$$\text{Concentration (mg/L)} = (\text{Cdialysate} \times 100) / \textit{in vivo} \text{ recovery},$$

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

Nonlinear regression analysis (SAAM II, version 1.2.1) was utilized to characterize parameters for a compartmental model that assumed linear distribution and elimination kinetics for meropenem in plasma and PF.

Data from the second dose of 1000 mg infused at a constant rate over 20 minutes, beginning at 480 min after the first dose, without an assumption of steady state, were analyzed. The plasma concentration-time data were assumed to reflect unbound levels in view of the negligible protein binding of this antibiotic (Robatel et al., 2003).

Visual inspection of the plasma and PF concentration-time data in individual patients suggested that the unbound AUC measured in the PF was less than that in the plasma, with the early concentration time-course in the PF being much lower than that in plasma. Modelling of each patient's plasma and unbound PF concentration-time data of meropenem was conducted in two phases. In the first phase the plasma concentration-time data were analyzed using a two-compartment open model. The four parameter estimates obtained were then fixed and a forcing function was used to estimate input and output rate constants for the PF compartment. The PF was described as one compartment with first-order entry and exit. It was assumed that meropenem distribution between the plasma and PF was governed by a passive diffusion processes. Therefore unbound distributional Cl was assumed to be equal. The differences in the corresponding AUCs were then considered to result from the instability of meropenem in PF observed in *ex vivo* degradation experiments (vide infra).

Thus, the model also included a first-order rate of loss (degradation) from the PF. The volume of the PF compartment was assumed to be equal to the volume measured postoperatively by drainage of the cavity. Because no drainage could be obtained from patient 14, a modest volume of 0.1 L was assumed.

Using point estimates of the parameters, the concentration-time profiles of meropenem in plasma and PF in each patient were simulated over three days of dosing (steady-state). Assuming that the dose was administered at a constant rate over 20 minutes, three meropenem regimens were simulated:

A. 1000 mg every 8 hr (the regimen used in the current study)
B. 750 mg every 6 hr (representing the same daily dose used in regimen A)
C. 1000 mg every 6 hr (representing a 33% increase in daily dose over regimens A and B)

The simulated profiles obtained over the last dosing interval on day 3 for each patient were examined. Steady state was confirmed by comparing concentrations at the beginning and end of the interval.

The *ex vivo* determination of meropenem stability in PF was considered only after this microdialysis study had shown that meropenem concentrations in PF were lower than those in plasma. Therefore this *ex vivo* stability assay was conducted using PF from patients distinct from those participating in the *in vivo* microdialysis study. Fresh PF collected from five patients with peritonitis was spiked with meropenem at an initial concentration of 25 μg/mL and incubated at 37°C for 8 hours. Samples were collected every 60 min and assayed for meropenem concentrations using LC-MS/MS as described above. Visual inspection of the concentration versus time profiles indicated first-order degradation. Individual linear regression analyses were conducted to estimate degradation rate constants.

$T_{>MIC}$ was calculated and used as surrogate parameters for antimicrobial efficiency against susceptible (MIC_{90} < 4 mg/L) and resistant microorganisms (MIC_{90} ≥ 16 mg/L), which were defined according to Clinical and Laboratory Standards Institute (CLSI, 2006).

4.6. PK – PD model

MIC_{90} and time-kill studies were performed with two clinical strains of *B. fragilis* with MIC_{90} 0.125 mg/L and 1 mg/L. Two replicates of each strain were used and arithmetical mean was presented in the results.

Growth and time-kill assays were performed in pre-reduced and cation-adjusted Wilkins-Chalgren Broth (Oxoid, Basingstoke, UK), containing vitamin K and haemin. Wilkins-Chalgren Agar (Oxoid, Basingstoke, UK) enriched with 5% sheep blood was used for plating experimental samples for colony number

determination. All media was pre-reduced in an anaerobic chamber before inoculation.

The MIC_{90} of metronidazole for both used isolates was determined by E-test (AB Biodisk, Solna, Sweden) as proposed by the manufacturer. Pre-reduced blood agar plates were used for susceptibility testing. Inoculated plates were incubated at 35ºC inside an anaerobic chamber (Bactron, Sheldon Manufacturing, Portland, OR, USA). Both *B. fragilis* strains were fully susceptible to metronidazole according to published breakpoints.

Both organisms were placed on the Wilkins-Chalgren Agar plates and incubated overnight at 35ºC in an anaerobic chamber. The microorganisms were diluted with pre-reduced sterile saline until the turbity of suspension matched a 0.5 McFarland Standard ($\sim 1\times10^8$ cfu/mL). The suspension (0.5 mL) was then used to inoculate into test flasks with Wilkins-Chalgren-Broth. This yields a starting inocula of approximately $1–5\times10^6$ cfu/mL. The actual size of each inoculum was also determined via colony counts.

Based on the microdialysis derived mean time-concentration curve from the muscle tissue after 500 mg of i/v metronidazole administration we simulated a similar curve in an *in vitro* model. We also performed similar experiments for tissue concentration-time profiles following 250 mg and 1000 mg doses of metronidazole by multiplying the 500 mg profile by factor 0.5 and 2, respectively, as a linear relationship exists between dose and plasma concentration for doses of 500 to 2000 mg. 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 of the drug concentration up to the level equivalent to the peak level in the ECF. 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 35ºC for 48 h and colony counts were performed visually. A reazurin indicator was used to ensure that conditions remained anaerobic throughout the experiments. Results were assessed by plotting $\log_{10}$ cfu against time. The killing rate over time was determined as bactericidal if a reduction of three 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.

4.7. Statistics

The data are presented as arithmetical means and standard deviation (SD), if not stated otherwise. PK data were compared using nonparametric two-tailed Mann-Whitney U-test, using Graph Pad Prism (San Diego, California, USA) software. $P < 0.05$ was considered as statistically significant.

The change in colony counts over time was determined by linear regression analysis of the time-kill plots. The time to $\geq$ 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, California, USA) software.

5. RESULTS

5.1. Metronidazole in perioperative prophylaxis (paper I)

Demographic characteristics of the patients are shown in Table 4. One patient had history of supraventricular ectopic beats, but had no medication on use, while another patient had mild mood disorder requiring citalopram 10 mg once daily. All women underwent gynaecologic surgery; four of them had laparoscopic procedures, one vaginal hysterectomy and one – myomectomy via laparatomy. Surgery was uneventful in all cases, no infectious complications were detected in postoperative period, and patients were discharged from hospital according to common routine. Data from patient 4 were excluded due to technical problems with microdialysis sampling (blood staining).

Table 4. Demographics of elective surgical patients receiving metronidazole as perioperative prophylaxis

	Patients					
	1	2	3	5	6	Mean (SD)
Age	49	38	30	46	37	42.5 (9.1)
Gender	F	F	F	F	F	
Weight, kg	68	86	53	62	45	62.8 (13.9)
Height, m	1.72	1.70	1.62	1.58	1.58	1.63 (0.1)
BMI, kg/m^2	23.0	29.8	20.2	24.8	18.2	23.5 (4.0)
I.v. fluids during the study period, mL	3000	2750	2000	2500	2500	2542 (332)

BMI – body mass index

Recovery of metronidazole from the muscle tissue ranged from 40.1 to 64.8% (mean value 50.0 (9.7) %).

The individual time vs. concentration curves of metronidazole in plasma and muscle tissue after a single dose of 500 mg are shown in Figure 4a and Figure 4b. Figure 4c compares mean concentrations in plasma and muscle tissue with mean MIC_{90} for the *B. fragilis* group, which was 0.25 (0.26) mg/L. Both, plasma and muscle mean metronidazole concentrations are substantially higher than MIC_{90} for whole observation period.

PK parameters, calculated from plasma and muscle tissue concentrations, are shown in Table 5. $AUC_{0–10}$ muscle /$AUC_{0–10}$ plasma and $AUC_{0–\infty}$ muscle / $AUC_{0–\infty}$ plasma ratios, 0.73 (0.16) and 0.91 (0.19) respectively, indicate good penetration of metronidazole into the muscle tissue. To assess antimicrobial efficiency of the drug surrogate parameters C_{max}/MIC_{90} ratio and $T_{>MIC}$ were calculated. C_{max}/MIC_{90} ratio was 65.8 (18.5) for plasma and 31.1 (6.2) for muscle tissue. The $T_{>MIC}$ and time over four times MIC_{90}, calculated by fitting

the data from plasma samples into the two-compartmental model, were 52.1 (13.5) and 33.2 (8.7) hours, respectively.

Table 5. Main PK parameters of metronidazole calculated from the blood and micro-dialysis samples of elective surgical patients. Data are mean (SD).

	$AUC_{0-\infty}$ mg/L×h	AUC_{0-10} mg/L×h	$T_{1/2\beta}$ h	C_{max} mg/L	t_{max} min	Cl mL/min	Vd L
Plasma	178.9 (39.6)	76.0 (19.4)	12.9 (4.9)	16.5 (4.6)	30[a]	46.6 (10.3)	45.4 (6.1)
Muscle	112.9 (46.9)	54.1 (10.7)		7.8 (1.5)	114 (74.7)		

AUC, area under the concentration curve; $T_{1/2\beta}$, elimination half-life; C_{max}, maximum concentration; T_{max}, time to maximum concentration; Cl, clearance; Vd, volume of distribution
a – time of the first blood sample

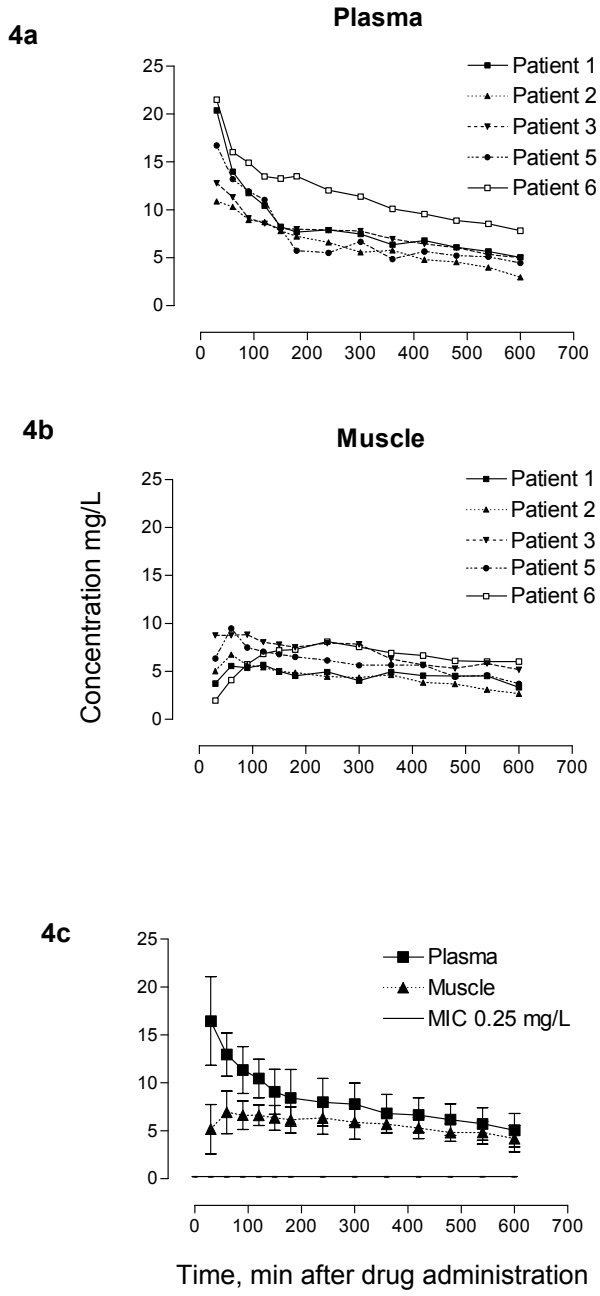

Figure 4. Individual time vs. concentration curves of metronidazole in plasma (4a) and muscle tissue (4b) after a single intravenous dose of 500 mg. Mean concentrations and SD are shown in panel 4c. Mean MIC_{90} for the *Bacteroides fragilis* group, detected by microbiology laboratory of Tartu University Hospital, is shown by solid line.

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5.2. Metronidazole in septic shock (paper II)

Demographic, laboratory and instrumental data of patients in septic shock are presented in Table 6. Causes of septic shock were, as follows: two cases of bronchopneumonia, two cases of peritonitis from the purulent cholecystitis and colonic perforation, one case of urosepsis and one case of *phlegmone* of upper extremity. Only two patients survived to ICU discharge.

Table 6. Demographics and clinical characteristics of patients in septic shock receiving metronidazole.

	Patient						Mean (SD)
	7	8	9	10	11	12	
Age	66	32	63	65	69	54	58.2 (13.8)
Gender	M	M	M	M	M	M	
Weight, kg	70	75	70	85	95	70	77.5 (10.4)
Height, m	1.75	1.80	1.70	1.70	1.75	1.70	1.70 (0.04)
BMI, kg/m^2	22.9	23.1	24.2	29.4	31.0	24.2	25.8 (3.5)
APACHE II (at admission)	16	5	15	22	44	24	21 (13.1)
Vasopressors during the study	D /N/Db	D / N	D / N	D / N	D / N	D / N	
I.v. fluids within12 h after inclusion, mL	1800	2475	2095	2170	3500	2280	2358 (627)
Urine output within 12 h after inclusion, mL	500	CVV HD	1950	1350	900	1350	1218 (532)
Fluid gain within 12 h after inclusion, mL	900	1825	0	700	2500	880	1134 (887)
Leucocytes count, at inclusion, mm^{-3}	15.5	5.53	17.3	15.7	18.2	29.5	
CRP, at inclusion, mmol/L	178	255	251	101	292	289	
Haemodynamic profile at inclusion							
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, $dynes \times sec \times cm^{-5}/m^2$	1444	1894	2144	1974	2159	2049	
PVRI, $dynes \times sec \times cm^{-5}/m^2$	176	128	341	325	510	406	

Table 6. Continuation

	Patient						Mean (SD)
	7	8	9	10	11	12	
Oxygen profile at inclusion							
D_{O2}, mL/min/m^2	557	442	471	490	559	624	
V_{O2}, mL/min/m^2	152	193	138	164	180	132	
FiO_2, %	50.0	40.0	45.0	45.0	50.0	40.0	
PaO_2, mmHg	86.0	80.0	176	155	180	114	
SvO_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	
ICU outcome	Died	Died	Surv.	Surv.	Died	Died	

APACHE – Acute Physiology and Chronic Health Evaluation; D – dopamine; N – noradrenaline; Db – 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 resitance index; D_{O2} – oxygen delivery; V_{O2} – oxygen consumption; Qs/Qt– shunt fraction; FiO_2 – fraction of oxygen; PaO_2 – oxygen partial pressure; SvO_2 – mixed venous blood saturation; Hgb – concentration of hemoglobin; CRP – C-reactive protein, CVVHD – continious venovenous haemodiafiltration; Surv. – survived

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 time vs. concentration curves of metronidazole in plasma and muscle tissue after a single dose of 500 mg are shown in Figure 5. The $AUC_{0–10}$ muscle /$AUC_{0–10}$ plasma ratio 0.88 (0.47) indicates a good penetration of metronidazole into the muscle tissue in patients in septic shock. Other PK parameters, calculated from plasma and muscle tissue time-concentration profiles of the drug, are shown in Table 7.

Table 7. Main PK parameters of metronidazole calculated from the blood and micro-dialysis samples of patients in septic shock. Data are mean (SD)

	$AUC_{0–10}$ mg/L×h	$t_{1/2}$ h	C_{max} mg/L	T_{max} min	CL mL/min	Vd L
Plasma	66.0(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)		

AUC, area under the concentration curve; $T_{1/2\beta}$, elimination half-life; C_{max}, maximum concentration; T_{max}, time to maximum concentration; Cl, clearance; Vd, volume of distribution
a – time of the first blood sample; b – calculated using noncompartmental model

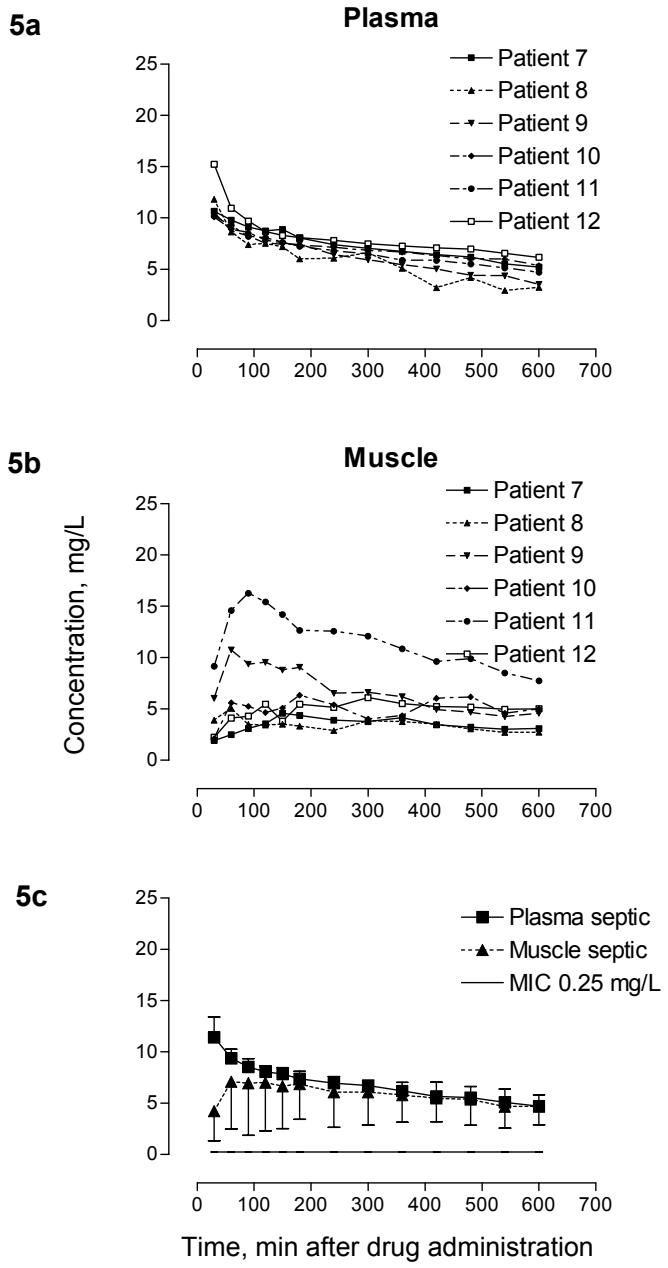

Figure 5. Time course of metronidazole concentrations in plasma and muscle tissue in septic shock patients after a single intravenous dose of 500 mg. The individual time course of metronidazole in plasma (5a) and in muscle tissue (5b); the patients are represented by individual lines. Figure 5c for the mean time course in plasma and muscle tissue compared to *Bacteroides fragilis* mean MIC_{90}.

The PD of metronidazole against two strains of *B. fragilis* are shown in Figure 6 and Table 8. All three simulated metronidazole doses were rapidly bactericidal against both strains of *B. fragilis* tested, with viable counts falling 5–6 logs to undetectable levels and remaining below this limit for the following 10 h. The control regimens (with no drug) exhibited approximately a 3-log increase in cfu/mL by 10 h test time. However, no regrowth on the control plates was detected after 24 h.

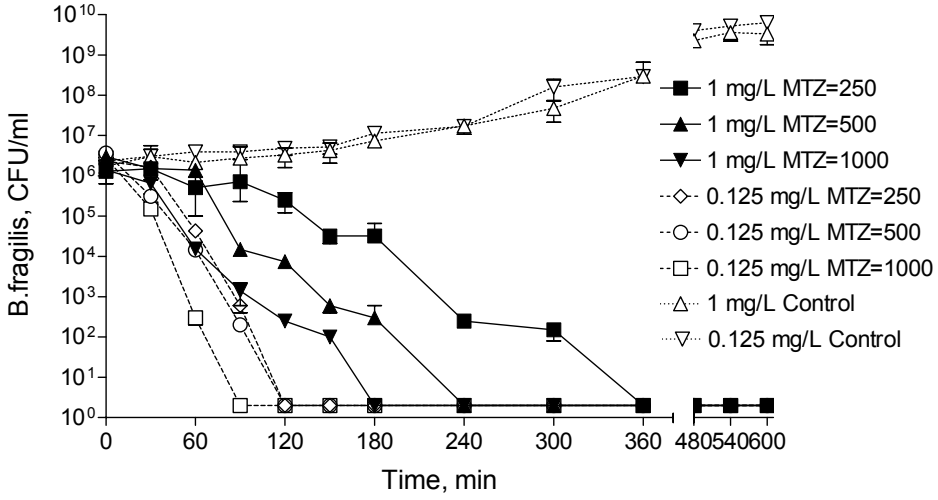

Figure 6. Time – killing curves of *Bacteroides fragilis* after in vitro simulation of time vs. concentration profile of metronidazole (MTZ) in the muscle tissue of patients in septic shock, at different doses.

Table 8. Static and dynamic antimicrobial activity measures of metronidazole obtained from PK-PD model.

Strain, MIC_{90}, mg/L	Metronidazole dose, mg	C_{max}/ MIC_{90}	AUC $_{0–10}$ /MIC_{90}	Time to kill 99,9%, h [a], (SD)	Time to eradication [b], h, (SD)
B.fragilis 0.125	250	28.4	231	1.41 (0.01)	2.53 (0.01)
B.fragilis 0.125	500	56.8	463	1.29 (0.08)	2.27 (0.08)
B.fragilis 0.125	1000	113	926	0.95 (0.03)	1.69 (0.02)
B.fragilis 1.0	250	3.55	29.0	3.47 (0.05)	6.49 (0.05)
B.fragilis 1.0	500	7.10	57.9	2.43 (0.12)	4.33 (0.08)
B.fragilis 1.0	1000	14.2	115	1.84 (0.00)	3.42 (0.00)

[a] time required to achieve a 99,9% kill of the inoculum
[b] time to required to decrease viable counts below the limit of detection

5.3. Metronidazole pharmacokinetics: elective surgical vs. septic patients (paper III)

Studied elective surgical patients substantially differ from septic patients. First, all these patients were women, while septic patients were men. Second, mean weight of elective patients was 62.8 (13.9) kg vs. 77.5 (10.4) kg of septic patients ($p<0.05$). Mean height also differ significantly 1.63 (0.1) m vs. 1.70 (0.04) m, respectively ($p<0.05$). PK parameters, recovery of microdialysis probes and surrogate parameters of antimicrobial activities presented in Tables 9 and 10. Mean time-concentration profiles are presented in figure 7.

Table 9. Comparison of PK parameters of metronidazole in plasma of elective surgical versus septic patients. Data are mean (SD)

	Healthy	Septic shock	p-value
C_{max}, mg/L	16.5 (4.6)	11.4 (2.0)	0.03
T_{max}, min	30	30	
AUC $_{0-10\,t}$, mg/L×h	76.0 (19.4)	66.6 (8.3)	0.54
AUC $_{o-\infty}$, mg/L×h	142 (54.0)	134 (39.5)	0.79
Vd, l	50.6 (20.0)	53.5 (4.0)	0.15
Cl, mL/min	49.0 (27.0)	56.2 (26.9)	0.69
$T_{1/2\beta}$, h	12.9 (4.9)	13.2 (5.3)	0.26

AUC, area under the concentration curve; $T_{1/2\beta}$, elimination half-life; C_{max}, maximum concentration; T_{max}, time to maximum concentration; Cl, clearance; Vd, volume of distribution

Table 10. Recovery, PK parameters and antimicrobial efficiency parameters of metronidazole in muscle tissue of elective surgical versus septic patients. Data are mean (SD)

	Healthy	Septic shock	p-value
Recovery, %	50.0 (9.7)	54.9 (20.0)	0.54
C_{max}, mg/L	7.8 (1.5)	8.2 (4.5)	0.66
T_{max}, min	114 (74.7)	140 (92.3)	0.70
$AUC_{0-10\,t}$, mg/L×h	54.1 (10.7)	57.9 (29.9)	0.79
$AUC_{0-\infty}$, mg/L×h	130 (53.4)	242 (173)	0.33
C_{max} / MIC_{90}	31.1 (6.2)	32.7 (18.1)	0.66
$T_{>MIC}$	23.3 (5.4)	36.2 (25.2)	0.79
AUC_{muscle} / AUC_{plasma} 0–10 h	0.73 (0.16)	0.88 (0.47)	0.79
AUC_{muscle} / AUC_{plasma} 0–∞	0.91 (0.19)	1.69 (0.83)	0.052

AUC, area under the concentration curve; $T_{1/2\beta}$, elimination half-life; C_{max}, maximum concentration; T_{max}, time to maximum concentration; Cl, clearance; Vd, volume of distribution

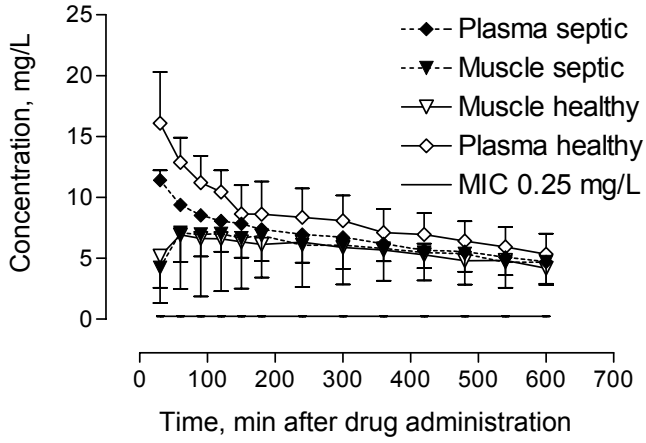

Figure 7. Mean concentration versus time curves in plasma and muscle tissue of elective surgical and septic patients compared to *Bacteroides fragilis* mean MIC_{90}.

5.4. Meropenem in severe peritonitis (paper IV)

Demographic, clinical and laboratory data of the patients in severe peritonitis and septic shock are presented in Table 11. The causes of peritonitis were perforation of duodenal ulcer in two cases, perforation of stomach due to cancer in one case, perforation of large bowel in two cases and large bowel wall necrosis in one case. Only half of the patients survived until discharge from ICU.

Table 11. Demographics and clinical characteristics of patients with severe secondary peritonitis and septic shock

	Patients						
	13	14	15	16	17	18	Mean (SD)
Age, y	52	81	65	77	59	60	65.7 (11.2)
Gender	F	M	M	M	M	M	
Weight, kg	65	50	100	80	67	70	70.3 (16.8)
APACHE II, at admission	9	14	21	34	12	12	16.7 (9.3)
MPI	41	28	38	32	34	30	33.8 (4.9)
Vasopressors	N/D	N/D/Db	N/D	N/D	D	N/D/Db	
Diuretics	Yes	Yes	No	Yes	No	Yes	
I.v. fluids during 24 h after inclusion., mL	13955	10495	11860	11800	9500	7495	10851 (2226)
Urine output 24 h after inclusion, mL	2100	4350	2300	360	2025	1700	2139 (1287)
Fluid gain, 24 h after inclusion, mL	10320	5995	7760	10630	6125	4545	7563 (2477)
Leucocytes count, at inclusion, mm^{-3}	8.3	18.4	3.7	2.5	5.3	19.6	
CRP, at inclusion, mmol/L	364	146	219	281	360	97	
Lactate, at inclusion, mmol/L	7.3	3.0	2.9	6.9	1.3	2.9	
Creatinine, μmol/L	96	123	98	332	49	47	
Creatinine clearance, mL/min	34.8	29.6	n.a.	0	134.7	60.9	52 (51)
ICU outcome	Surv.	Died	Died	Died	Surv.	Surv.	

APACHE – Acute Physiology and Chronic Health Evaluation score; MPI – Mannheim Peritonitis Index; N – norepinephrine; D – dopamine; Db – dobutamine; CRP – C-reactive protein; Surv. – survived

Microdialysis probes recoveries exhibited wide inter patient variability, ranging from 38.5% to 90.3% (mean value 77.2 (19.4)%). The selected pharmacokinetic model with passive inter-compartment distribution and elimination from a peripheral compartment provided adequate fitting in all subjects (Figure 8), even in patient 16 who exhibited a rather erratic profile possibly because of anuria. Degradation half-lives in the PF ranged between 5.6 min and 227.7 min, with an outlier value (288.8 min) in patient 14. Accordingly the steady-state PF to plasma concentration ratio was equal to unity in this patient, whereas it varied between 0.60 and 0.82 in others. The model parameters are given in Table 12. The relatively short terminal phase plasma half-life of meropenem (221 (117) min) confirms that steady-state levels in plasma would be reached by the second day of continued dosing. That the decline in PF concentrations of this antibiotic

appears to mirror that in plasma suggests that steady state would also be achieved in this compartment by Day 2.

The AUC_{ss}, $Cmax_{ss}$, $Cmin_{ss}$, and $T_{>MIC}$ were calculated from simulated plasma and PF profiles for each of the 6 study patients. Table 13 summarizes the data predicted by the model after three distinct multiple dosing regimens. Figure 9 shows $T_{>MIC}$, expressed as % of dosing interval, during which plasma and PF levels of meropenem exceeded the MIC_{90} for susceptible and resistant strains of microorganisms. Where an MIC_{90} of 4 mg/l is assumed, the $T_{>MIC}$ was at least 87% (average for the 6 patients) for any regimen, regardless of whether plasma or PF levels were referenced. However, where an MIC_{90} of 16 mg/l was considered, the average $T_{>MIC}$ was as low as 55% for plasma (in the 1000 mg q8h regimen), and 43% for PF in the same regimen.

The concentration of meropenem in the PF incubates declined mono-exponentially, with a mean $T_{1/2}$ of 948 (264) min (range: 726 – 1332).

Table 12. Individual and mean pharmacokinetic metrics and parameters of meropenem

	Patients						
	13	14	15	16	17	18	Mean (SD)
C_{max} pl, mg/L	68.5	93.0	94.7	120.5	71.5	68.6	86.1 (20.7)
C_{max} pf, mg/L	16.9	28.5	39.2	75.9	31.0	29.6	36.8 (20.4)
Vc, L	5.99	7.50	5.27	4.25	10.15	9.50	7.11 (2.36)
Vss, L	26.5	27.6	17.8	17.8	24.1	29.1	23.8 (4.94)
$T_{1/2}\alpha$, min	3.9	9.2	5.6	5.1	11.9	7.2	7.15 (2.96)
$T_{1/2}\beta$, min	187	255	123	421	96	241	221 (117)
CL, L/min	0.107	0.085	0.116	0.033	0.242	0.090	0.112 (0.07)
Vpf, L	0.6	0.1	0.4	0.2	0.5	0.6	0.400 (0.21)
kdeg, per min	0.00250	0.00024	0.01231	0.00349	0.0107	0.0071	0.0061 (0.0048)
Cldeg, L/min	0.0015	0.000024	0.0049	0.00070	0.0053	0.0043	0.0027 (0.0023)
$T_{1/2}$ deg, min	27.7	288.8	5.6	19.9	6.5	9.8	59.7 (113)
CLin, Clout, L/min	0.0026	0.0090	0.0117	0.0031	0.0080	0.0090	0.0072 (0.0036)
Cpfss/Cplss	0.63	1.00	0.70	0.82	0.60	0.68	0.74 (0.15)

C_{max} pl – observed maximum concentration in plasma; C_{max} pf – observed maximum concentration in peritoneal fluid; Vc – volume of central compartment; Vss – volume of distribution at steady state; Cl – clearance; Vf – volume of peritoneal fluid; kdeg – degradation rate constant; Cldeg – clearance by degradation; $T_{1/2}$ deg – degradadtion half-life; Clin,Clout – clearance into and out of peritoneal fluid; Cpfss/Cpss – ratio of peritoneal fluid to plasma concentrations at steady state.

Table 13. Mean simulated area under the concentration-time curves and minimum and maximum concentrations with three different meropenem dosing regimens at steady state

	Plasma Mean (SD)			Peritoneal fluid Mean (SD)		
Regimen	AUC (0–24) mg×min/L	C_{min}ss mg/L	C_{max}ss mg/L	AUC (0–24) mg×min/L	C_{min}ss mg/L	C_{max}ss mg/L
3000 mg/day	37525	12.5	92.8	29471	11.9	32.3
(1000 mg q8h)	(26914)	(15.6)	(32.9)	24523	14.0	19.9
3000 mg/day	37525	14.9	74.8	29471	14.0	28.6
(750 mg q6h)	(26914)	(17.1)	(29.6)	(24523)	(15.4)	(19.1)
4000 mg/day	50033	19.9	99.8	39294	18.7	38.1
(1000 mg q6h)	(35885)	(22.8)	(39.4)	(32698)	(20.5)	(25.4)

AUC (0–24) – area under the concentration-time curve; C_{min}ss – minimum (trough) concentration in plasma and PF before the administration of next meropenem dose at steady state; C_{max}ss – maximum concentration of meropenem in plasma and PF after dose administration at steady state

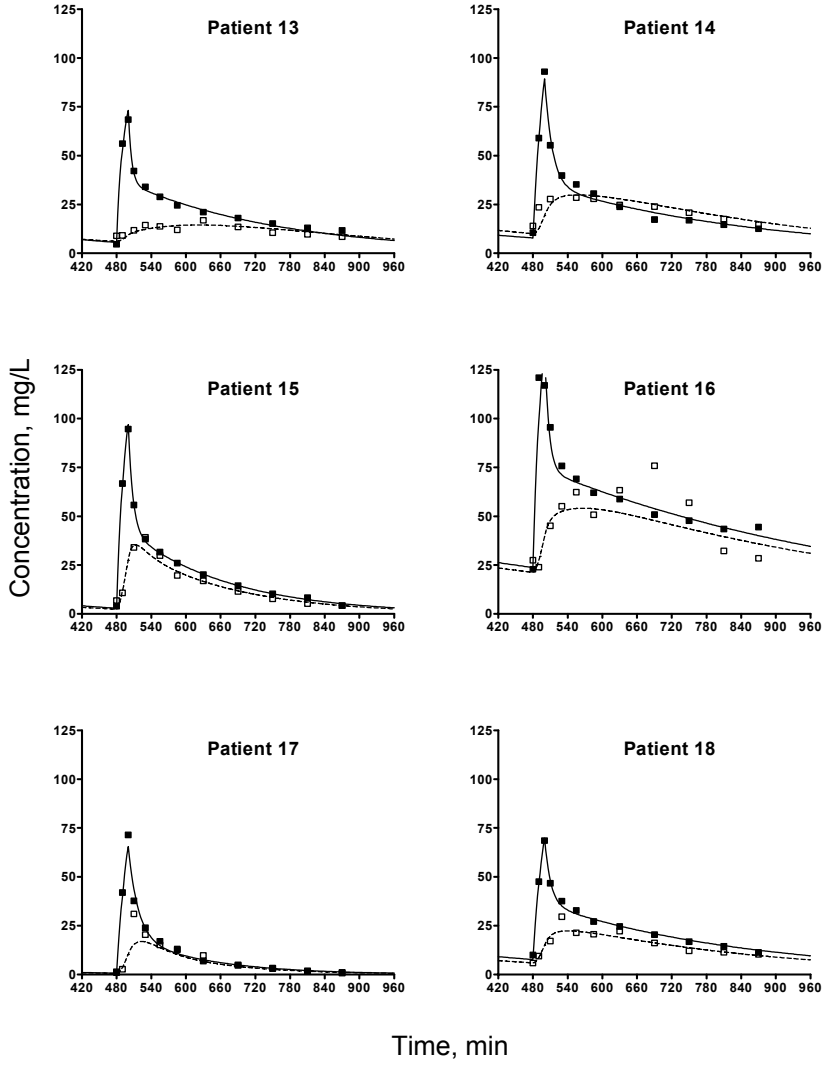

Figure 8. Time course of meropenem in plasma and peritoneal fluid in individual patients after a intravenous dose of 1000 mg infused over 20 min. Closed and open symbols represent measured concentrations in plasma and peritoneal fluid, respectively; solid and dashed lines represent computer predicted functions for plasma and peritoneal fluid concentrations, respectively.

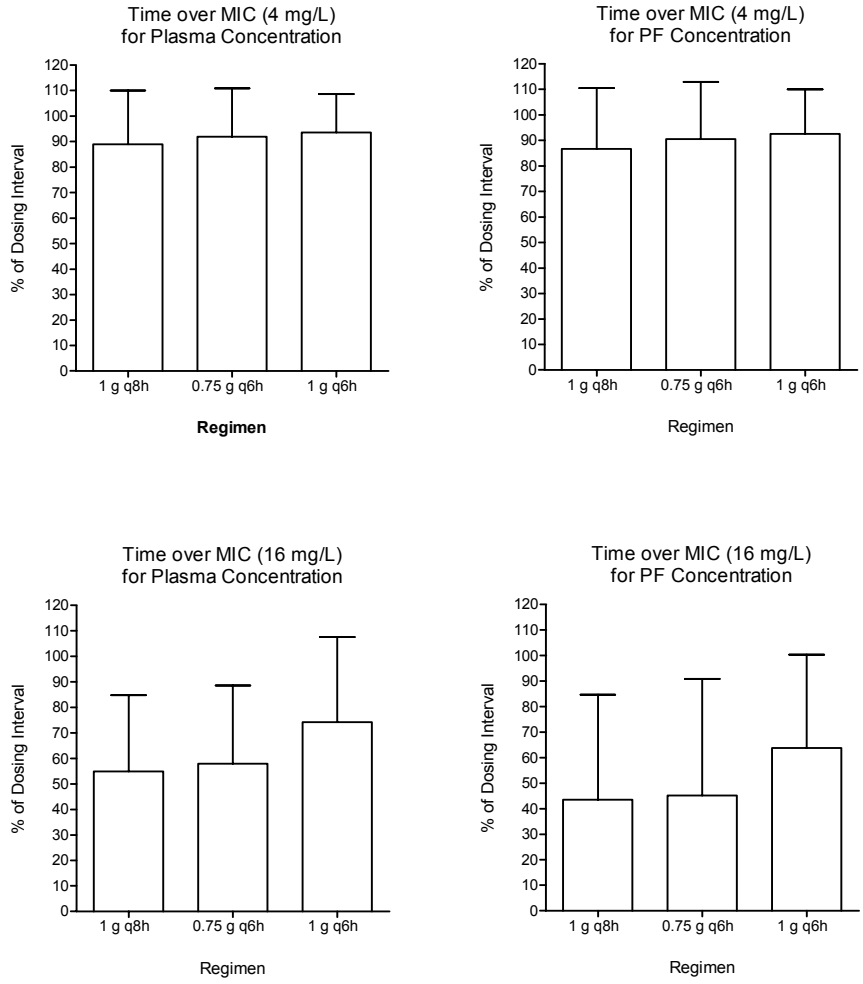

Figure 9. Percentage of time (mean and SD) during which meropenem concentrations in plasma and in peritoneal fluid are greater than the MIC_{90} for susceptible and resistant microorganisms. Three regimens are represented in simulations representing steady-state conditions for the six patients in the study.
$T_{>MIC}$ in plasma and peritoneal fluid is expressed as a percentage of dosing interval. MIC_{90}s for susceptible (4 mg/L) and resistant (16 mg/L) bacteria (CLSI, 2006) are represented.

6. DISCUSSION

Present investigation demonstrates that a remarkable inter-individual variability in the target site concentration of antimicrobial drugs exist in patients suffering from septic shock. However, the target-site concentration of studied antibiotics is in most cases high enough to kill susceptible microorganisms. On the other hand, the patients with lowest concentrations of meropenem in our study might be at risk for incomplete antimicrobial killing.

6.1. Methodological considerations

The concentration in the dialysate is never exactly equal to the concentration in periprobe fluid (Plock and Cloft, 2005). Recovery is the concentration in dialysate as a proportion of the concentration in the tissue surrounding the microdialysis probe. The measuring of recovery of the probes *in vivo* is of paramount importance in microdialysis experiment, when absolute numbers are of importance. There are some methods to estimate the *in vivo* recovery of the probe (Stenken J, 1999). We employed retrodialysis or delivery method, which have a reasonably good prediction error < 15% (Ståhle et al., 1991; Muller, 1995) and is not very time consuming as golden standard: no-net flux method (Stenken J, 1999). Moreover, the retrodialysis is at least as accurate as no net flux method in tissues without active transport processes for drugs as muscle tissue and peritoneal cavity (Song and Lunte, 1999). Recovery is dependent on velocity of diffusion, composition of perfusate, perfusion flow rate and tortuosity of sample matrix. The diffusion itself depends on temperature, weight cut-off and membrane area and concentration gradient (Ståhle et al., 1991; Plock and Cloft, 2005). We have controlled majority of these factors during the sampling, except for tissue tortuosity and concentration gradient.

Tissue tortuosity is dependent on tissue composition and blood flow at some extent, higher blood flow increases tissue fluid content and increases the recovery (Song Y and Lunte, 1999, Benveniste and Hüttemeier, 1990). Blood flow to peripheral tissues in septic shock conditions is known to be changed. The patients' condition through the study with metronidazole was stable and noradrenaline infusion adjustments were minimal, the muscle blood flow in well resuscitated septic shock patients are higher compared to non-septic patients and healthy volunteers measured by means of near infrared spectroscopy (Girardis, 2003). The recovery of metronidazole in patients with septic shock shows greater variability compared to elective surgical patients (Table 10). This, at least in part, might be explained by muscle blood flow variability. Patients with severe peritonitis and septic shock were included in resuscitation phase. The alterations in blood flow and noradrenaline infusion

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adjustments might influence the recovery also in these patients, as the splanchnic blood flow increases in septic shock during hypotension and after vasopressor therapy (Ruokonen et al., 1993; Takala, 1997).

Concentration gradient influences the recovery only when the substance is present in periprobe fluid during calibration of the probe, as it was in experiment with meropenem. In situation, when retrodialysis performed after the first application of the study drug, the drug concentration in perfusate should exceed the tissue concentration by factor of 10 (Plock and Cloft, 2005). We were unable to achieve recommended concentration gradient in four patients, patient 13, 14, 16 and 18, but the concentration of meropenem in perfusate of patient 16 was less than twice higher than in tissue. The concentration of meropenem in perfusate of patients 13, 14 and 18 were at least 3.5 times higher than in periprobe tissue. Therefore, we incorporated the predicted concentration in PF in calculation of recovery.

We generate a trauma introducing a probe into the tissues. Trauma might be qualified as minor, but still it may induce inflammatory response, including periprobe oedema and lymphocyte infiltration. Tissue trauma has shown not to influence upon recovery values and quality of microdialysis samples (Ault, 1994). Comparison of mathematical models with *in vivo* calibration revealed that fitting the model with *in vivo* calibration technique were possible only increasing the volume of ECF, which corresponds to tissue oedema formation (Dykstra et al., 1992, 1993). But it seems that such changes will not affect the probe recovery, as there were no changes in recovery of microdialysis probe in period of 23 days (Martin-Fardon et al., 1997).

6.2. Metronidazole in perioperative prophylaxis

Observed PK data of metronidazole (C_{max}, V_{ss}, Cl and AUC), calculated based on plasma samples, are well in concordance with previous studies (review by Lamp et al., 1999). Although only female patients are included in present study, it would very unlikely influence the results. There is one study showed higher clearance in healthy women compared to men, but only only by 12%, which is hardly clinically relevant (Carcas et al., 2001).

For PK calculations we used a two-compartment model. The model presumes C_{max} in muscle tissue is approximately equal to plasma concentration at T_{max} muscle tissue. However, as seen from microdialysis samples, that was not the case. There are several possible explanations for this. First, plasma total concentration of metronidazole was measured without estimation of protein binding. Second, the microdialysis probe can be considered as a different compartment; and the rate of diffusion into the probe could be different from the rate of diffusion from capillaries into the interstitial space. Third, the recovery from the tissue may not be stable over the time, and this could explain

underestimation of tissue concentration. Fourth, metronidazole might be distributed intracellularly (Hand et al., 1987), and thereby a three-compartmental model could be considered. Metronidazole concentration in human polymorphonuclear leukocytes has shown to achieve the level of interstitial concentration (Hand, 1987). Thus, most likely the intracellular and interstitial compartments could be combined for modelling of metronidazole PK. In our patients we observed that final equilibrium between plasma and muscle concentrations is achieved about 4 hours after the drug administration. From this time point forward the interstitial concentration were in the same range as unbound concentration in plasma.

It has been suggested that metronidazole exhibits a concentration dependent killing effect against anaerobic pathogens (Nix et al., 1995). We found that after single 500 mg intravenous dose the maximum muscle tissue concentration of metronidazole was 7.8 (1.5) mg/L. in literature, the MIC_{90} for metronidazole has been reported as variable as <0.25 – 8 mg/L (Mandell, 1990). The product information of metronidazole quotes MIC_{90} for *B. fragilis* group 0.5 – 1.0 mg/L. Thus, it could be speculated that there exists the possibility that sufficient target concentration of the drug could not be achieved. Therefore we analysed the data from our local hospital microbiology laboratory. The MIC_{90} of "local" *B. fragilis* ranged from 0.016 to 1.0 mg/mL with mean value of 0.25 mg/L. The calculated muscle C_{max}/MIC_{90} ratio (31.1 (6.2)) suggests that in our patients the effective tissue concentration was most likely achieved. These data emphasize the importance of assessment of MIC_{90} in "local" *B. fragilis* groups.

6.3. Metronidazole in septic shock

In the present study, concentration of protein-unbound metronidazole in interstitium of muscle tissue has been measured for the first time in patients with septic shock. Using a microdialysis technique we found that the mean maximum concentration of the drug in muscle tissue of these patients is similar to that observed in setting of perioperative prophylaxis in elective surgery.

Few studies on PK of metronidazole have been performed in critically ill patients. In one study, patients with renal or liver disease expressed prolonged Cl and the $T_{1/2}$ of the drug ($T_{1/2}$ in range from 7.98 to 42.4 h) (Plaisance et al., 1988). Our results neither prove nor refute these findings, as none of our patients had impaired liver function.

The AUC_{0-8} skin /AUC_{0-8} plasma as measure of metronidazole tissue penetration in healthy volunteers was 0.672 (0.196) (Bielecka-Grzela and Klimowicz, 2003). AUC_{0-10} muscle /AUC_{0-10} plasma ratio in our septic patients was 0.88 (0.47), which suggests that tissue penetration of metronidazole in septic shock is at least as good as in healthy volunteers.

Metronidazole appears to exhibit a peak concentration dependent killing effect against anaerobic pathogens Nix et al., 1995). The MIC_{90} of *B. fragilis* from <0.25 to 8 mg/L has been reported (Mandell, 1990). Thus, it could be speculated that there is a possibility that the sufficient target concentration of the drug could not be achieved. Therefore we performed PK – PD model with two different *B. fragilis* strains, isolated in the microbiology laboratory of our hospital. Among the number of isolated strains, one with the lowest and one with the highest MIC_{90} for metronidazole, were chosen. The model is based on the interstitial concentration of metronidazole, measured in the muscle tissue of the septic patients. Results of the experiments confirmed the high efficiency of the drug against *B. fragilis* strains. Eradication time for the B. fragilis with the highest MIC_{90} was only 6.49 (0.05) h for the lowest dose studied and no regrowth were detected within the next 24 h.

Important limitation is that metronidazole concentration in the test flasks was not controlled during *in vitro* experiments. However, to the best of our knowledge metronidazole is chemically stable and not metabolised 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 *in vitro* experiments. At 3 hours the volume of broth in the test flask was increased by 15%, while the count of bacteria was decreased at least by 10 times (Fig 6); at 6 hours 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 rather marginal in our experiments.

6.4. Metronidazole pharmacokinetics: elective surgical vs. septic patients

We established that C_{max} of metronidazole in plasma of septic shock patients appears to be lower compared to elective surgical patients. The maximal muscle tissue concentration after i/v administration of 500 mg of metronidazole is similar in surgical patients undergoing gynaecological procedures and patients suffering from septic shock. Furthermore, other PK characteristics and antimicrobial efficiency parameters in muscle tissue of septic patients were also not different from that we observed in elective surgical patients.

Increased Vd due to capillary leakage and interstitial oedema as well as the difference in haemodynamic profile and application of haemodialysis in septic patients most likely explain the difference in maximal plasma concentrations. These aspects are probably causative for wide variation of the data in septic patients, which is noteworthy (Fig 6b).

From fitting the plasma concentrations into the two-compartment model we observed $T_{1/2}$ of metronidazole in septic patients similar to elective surgical patients. However, our results (13.2 (5.3) h in septic patients) are somewhat different from that reported on volunteers (6–10 h) (Lamp et al., 1999). Therefore, it is difficult to conclude whether the $T_{1/2}$ of metronidazole is prolonged or not in septic shock.

Taking studies on metronidazole together, our clinical and experimental data suggest PK of metronidazole are not significantly altered by septic shock. The achieved target-site concentration is sufficient to kill anaerobic bacteria. Our data allow speculating that it would be enough to administer metronidazole twice or even once daily in patients with septic shock. However, such change of clinical practice needs studies with multiple dosing regimes as described by Lewis et al. They established the PK – PD model after oral administration of metronidazole to determine whether the newer extended release oral metronidazole, twice or once daily, had the same efficiency compared with immediate release oral metronidazole three times a day. Similar to us, the authors obtained rapid bactericidal activity (≥ 99.9% reduction) by 12 h with both regimens and no regrowth during the next 48 h (Lewis et al., 2000).

6.5. Meropenem in severe peritonitis

The PK of meropenem in critically ill patients exhibiting major disorders in circulation and renal function has already been described in a number of studies (Lovering et al., 1995; Kitzes-Cohen et al., 2002; Novelli et al., 2005). Our results are in general agreement with previously reported values. Tissue distribution of meropenem has also been previously investigated using tissue biopsies (Condon et al., 1997; Gal et al., 1997). Only one distribution study was conducted with microdialysis to investigate meropenem distribution in lung extracellular fluid (Tomaselli et al., 2004).

According to classical theory of pharmacokinetic principles, where passive diffusion of drug between blood and tissue predominates, distributional Cl that oppose each other are equal and therefore steady-state unbound drug concentrations in tissue ECF and blood during continued dosing should be equivalent (Rowland and Tozer, 1995). A major exception to this is noted for organs protected by physiological barriers such as the brain. In addition, tissue and blood AUCs measured as unbound levels following a single dose should also be equal. However such was not the case for meropenem in PF. Redistributed and shunted mesenteric blood flow as may be anticipated in critical care patients with septic shock (Takala, 1997; Ruokonen et al., 1993) may decrease the rate of meropenem PF distribution and therefore delay the PF peak concentration. But blood flow alone has no effect on the unbound tissue to plasma concentrations ratio at steady-state nor on the corresponding AUC after single

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dose administration (Rowland and Tozer, 1995). Although total meropenem concentrations were not determined in the present study its protein binding is only 2% (Robatel, 2003) and is very unlikely to contribute to the observed discrepancy between plasma and PF AUCs. The presence of a physiological barrier with active efflux transport systems in the peritoneal cavity has never been described, and this was therefore not considered to contribute to the observed reduced meropenem PF concentrations. Alternatively, meropenem degradation in PF was considered to be a possible factor, and indeed assessment of this experimentally *ex vivo*, in the PF of additional patients indicated that this might be contributing to the difference in AUCs.

Accordingly, the model assumed that passive diffusion was the only mechanism of distribution of this drug, and that loss of meropenem (by metabolism or other degradative means) occurred in PF. In the compartmental modelling the values of the distributional clearances between plasma and PF (as well as the degradation clearance in PF) are directly dependent on the volume of the PF. However, the ratio of AUCs (AUCpf/AUCpl), as well as the rate constant for degradation, is independent of the volume of the PF. Estimates of these volumes were made based on recovered fluid from the peritoneal cavity following the study, although no direct measurements were made during the study period. To the extent that uncertainty in these volumes exists, a corresponding uncertainty in the distributional clearances is present. Nevertheless an accurate estimate of this volume was not needed to characterize the AUC ratio nor the degradation rate constant determined in the modelling. A pharmacokinetic model consistent with a peripheral degradation hypothesis was therefore successfully developed to describe the data. The decay of meropenem plasma concentrations with time was clearly bi-exponential and therefore the parameters of a two-compartment open model were fit to the data. PF concentrations were obviously not a part of the central compartment and it was found that the time of peak levels in the peripheral compartment was earlier than the T_{max} for PF concentrations. The final model therefore incorporated a third compartment corresponding to PF.

From complementary studies conducted *ex vivo*, it was confirmed that meropenem is not stable in the PF of patients with peritonitis. Whether this degradation is mediated by enzymes or by other means is not clear, but it could be enzymatically mediated since we have also observed that meropenem in aqueous solution was stable at 37°C for 8h. However, the degradation rate constants determined *ex vivo* in PF in different patients with peritonitis were much less (approximately 7-fold on average) than those determined *in vivo*. A separate group of patients was examined for the *ex vivo* experiments, possibly contributing to the observed differences. Patients participating in the *in vivo* study were more critical (only 2 of 6 survived) than those not in septic shock who provided PF fluid for the *ex vivo* study (all survived). Other unknown sources of interpatient variability or differences in the *in vivo* and *ex vivo* conditions may also have existed.

Meropenem is considered to exhibit time-dependent killing (Mouton et al., 2000). This implies that antibacterial efficacy requires concentrations to be maintained at a certain minimal level for a sufficiently long period of time. This issue was addressed using simulations of the time-course of antibiotic levels in plasma or at the target site (Table 13). For all regimens, the mean minimum and maximum levels in both plasma and PF were above the MIC_{90} for susceptible strains (4 mg/l), although both the minimum plasma and PF levels were less than 4 mg/l in 2 of the 6 patients. Considering PF levels to be the target site, where an MIC_{90} of 16 mg/l was assumed (resistant strains), average steady-state maximum levels were higher than this value for all 3 regimens (3 and 4 g/day), although not all patients dosed at 3 g/day reached this value. However all of the patients exhibited maximum PF levels of meropenem that exceeded the MIC_{90} when dosed at 4 grams per day (1g every 6 hr). Therefore peritoneal concentrations of meropenem in patients suffering from severe secondary peritonitis associated with septic shock are generally sufficient to produce bacterial killing effect on susceptible bacteria using a regimen of 1000 mg every 8 hr. Yet in view of the potential role of instability of this antibiotic in PF, and in view of the observation that levels of this drug in PF may not be sufficient to control infections caused by resistant strains in all patients, the use of higher daily doses of this drug, e.g., 4 g/day may be warranted. However, it should also be mentioned that despite good penetration and relatively high peritoneal concentrations of this agent, the mortality is still high indicating that other factors, such systemic inflammatory response and immune status of the patient, play role in pathophysiology of peritonitis.

7. CONCLUSIONS

1. The intravenous administration of 500 mg metronidazole to the women in perioperative settings as prophylaxis leads to plasma and muscle tissue concentrations, which are much greater than MIC_{90} for common anaerobic pathogens causing surgical wound infection.
2. The intravenous administration of 500 mg metronidazole to the patients in septic shock with different aetiologies results in plasma and muscle tissue concentrations, which are much greater than MIC_{90} for common anaerobic pathogens causing severe sepsis and septic shock.
3. PK-PD modelling by the means of *in vitro* simulation of time-concentration profile obtained from muscle tissue of patients suffering from septic shock shows excellent ability of metronidazole to eradicate the *Bacteroides fragilis* with no regrowth after 24 hours.
4. The intravenous administration of 500 mg metronidazole to the patients in septic shock with different aetiologies results in lower peak plasma concentration than in elective surgical patients, but the concentration in muscle tissue is almost identical.
5. The intravenous administration of single dose of 1000 mg meropenem to the patients with severe secondary peritonitis associated with septic shock leads to peritoneal concentration, which is sufficient to produce antimicrobial killing effect against susceptible bacteria. Peritoneal concentration is lower than plasma concentration and therefore using plasma concentration instead of peritoneal could lead to overestimation of antimicrobial effect.
6. Compartmental analysis confirmed that at steady state meropenem dosage regimen 1000 mg three times daily results in sufficient $T_{>MIC}$ in peritoneal fluid to kill susceptible bacteria.

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9. SUMMARY IN ESTONIAN

METRONIDASOOLI JA MEROPENEEMI FARMAKOKINEETIKA SEPTILISES ŠOKIS PATSIENTIDEL

Sepsis – infektsiooniga kaasnev generaliseerunud põletikureaktsioon – on sageli esinev sündroom, mis on üheks peamiseks surevuse põhjuseks tänapäeva intensiivravis. Sepsise haigestumus on viimase 30 aasta jooksul tõusnud 82.7 kuni 240.4 juhuni 100 000 elaniku kohta aastas. Hoolimata ravitulemuste mõningasest paranemisest viimase dekaadi jooksul, püsib sündroomi surevus väga kõrgena, ulatudes raske sepsise ja septilise šoki korral vastavalt 32.2 ja 54.1 protsendini (Martin jt., 2003, Vincent jt., 2006).

Sepsise ravis on olulisemateks printsiipideks infektsioonikolde (kirurgiline) saneerimine, antibakteriaalne ravi ja infusioonravi. Kuigi penitsilliini avastamine ja kasutuselevõtt tõid esialgu olulise murrangu infektsioonhaiguste ravis, on mikroobide kiire evolutsioon ja sellest tulenev ravimresistentsus põhjustanud olulisi tagasilööke infektsioonide ravis. Üheks võimalikuks ebaõnnestumise põhjuseks võib olla antibiootikumi ebaadekvaatne kontsentratsioon infektsioonikoldes. Kaasaegsete antibiootikumide annustamise soovitused tuginevad väga paljus tervetel vabatahtlikel tehtud uuringutele. Samas on teada, et patofüsioloogilised muutused kriitilises seisundis oleval patsiendil võivad olulisel määral mõjutada ravimite farmakokineetikat. Neeru- ja maksafunktsiooni häired põhjustavad häireid ravimite metabolismis ja eliminatsioonis, agressiivne infusioonravi mõjutab ravimi jaotusruumala. On võimalik, et nende muutuste tagajärjel suureneb hüdrofiilsete ravimite (siia hulka kuulub enamus antibiootikume) ümberjaotusruumala, suureneb ravimite renaalne kliirens ja seega langeb kontsentratsioon infitseeritud koes. Madalam kontsentratsioon võib olla ebapiisav bakteritsiidseks toimeks, ja see omakorda tingib nii ravi ebaõnnestumise kui ka selekteerib välja resistentsed bakterid.

Mikrodialüüs on üle 30 aasta kasutusel olnud koeproovide kogumismeetod, mis põhineb madalmolekulaarsete ainete difusioonil läbi dialüüsimembraani. Mikrodialüüsi on kasutatud erinevate kudede ja elundite (nt. lihaskude, nahaalune rasvkude, luukude, kasvajad, aju, maks, neerud, süda, kopsud, jt.) uurimiseks, muuhulgas ka farmakokineetilisteks uuringuteks. Metoodika abil on võimalik määrata antibiootikumi kontsentratsioon ravimi toimepunkti lähedal, st. märklaudkudedes. Seni on vaid üksikuid andmeid antibiootikumide farmakokineetikast kriitilises seisundis haigetel.

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Uurimuse eesmärgid

Uurimistöö peamiseks eesmärgiks oli kindlaks teha kahe erineva antibiootikumi farmakokineetika septilises šokis patsientidel. Täpsemalt olid eesmärgid järgmised:

1. Kindlaks teha metronidasooli kontsentratsioon lihaskoes kirurgilistel patsientidel, kellel metronidasooli kasutati perioperatiivseks profülaktikaks günekoloogiliste operatsioonide puhul.
2. Kindlaks teha metronidasooli kontsentratsioon lihaskoes septilises šokis haigetel.
3. Hinnata *in vitro* PK/PD mudeli abil, kas metronidasooli lihaskoe kontsentratsioon septilises šokis olevatel patsientidel ja selle muutus ajas avaldab antimikroobset toimet.
4. Hinnata metronidasooli farmakokineetika erinevusi kirurgilistel patsientidel ja septilises šokis olevatel inimestel.
5. Selgitada, kas meropeneemi standardannuse intravenoosne manustamine raske sekundaarse peritoniidi ja septilises šokis patsientidele tagab antimikroobseks toimeks piisava ravimi kõhuõõnesisese kontsentratsiooni. Võrrelda viimast meropeneemi plasmakontsentratsiooniga.
6. Kasutades mitmekambrilist mudelit töötada välja meroneemi optimaalne annustamisskeem raske peritoniidi ravis.

Patsiendid ja metoodika

Esimeses alauuringus olid uuritavateks patsientideks kuus naishaiget, kellel kasutati metronidasooli perioperatiivseks kemoprofülaktikaks plaanilises günekoloogilises kirurgias. Uuringusse ei kaasatud patsiente, kellel esines aktiivne põletikuline protsess (kõrge leukotsütoos) või kes olid ülekaalulised (kehamassi indeks, KMI>30).

Teises alauuringus olid uuritavateks kuus meespatsienti, kes viibisid ravil Tartu Ülikooli Kliinikumi üldintensiivravi osakonnas ning kellel esines septiline šokk. Uuringusse ei kaasatud patsiente, kelle KMI oli üle 35, ning patsiente, kes olid viimase 48 tunni jooksul saanud metronidasoolravi.

Kolmanda uuringu patsientideks olid kuus patsienti, keda raviti Tartu Ülikooli Kliinikumi üldintensiivravi osakonnas raske sekundaarse peritoniidi (vastavalt Mannheimi Peritoniidi Indeksile) ja septilise šoki tõttu. Uuringusse ei kaasatud patsiente, kes olid viimase 48 tunni jooksul saanud meropeneemi.

Plasma ja mikrodialüüsi proove võeti vastavalt ajagraafikule, kahe annuse vahelisel perioodil. Metronidasooli puhul uuriti esimese annuse farmakokineetikat. Meropeneemi puhul osutus esimese annuse uurimine eetilistel kaalutlustel võimatuks, seega viidi uuring läbi peale teist annust. Vereanalüüsid võeti kas veeni või arterisse paigaldatud kanüülist.

Metronidasooli kontsentratsioon määrati kõrgsurve vedelikkromatograafia (HPLC) ja ultraviolettkiirguse neeldumise abil, meropeneemi HPLC – tandem mass-spektromeetria abil.

Tulemused

Metronidasooli piikkontsentratsioon vereplasmas oli septilise šokis patsientidel oluliselt madalam kui tervetel naistel (11.4(2.0) vs. 16.5(4.6) mg/L, $p<0.05$). Teised farmakokineetilised parameetrid ei erinenud oluliselt plaaniliste kirurgiliste ja septilises šokis haigete vahel (Tabelid 5, 7, 9 ja 10). Märkimisväärne on, et lihaskoe kontsentratsiooni muutused ajas on praktiliselt identsed mõlemas rühmas. Kontsentratsioonide ja MIC_{90} võrdlus näitas, et nii $T_{>MIC}$ kui ka C_{max}/MIC_{90} on piisavad bakteritsiidseks toimeks. Septilises šokis patsientidel saadud metronidasooli lihaskoe kontsentratsiooni muutuste ajas *in vitro* modelleerimine kinnitas ravimi head tapmisvõimet *B. fragilise* suhtes (Tabel 8 ja Joonis 6).

Meropeneemi keskmine piik-kontsentratsioon vereplasmas oli 86.1(20.7) ja kõhuõõnes 36.8 (20.4) mg/L. Teised farmakokineetilised ja farmakodünaamilised parameetrid on esitatud tabelis 12. Joonisel 9 on näha, et kõikide annustamisskeemide puhul püsis ravimi kontsentratsioon plasmas ja kõhukoopas vähemalt 87% annustevahelisest ajast tundlike bakterite puhul, aga resistentsete akterite puhul oli see aeg kõhukoopas vaid 43%. Kõhukoopa ja vereplasma kontsentratsioonide võrdleval analüüsil selgus, et meropeneem kõhukoopas ei käitu kahekambrilise mudelile vastavalt, seega kas toimib meropeneemi lagunemine või aktiivne transport kõhukoopast välja. *Ex vivo* katse selgitas, et infitseeritud peritoneaalvedelikus meropeneem laguneb 30% ulatuses..

Järeldused

1. 500 mg metronidasooli intravenoosne manustamine plaanilistele kirurgilistele haigetele tagab vereplasma ja lihaskoe raviminivoo, mis on oluliselt kõrgem kui haavainfektsiooni põhjustavate tavaliste anaeroobsete bakterite MIC_{90} ja seega piisav perioperatiivseks antibakteriaalseks profülaktikaks.
2. 500 mg metronidasooli intravenoosne manustamine erineva etioloogiaga septilises šokiga patsientidele tagab plasma ja lihaskoe kontsentratsiooni, mis on oluliselt kõrgem kui rasket sepsist ja septilist šokki põhjustavate anaeroobsete bakterite MIC_{90}.
3. Modelleerides septilises šokis olevate patsientide lihaskoe metronidasooli kontsentratsiooni ajamuutust *in vitro* PK-PD mudelis, selgus metronidasooli suurepärane *B. fragilise* vastane efekt, ilma taaskasvu tekkimiseta 24 tunni jooksul.
4. Metronidasooli intravenoosne manustamine erineva etioloogiaga septilises šokis patsientidele annuses 500 mg resulteeris madalamas plasma piik-

kontsentratsioonis kui plaanilistel kirurgilistel patsientidel. Lihaskoe kontsentratsioonid olid aga praktiliselt identsed.

5. Ühekordne 1000 milligrammi meropeneemi intravenossne manustamine raske sekundaarse peritoniidi tõttu septilises šokis olevatele patsientidele tagas ravimi kontsentratsiooni kõhuõõnes, mis on piisav antimikroobse toime avaldamiseks tundlikele bakteritele.
6. Kahekambriline mudel kinnitas, et stabiilses seisundis meropeneemi manustamine 1000 mg kolm korda päevas tagab $T_{>MIC}$ peritoneaal vedelikus, mis on piisav antibakteriaalse toime avaldamiseks tundlikele bakteritele.

Töö kokkuvõttes järeldame, et kahe antibiootikumi – metronidasooli ja meropeneemi –farmakokineetika ei ole septilise šoki korral märkimisväärselt erinev normaalsest farmakoloogiast. Tootjajuhendi järgne manustamine tagab ravimi toimeks piisava kontsentratsiooni infektsioonikoldes.

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11. PUBLICATIONS

I

Karjagin, J.; Pähkla, R.; Starkopf, J. (2004).
Perioperative penetration of metronidazole into muscle tissue:
a microdialysis study. European Journal
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II

Karjagin, J.; Pähkla, R.; Starkopf, J. (2004).
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III

Karjagin, J.; Pähkla, R.; Karki, T.; Starkopf, J. (2005). Distribution of metronidazole in muscle tissue of patients with septic shock and its efficacy against *Bacteroides fragilis in vitro*. Journal of Antimicrobial Chemotherapy, 55(3), 341–346.

IV

Karjagin, J.; Lefeuvre, S.; Oselin, K.; Marchand, S.; Tikkerberi, A.;
Starkopf, J.; Couet, W.; Sawchuk, RJ. Pharmacokinetics
of meropenem determined by microdialysis in the peritoneal fluid
of patients with severe peritonitis associated with septic shock.
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Pharmacokinetics of Meropenem Determined by Microdialysis in the Peritoneal Fluid of Patients With Severe Peritonitis Associated With Septic Shock

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Our objective was to describe the pharmacokinetics of meropenem in the peritoneal fluid (PF) of six patients with severe peritonitis and septic shock and to relate measured concentrations to the minimum inhibitory concentration of bacteria. Microdialysis catheters were placed into the peritoneal space during surgery. Meropenem concentrations in plasma and in PF were analyzed using compartmental modeling. Meropenem areas under the concentration–time curve were lower in PF than in plasma (average ratio, 73.8 ± 15%) because of degradation confirmed *ex vivo*. Compartment modeling with elimination from a peripheral compartment described the data adequately, and was used to simulate steady-state concentration profiles in plasma and PF during various dosing regimens. At the currently recommended dosing regimen of 1 g infused over 20 min every 8 h, PF concentrations of meropenem in patients with severe peritonitis associated with septic shock reach values sufficient for antibacterial effects against susceptible, but not always against intermediately susceptible, bacteria.

Sepsis and septic shock are the major causes of mortality in intensive care. Abdominal infections are the second major reason for sepsis and septic shock after chest-related diseases.[1] Overall mortality of secondary peritonitis is 22.6%.[2] Septic shock develops in 11% of patients with peritonitis and results in a significant increase in the relative risk for death.[3] Severe peritonitis, defined as a Mannheim Peritonitis Index of 21–29, has a mortality rate of 22.5%, whereas a Mannheim Peritonitis Index above 29 is associated with mortality of 59.1%.[4]

The most important component of peritonitis management by far is the surgical control of the source of infection. Kirschner[5] reported reduction of mortality from abdominal sepsis from 90 to 40% in the early twentieth century with surgical intervention. In addition, antimicrobial therapy is an essential part of the management of peritonitis, although the therapy must often begin empirically. Carbapenems, as a first choice for empiric therapy, are widely used in the treatment of severe secondary peritonitis associated with severe sepsis and septic shock. The concentrations of meropenem in plasma[6–8] or intra-abdominally through tissue biopsy[9,10] have been evaluated in only a few studies, whereas there are no studies in patients with severe secondary peritonitis associated with severe sepsis or septic shock.

Microdialysis is a well-known sampling technique, allowing the measurement of the concentration of drug continuously and, more importantly, at the infection site.[11] It has been used on many occasions for investigating the kinetics of antibiotics in different tissues: muscle, sub- and intracutaneous tissue, lung, brain, and so on, in both animals and humans.[11–14] It has also been used to monitor intraperitoneal concentrations of ischemic markers, such as lactate, mainly in pigs,[15,16] but also in rats[17,18] and humans.[19] Recently, we have for the first time used microdialysis to investigate the distribution of an antibiotic in the peritoneal fluid (PF) of rats with and without peritonitis.[20]

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The rationale of this study is the need for objective evaluation of the concentrations of antimicrobial drug at the infection site in patients during therapy. The specific aims were to (a) evaluate the intraperitoneal fluid and plasma concentrations of meropenem, (b) describe its pharmacokinetics in patients with severe secondary peritonitis associated with septic shock, and (c) relate the measured concentrations to the minimum inhibitory concentration (MIC) of susceptible and intermediately susceptible microorganisms.

RESULTS

Patients' characteristics are described in **Table 1**. Microdialysis probe recoveries exhibited wide interpatient variability, ranging from 38.5 to 90.3% (mean value: 77.2 ± 19.4%). The selected pharmacokinetic model with passive intercompartment distribution and elimination from a peripheral compartment provided adequate fitting in all subjects (**Figure 1**), even in patient 4, who exhibited a rather erratic profile possibly because of anuria. Degradation half-lives in the PF ranged between 5.6 and 227.7 min, with an outlier value (288.8 min) in patient 2. Accordingly, the steady-state PF to plasma concentration ratio was equal to unity in this patient, whereas it varied between 0.60 and 0.82 in others. The model parameters are given in **Table 2**. The relatively short terminal phase plasma half-life of meropenem (221 ± 117 min) confirms that steady-state levels in plasma would be reached by the second day of continued dosing. That the decline in PF concentrations of this antibiotic appears to mirror that in plasma suggests that steady state would also be achieved in this compartment by day 2.

The area under the concentration–time curve (AUC) over a 24-h period at steady state (AUC_{ss}), peak plasma concentration at steady state ($C_{max,ss}$), trough plasma concentration at steady state ($C_{min,ss}$), and time over MIC (time > MIC) were calculated from simulated plasma and PF profiles for each of the six study patients. **Table 3** summarizes the data predicted by the model after three distinct multiple dosing regimens. **Figure 2** shows time > MIC, expressed as percentage of dosing interval, during which plasma and PF levels of meropenem exceeded the MIC for susceptible and intermediately susceptible strains of microorganisms. Where an MIC of 4 mg/l is assumed, the time > MIC was at least 87% (average for the six patients) for any regimen, regardless of whether plasma or PF levels were referenced. However, where an MIC of 16 mg/l was considered, the average time > MIC was as low as 55% for plasma (in the 1-g q8h regimen) and 43% for PF in the same regimen.

The concentration of meropenem in the PF incubates declined monoexponentially, with a mean half-life of 948 ± 264 min (range: 726–1,332 min).

DISCUSSION

The pharmacokinetics of meropenem in critically ill patients exhibiting major disorders in circulation and renal function has already been described in a number of studies.[6,7,21] Our results are in general agreement with values reported previously. Tissue distribution of meropenem has also been investigated previously using tissue biopsies.[9,10] In particular, Condon *et al.*[9] studied the penetration of meropenem into different tissues and fluids during elective abdominal surgery. Only one distribution study was conducted with micro-

Table 1 Patients' characteristics

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Means ± SD
Age (years)	52	81	65	77	59	60	65.7 ± 11.2
Gender	F	M	M	M	M	M	
Weight (kg)	65	50	100	80	67	70	70.3 ± 16.8
APACHE II, at admission	9	14	21	34	12	12	16.7 ± 9.3
MPI	41	28	38	32	34	30	33.8 ± 4.9
Vasopressors during the study	Nor/Dopa	Nor/Dopa/Dobu	Nor/Dopa	Nor/Dopa	Dopa	Nor/Dopa/Dobu	
Diuretics	Furosemide	Furosemide		Torasemide		Torasemide	
Volume replacement during 24 h after inclusion (ml)	13,955	10,495	11,860	11,800	9,500	7,495	10,851 ± 2,226
Urine output during 24 h after inclusion (ml)	2,100	4,350	2,300	360	2,025	1,700	2,139 ± 1,287
Fluid gain during the 24 h after inclusion (ml)	10,320	5,995	7,760	10,630	6,125	4,545	7,563 ± 2,477
Leukocyte count, at inclusion (mm^{-3})	8.3	18.4	3.7	2.5	5.3	19.6	9.6 ± 7.5
CRP, at inclusion (mmol/l)	364	146	219	281	360	97	245 ± 111
Lactate, at inclusion (mmo/l)	7.3	3.0	2.9	6.9	1.3	2.9	4.1 ± 2.4
Creatinine (μmol/l)	96	123	98	332	49	47	124 ± 106
Creatinine clearance (l/min)	0.035	0.030	NA	0	0.135	0.061	0.052 ± 0.051
ICU outcome	Survived	Died	Died	Died	Survived	Survived	

APACHE, Acute Physiology and Chronic Health Evaluation score; CRP, C-reactive protein; Dobu, dobutamine; Dopa, dopamine; F, female; ICU, intensive care unit; M, male; MPI, Mannheim Peritonitis Index; NA, not available; Nor, norepinephrine.

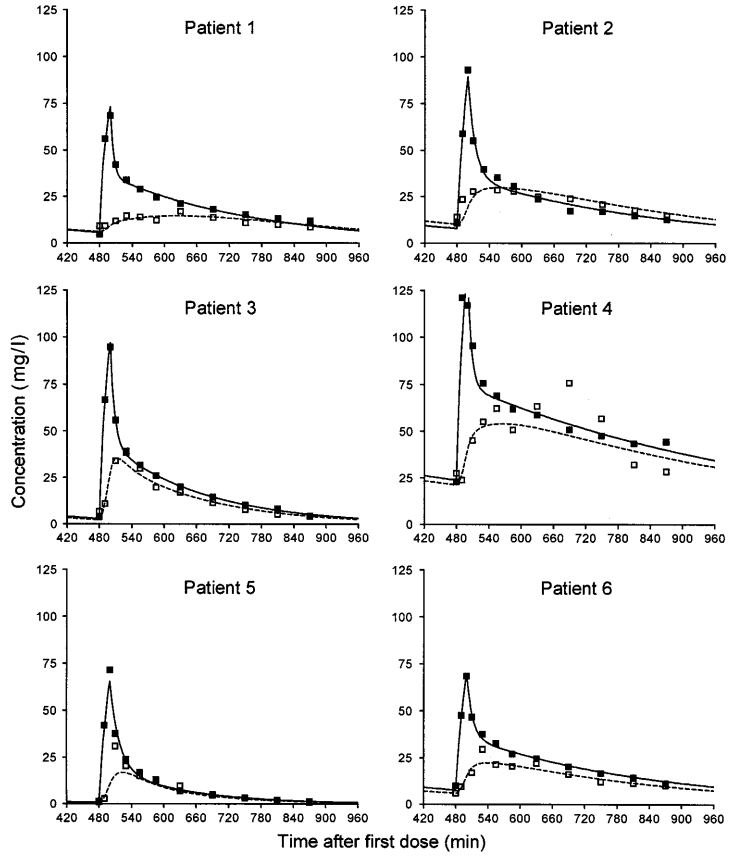

Figure 1 Time course of meropenem in plasma and PF in individual patients after the second intravenous dose of 1 g infused over 20 min. Closed and open symbols represent measured concentrations in plasma and PF, respectively; solid and dashed lines represent computer-predicted functions for plasma and PF concentrations, respectively.

dialysis to investigate meropenem distribution in lung extracellular fluid.[13] The major advantage of microdialysis is that it allows multiple determinations of unbound, pharmacologically active levels at a target site in the same patient, whereas tissue biopsies provide only a single ratio of tissue to plasma concentrations at a certain time. However, as illustrated by the results of this study, where the rate of equilibration of drug with the target site is slow, this ratio varies with time making its interpretation difficult.

The relative microdialysis recoveries of meropenem from PF were quite high in five of the six patients. This was possibly due to the use of 30-mm-long membranes and the relatively low perfusate flow rates used (1 μl/min). Recovery averaged $85 \pm 4.9\%$ (coefficient of variation = 5.8%) for five of the patients. The recovery in patient 4 was only 38%, for reasons unknown. Perhaps the probe placement was such that a portion of the dialysis membrane was not in contact with PF, or was partially obstructed by tissue in the peritoneal cavity.

According to the classical theory of pharmacokinetic principles, where passive diffusion of drug between blood and tissue predominates, distributional clearances that oppose each other are equal and, therefore, steady-state unbound drug concentrations in tissue extracellular fluids and plasma during continued dosing should be equivalent.[22] In addition, tissue and plasma AUCs measured as unbound levels following a single dose should also be equal. However, this was not the case for meropenem in PF. Redistributed and shunted mesenteric blood flow as may be anticipated in critical care patients with septic shock[23,24] may decrease the

Table 2 Individual and mean pharmacokinetic parameters

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Mean ± SD	%CV
$C_{max,p}$ (mg/l)	68.5	93.0	94.7	120.5	71.5	68.6	86.1 ± 20.7	—
$C_{max,pf}$ (mg/l)	16.9	28.5	39.2	75.9	31.0	29.6	36.8 ± 20.4	—
V_c (l)	5.99	7.50	5.27	4.25	10.15	9.50	7.11 ± 2.36	33.2
V_{ss} (l)	26.5	27.6	17.8	17.8	24.1	29.1	23.8 ± 4.94	20.7
$t_{1/2}\alpha$ (min)	3.9	9.2	5.6	5.1	11.9	7.2	7.15 ± 2.96	41.5
$t_{1/2}\beta$ (min)	187	255	123	421	96	241	221 ± 117	52.9
CL (l/min)	0.107	0.085	0.116	0.033	0.242	0.090	0.112 ± 0.07	62.3
V_{pf} (l)	0.6	0.1	0.4	0.2	0.5	0.6	0.400 ± 0.21	524
k_{deg} (min^{-1})	0.00250	0.00024	0.01231	0.00349	0.0107	0.0071	0.0061 ± 0.0048	79.1
CL_{deg} (l/min)	0.0015	0.000024	0.0049	0.00070	0.0053	0.0043	0.0027 ± 0.0023	82.9
$t_{1/2deg}$ (min)	27.7	288.8	5.6	19.9	6.5	9.8	59.7 ± 113	188
CL_{in}, Cl_{out}	0.0026	0.0090	0.0117	0.0031	0.0080	0.0090	0.0072 ± 0.0036	49.8
$C_{pf,ss}/C_{p,ss}$	0.63	1.00	0.70	0.82	0.60	0.68	0.74 ± 0.15	20.2

CL, clearance; CL_{deg}, clearance by degradation; CL_{in}, CL_{out}, clearance into and out of peritoneal fluid; $C_{max,p}$, observed maximum concentration in plasma; $C_{max,pf}$, observed maximum concentration in peritoneal fluid; $C_{pf,ss}/C_{p,ss}$, ratio of peritoneal fluid to plasma concentrations at steady state; CV, coefficient of variation; k_{deg}, degradation rate constant; $t_{1/2}\alpha$, half-life α; $t_{1/2}\beta$, half-life β; $t_{1/2deg}$, degradation half-life; V_c, volume of central compartment; V_{pf}, volume of peritoneal fluid; V_{ss}, volume of distribution at steady state.

Table 3 Mean simulated areas under the concentration–time curve and minimum and maximum concentrations with three different dosing regimens at steady state

	Plasma mean (SD)			Peritoneal fluid mean (SD)		
Regimen	$AUC_{p(0-24)}$ (mg min/l)	$C_{min,p}$ (mg/l)	$C_{max,p}$ (mg/l)	$AUC_{pf(0-24)}$ (mg min/l)	$C_{min,pf}$ (mg/l)	$C_{max,pf}$ (mg/l)
3 g/day	37,525	12.5	92.8	29,471	11.9	32.3
(1 g q8h)	(26,914)	(15.6)	(32.9)	(24,523)	(14.0)	(19.9)
3 g/day	37,525	14.9	74.8	29,471	14.0	28.6
(0.75 g q6h)	(26,914)	(17.1)	(29.6)	(24,523)	(15.4)	(19.1)
4 g/day	50,033	19.9	99.8	39,294	18.7	38.1
(1 g q6h)	(35,885)	(22.8)	(39.4)	(32,698)	(20.5)	(25.4)

$AUC_{p(0-24)}$, area under the plasma concentration–time curve at steady state; $AUC_{pf(0-24)}$, area under the peritoneal fluid concentration–time curve at steady state; $C_{max,p}$, maximum (peak) concentration in plasma at steady state; $C_{max,pf}$, maximum (peak) concentration in peritoneal fluid at steady state; $C_{min,p}$, minimum (trough) concentration in plasma at steady state; $C_{min,pf}$, minimum (trough) concentration in peritoneal fluid at steady state.

rate of meropenem PF distribution and therefore delay the PF peak concentration. However, blood flow alone has no effect either on the unbound tissue to plasma concentrations ratio at steady state or on the corresponding AUC after single dose administration.[22] Although unbound plasma concentrations of meropenem were not determined in this study, its plasma protein binding has been reported to be only 2%,[25] and thus cannot contribute to the observed discrepancy between plasma and PF AUCs. The presence of a physiological barrier with active efflux transport systems in the peritoneal cavity could explain the lower AUCs determined in this liquid. However, such systems have never been described and this was therefore not considered to contribute to the observed reduced meropenem PF concentrations. Alternatively, meropenem degradation in PF was considered to be a possible factor, and indeed assessment of this experimentally *ex vivo*, in the PF of additional patients, indicated that this was more likely contributing to the difference in AUCs. Accordingly, the model assumed that passive diffusion was the only mechanism of distribution of this drug and that loss of meropenem (by metabolism or other degradative means) occurred in PF. In the compartmental modeling, the values of the distributional clearances between plasma and PF (as well as the degradation clearance in PF) are directly dependent on the volume of the PF. However, the ratio of AUCs (AUC_{pf}/AUC_p), as well as the rate constant for degradation, is independent of the volume of the PF. Estimates of these volumes were made based on recovered fluid from the peritoneal cavity following the study, although no direct measurements were made during the study period. To the extent that uncertainty in these volumes exists, a corresponding uncertainty in the distributional clearances is present. Nevertheless, an accurate estimate of this volume was not needed to characterize either the AUC ratio or the degradation rate constant determined in the modeling. A pharmacokinetic model consistent with a peripheral

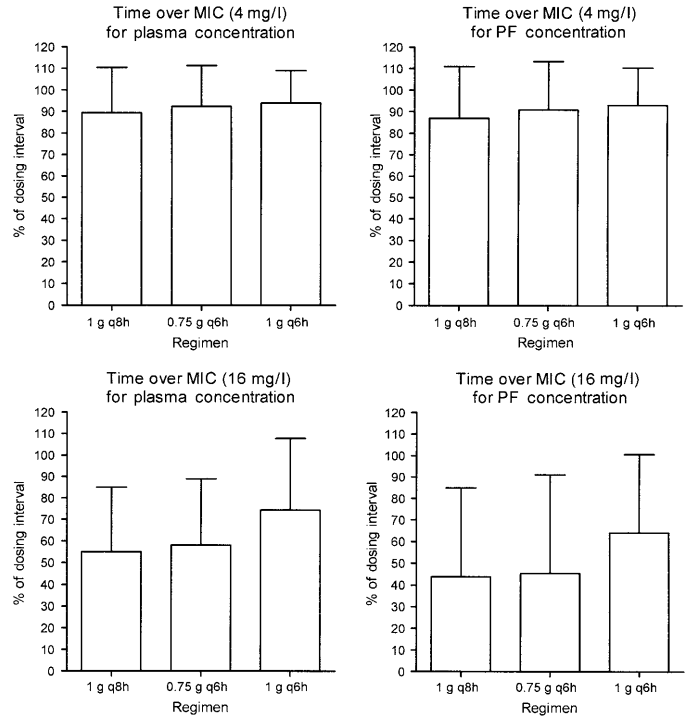

Figure 2 Percentage of time (mean and SD) during which meropenem concentrations in plasma and PF are greater than the MIC for susceptible and intermediately susceptible microorganisms. Three regimens are represented in simulations, representing steady-state conditions for the six patients in the study. Time over MIC in plasma and PF is expressed as a percentage of dosing interval. MICs for susceptible (4 mg/l) and intermediately susceptible (16 mg/l) bacteria (according to the clinical and laboratory standards institute (CLSI)) are represented.

degradation hypothesis was therefore successfully developed to describe the data. The decay of meropenem plasma concentrations with time was clearly biexponential, and therefore the parameters of a two-compartment open model were fit to the data. PF concentrations were obviously not a part of the central compartment, and it was found that the time of peak levels in the peripheral compartment was earlier than the time to maximum concentration (t_{max}) for PF concentrations. The final model therefore incorporated a third compartment corresponding to PF.

From complementary studies conducted *ex vivo*, it was confirmed that meropenem is not stable in the PF of patients with peritonitis. Whether this degradation is mediated by enzymes or by other means is not clear, but it could be enzymatically mediated, as we have also observed that meropenem in aqueous solution was stable at 37°C for 8 h. However, the degradation rate constants determined *ex vivo* in PF in different patients with peritonitis were much less (~7-fold on average) than those determined *in vivo*. A separate group of patients was examined for the *ex vivo* experiments, possibly contributing to the observed differences. Patients participating in the *in vivo* study were more critical (only two of six survived) than those not in septic shock who provided PF fluid for the *ex vivo* study (all survived). Other unknown sources of interpatient variability or differences in the *in vivo* and *ex vivo* conditions may also have existed.

Interestingly, comparable AUCs were estimated in plasma and PF during a recent microdialysis study conducted with imipenem in rats with peritonitis obtained by cecal ligation and puncture.[20] This was unexpected, as imipenem is less stable than meropenem and in particular more sensitive to hydrolysis by dehydropeptidase I.[24,26] It may be hypothesized that digestive or pancreatic enzymes, depending on the origin of the peritonitis, could be released in the PF and be responsible for meropenem degradation with the rather large associated interpatient variability. Therefore, the established experimental model of peritonitis in rats obtained by cecal ligation and puncture may not adequately reflect the patient's disease.

Meropenem is considered to exhibit time-dependent killing.[27] This implies that antibacterial efficacy requires concentrations to be maintained at a certain minimal level for a sufficiently long period of time. This issue was addressed using simulations of the time course of antibiotic levels in plasma or at the target site (**Table 3**). For all regimens, the mean minimum and maximum levels in both plasma and PF were above the MIC for susceptible strains (4 mg/l), although both the minimum plasma and PF levels were less than 4 mg/l in two of the six patients. Considering PF levels to be the target site, where an MIC of 16 mg/l was assumed (intermediately susceptible strains), average steady-state maximum levels were higher than this value for all three regimens (3 and 4 g/day), although not all patients dosed at 3 g/day reached this value. However, all patients exhibited maximum PF levels of meropenem that exceeded the MIC when dosed at 4 g/day (1 g every 6 h). Therefore, peritoneal concentrations of meropenem in patients suffering from severe secondary peritonitis associated with septic shock are generally sufficient to produce bacterial killing effect on susceptible bacteria using a regimen of 1 g every 8 h. Yet in view of the potential role of instability of this antibiotic in PF and in view of the observation that levels of this drug in PF may not be sufficient to control infections caused by intermediately susceptible strains in all patients, the use of higher daily doses of this drug, *e.g.* 4 g/day, may be warranted. However, it should also be mentioned that despite good penetration and relatively high peritoneal concentrations of this agent, mortality is still high indicating that other factors, such as systemic inflammatory response and immune status of the patient, play a role in pathophysiology of peritonitis.

In conclusion, microdialysis has been used successfully and for the first time to characterize the distribution of meropenem as a representative antibiotic in the PF of patients with severe peritonitis. A pharmacokinetic model with elimination from a peripheral compartment, consistent with meropenem instability assessed *ex vivo* in the PF, adequately described the data and may be used to simulate expected concentration profiles in plasma and PF of individual patients receiving meropenem and related penem antibiotics on a variety of dosing regimens.

METHODS

The ethics committee of the University of Tartu approved this study. Because the patients were not conscious, their relatives received a detailed description of study protocol and provided written informed consent. The study was performed in accordance with the Declaration of Helsinki Principles.

Patients. From November 2004 to November 2005, six patients with severe secondary peritonitis associated with septic shock were admitted to the general intensive care unit of Tartu University Hospital and included in this study. The causes of peritonitis were perforation of duodenal ulcer in two cases, perforation of stomach due to cancer in one case, perforation of large bowel in two cases, and large bowel wall necrosis in one case. Septic shock was diagnosed according to American College of Chest Physicians and Society of Critical Care Medicine consensus conference criteria.[28] Secondary peritonitis was defined as severe when the Mannheim Peritonitis Index is $\geqslant 19$. Patients who received meropenem during the 48 h before the study were excluded. Because it is essential to initiate antimicrobial treatment as soon as the primary diagnosis is made (*i.e.*, before intensive care unit admittance—in emergency room, operating theater, and so on), we were able to commence the study only after the second dose of meropenem.

Microdialysis. Microdialysis catheters (Gastrointestinal CMA 62 catheters with 30-mm-long polyamide membrane with a 20-kDa molecular weight cutoff; CMA, Solna, Sweden) were placed into the peritoneal cavity during the operation. Catheters were placed as close as possible to the source of peritonitis, *e.g.*, the perforation. Microdialysis was performed using a CMA 107 microdialysis pump and perfusion fluid containing Na^+ (154 mmol/l) and Cl^- (154 mmol/l), which was perfused at a rate of 1 μl/min. Two baseline samples (20 min and approximately 20 μl each) were taken before the second planned meropenem dose. Thereafter, 1 g of meropenem (AstraZeneca, Macclesfield, UK) was given intravenously by constant-rate infusion over 20 min. During the next 7 h, microdialysis samples were collected as follows: approximately 20, 40 min, 1, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 h. Samples were immediately frozen and stored at -80°C until analysis. Probe recoveries were estimated individually using the retrodialysis by drug method[29] at the end of the experiment over a 75-min period. Probes were perfused with a 50 mg/l solution of meropenem in 0.9% NaCl solution at a flow rate of 1 μl/min. Three microdialysate samples were collected over 45 min with 15-min intervals. Meropenem concentrations were determined in the perfusates (C_{in}) and dialysates (C_{out}) to determine the *in vivo* relative recovery by loss, expressed as a fraction ($RL_{in\ vivo}$) and calculated over each interval as

$$RL_{in\,vivo} = [(C_{in} - C_{out})/C_{in}] + C_{pf*}/C_{in}$$

where C_{in} and C_{out} are the perfusate and dialysate concentrations of meropenem and C_{pf*} is the estimated concentration of meropenem in the dialysate at the midpoint of the corresponding retrodialysis period during the post-study time frame, before the next dose was administered. Values of C_{pf*} were determined by extrapolation of the log-linear decrease of the concentrations measured in each patient. The *in vivo* recovery used to correct dialysate concentrations was taken as the mean of the last two determinations in each patient. At the end of this retrodialysis period, the microdialysis probe was removed.

Plasma sampling. Blood samples (6 ml) from an arterial line located either in the radial or cubital artery were taken exactly at the midpoint of the respective microdialysis collection interval. Blood was drawn into Vacutainer test tubes with Li-heparin. The samples were centrifuged at 2,000 *g* for 10 min at 4°C, and plasma was separated and stored in Eppendorf tubes at -80°C until analysis.

Meropenem assays: plasma samples. Meropenem trihydrate was obtained from AstraZeneca Pharmaceuticals (Tallinn, Estonia). A stock solution of meropenem was prepared by dissolving meropenem trihydrate in distilled water (5.0 mg/ml) and stored at -80°C. Plasma sample extraction with methanol was performed as described by Carlucci *et al.*[30] Quantitative analysis of total (free plus bound) meropenem levels was performed over the concentration range of 0.1–150 μg/ml (nine samples) in plasma. The high-performance liquid chromatography system consisted of a Waters 717 plus Autosampler (Waters Millipore, Milford, MA), Alltech 426 HPLC pump (Alltech Associates, Deerfield, IL), and a Waters 486 Tunable absorbance detector (Waters Millipore). The autosampler temperature was set at 4°C. Chromatography was performed on an analytical column, Inertsil ODS-3 C_{18} (150 mm × 4.6 mm inner diameter, 5 μm; GL Science, Tokyo, Japan), protected by a Platinum

C_{18} precolumn (7.5 mm × 4.6 mm inner diameter, 5 μm; Alltech Associates). Chromatography software Kromex, version 32S (Akrom-EX; Alltech, Tartu, Estonia), was used for data acquisition. A 100 μl portion of extracted plasma was transferred to the autosampler vial and 40 μl was injected using a flow rate of 0.7 ml/min in isocratic elution with a mobile phase composed of 0.05 M phosphate buffer (pH 3), acetonitrile, and methanol (86:12:2; by vol.). The absorbance of meropenem was measured at 302 nm.

Meropenem assays: microdialysis samples. Meropenem was quantified in PF by high-performance liquid chromatography coupled with tandem mass spectrometry. Dialysate samples were injected directly using a temperature-controlled (4°C) autosampler. The high-performance liquid chromatography column (Xterra MS C_{18} (150 mm × 3.9 mm, 5 μm); Waters, Saint Quentin en Yvelines, France), was connected to the tandem mass spectrometry (MS/MS) system (Quattro Micro Mass Spectrometer; Waters) equipped with an electrospray ionization interface. The mobile phase was composed of a mixture of solvent A (water/formic acid, 99.9:0.1, v/v) and solvent B (acetonitrile/formic acid, 99.9:0.1, v/v), delivered at a flow rate of 0.8 ml/min in the following gradient mode: 95–5% of A and 5–95% of B from 0 to 10 min, followed by 5–95% of A and 95–5% of B from 10.10 to 15 min. The flow was split, with one-fourth entering the mass spectrometer. Tandem mass spectrometry (MS/MS) detection in positive-ion mode by multiple reaction monitoring (nitrogen as desolvation gas (300 l/h) and as cone gas (100 l/h), argon as collision gas; temperature at 120°C; the desolvation temperature at 300°C; the cone voltage 20 V; and the capillary voltage 3.5 kV) allowed detection of meropenem (m/z384.3 → 340.1). Calibration curves were linear in the 0.03–1 μg/ml range. Within- and between-day accuracy and precision at three concentrations (0.03, 0.25, and 0.75 μg/ml) were within acceptable limits (<15%). The limit of quantification was considered to be 0.03 μg/ml.

Pharmacokinetic modelling. Nonlinear regression analysis (SAAM II, version 1.2.1, University of Washington, Seattle, WA) was utilized to characterize parameters for a compartmental model that assumed linear distribution and elimination kinetics for meropenem in plasma and PF. Data from the second dose of 1,000 mg infused at a constant rate over 20 min, beginning at 480 min after the first dose, without an assumption of steady state, were analyzed. The plasma concentration–time data were assumed to reflect unbound levels in view of the negligible protein binding of this antibiotic.[25] Visual inspection of the plasma and PF concentration–time data in individual patients suggested that the unbound AUC measured in the PF was less than that in the plasma, with the early concentration–time course in the PF being much lower than that in the plasma. Modeling of each patient's plasma and unbound PF concentration–time data of meropenem was conducted in two phases. In the first phase, the plasma concentration–time data were analyzed using a two-compartment open model. The four parameter estimates obtained were then fixed and a forcing function was used to estimate input and output rate constants for the PF compartment. The PF was described as one compartment with first-order entry and exit. It was assumed that meropenem distribution between the plasma and PF was governed by a passive diffusion processes. Therefore, unbound distributional clearances were assumed to be equal. The differences in the corresponding AUCs were then considered to result from the instability of meropenem in PF observed in *ex vivo* degradation experiments (see below). Thus, the model also included a first-order rate of loss (degradation) from the PF. The volume of the PF compartment was assumed to be equal to the volume measured postoperatively by drainage of the cavity. Because no drainage could be obtained from patient 2, a modest volume of 0.1 l was assumed. In both modeling phases, weighting of the data assumed a constant coefficient of variation based on the assay precision, and the variance model selected was associated with the data rather than the fitted function.

Meropenem stability in PF. The *ex vivo* determination of meropenem stability in PF was considered only after this microdialysis study had shown that meropenem concentrations in PF were lower than those in plasma. Therefore, this *ex vivo* stability assay was conducted using PF from patients distinct from those participating in the *in vivo* microdialysis study. Fresh PF collected from five patients with peritonitis was spiked with meropenem at an initial concentration of 25 μg/ml and incubated at 37°C for 8 h. Samples were collected every 60 min and assayed for meropenem concentrations using liquid chromatography tandem mass spectroscopy as described above. Visual inspection of the concentration versus time profiles indicated first-order degradation. Individual linear regression analyses were conducted to estimate degradation rate constants.

Simulations. Using point estimates of the parameters, the concentration–time profiles of meropenem in plasma and PF in each patient were simulated over 3 days of dosing (steady state). Assuming that the dose was administered at a constant rate over 20 min, the following three meropenem regimens were simulated:

(1) 1,000 mg every 8 h (the regimen used in this study);
(2) 750 mg every 6 h (the same daily dose used in regimen A);
(3) 1,000 mg every 6 h (a 33% increase in daily dose over regimens A and B).

The simulated profiles obtained over the last dosing interval on day 3 for each patient were examined. Steady state was confirmed by comparing concentrations at the beginning and end of the interval. Susceptible and intermediately susceptible microorganisms were defined according to the Clinical and Laboratory Standards Institute (CLSI) whose MICs were lower than 4 or 16 mg/l, respectively.[31] All results are presented as mean ± SD if not stated otherwise.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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Teadustegevus

Mitmeelundilise puudulikkuse preventsioon ja ravi. Mikrodialüüsi kasutamine intensiivravis. Uuringuobjektiks on olnud erinevate antimikrobiaalsete ravimite farmakokineetika sepsise ja septilise šoki puhul. 10 publikatsiooni

Organisatsioonid

Eesti Anestesioloogide Seltsi liige

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