



■ INFECTION

Single-dose pharmacokinetics of meropenem in porcine cancellous bone determined by microdialysis

AN ANIMAL STUDY

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Objectives

Meropenem may be an important drug in the treatment of open tibial fractures and chronic osteomyelitis. Therefore, the objective of this study was to describe meropenem pharmacokinetics in plasma, subcutaneous adipose tissue (SCT), and cancellous bone using microdialysis in a porcine model.

Methods

Six female pigs were assigned to receive 1000 mg of meropenem intravenously over five minutes. Measurements of meropenem were obtained from plasma, SCT, and cancellous bone for eight hours thereafter. Microdialysis was applied for sampling in solid tissues. The meropenem concentrations were determined using ultra-high-performance liquid chromatography.

Results

The penetration of meropenem into cancellous bone, expressed as the ratio of plasma to cancellous bone area under the concentration-curve from zero to the last measured value, was incomplete and delayed. The time with concentration above the minimal inhibitory concentration ($T_{>MIC}$), for an MIC of 0.5 $\mu\text{g/ml}$, was shorter for cancellous bone in comparison with both plasma and SCT. For MICs above 0.5 $\mu\text{g/ml}$, $T_{>MIC}$ in cancellous bone was only shorter than SCT. Considering an MIC of 4 $\mu\text{g/ml}$, no animals achieved the target of 40% $T_{>MIC}$ in plasma and cancellous bone, while less than 20% achieved it in SCT.

Conclusion

The main finding of this study was short $T_{>MIC}$ in cancellous bone after intravenous administration of 1000 mg meropenem. Consequently, in order to achieve sufficient tissue concentration in the cases of open tibial fractures and chronic osteomyelitis, supplemental application of meropenem may be necessary.

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Article focus

- This study aimed to describe meropenem pharmacokinetics in plasma, subcutaneous adipose tissue, and cancellous bone.

Key messages

- The main finding of this study was short time with concentration above the minimal inhibitory concentration ($T_{>MIC}$) in all tissues after intravenous administration of 1000 mg meropenem.
- In order to achieve sufficient tissue concentration, supplemental application of meropenem may be required.

Strengths and limitations

- This is the first study to investigate meropenem cancellous bone pharmacokinetics using microdialysis, which provided us with a high-resolution concentration-time profile.
- This is an experimental study using healthy young pigs, which did not suffer from either open tibial fracture or chronic osteomyelitis.

Introduction

Treatment of bone infections is complex and relies on a combination of surgical

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debridement and prolonged antimicrobial therapy.¹ Moreover, treatment failure is common and may partly be explained by an incomplete and heterogeneous tissue distribution of antimicrobials, which has been demonstrated for different combinations of tissue and drug.²⁻⁹ In the specific cases of open tibial fractures and chronic osteomyelitis, the literature is inconclusive regarding choice and application of antimicrobial treatment.¹⁰⁻¹³ Recent studies suggest the use of systemic meropenem in combination with vancomycin as first-line therapy for patients with open tibial fractures or chronic osteomyelitis.^{10,13}

Antimicrobial efficacy relies on its activity against the infectious microorganism and sufficient exposure at the target site. Evaluation of local antimicrobial tissue concentrations following antimicrobial administration is therefore important for improving both the prevention and treatment of infections.¹⁴ Particularly for bone, determination of antimicrobial concentrations has been a difficult task.^{15,16} However, in recent decades, microdialysis has emerged as a promising tool for continuous sampling of the unbound antimicrobial concentrations of the extracellular fluid in the bone.^{2,3,5-9} Currently, meropenem bone pharmacokinetics is poorly described and has not been assessed by means of microdialysis, whereas vancomycin bone pharmacokinetics has been described under different conditions.²⁻⁴ The objective of this study was therefore to describe meropenem pharmacokinetics in plasma, subcutaneous adipose tissue (SCT), and cancellous bone using microdialysis in a porcine model. The primary endpoints were the time with concentration above the minimal inhibitory concentration ($T_{>MIC}$) and the tissue penetration.

Materials and Methods

This study was conducted at the Institute of Clinical Medicine, Aarhus University Hospital, Denmark. The study was approved by the Danish Animal Experiments Inspectorate and was carried out according to existing laws. Chemical analyses were performed at the Department of Biochemistry, Aarhus University Hospital, Denmark.

Microdialysis. Briefly, microdialysis is a minimally invasive probe-based technique, which is based on diffusion of water-soluble molecules across a semipermeable membrane at the tip of the probe. Due to the continuous perfusion of the microdialysis probe, equilibrium across the semipermeable membrane will never occur. Consequently, the concentration in the dialysate will only represent a fraction of the actual tissue concentration. This fraction is referred to as the relative recovery (RR) and can be determined by various calibration methods. Determination of RR is a prerequisite when evaluating absolute antimicrobial tissue concentrations. In this study, retrodialysis by drug was applied for calibration.¹⁷

An in-depth description of microdialysis can be found elsewhere.¹⁸

Microdialysis equipment from M Dialysis AB (Stockholm, Sweden) was used. Specifically, the probes used were CMA 63 (membrane length 30 mm with a 20 kilodalton molecule cutoff), and CMA 107 precision pumps produced a flow rate of 2 μ l/minute.

The RR was calculated by using the following equation:

$$RR(\%) = 100 \times \left(1 - \frac{C_{out}}{C_{in}} \right)$$

where C_{out} is the concentration in the dialysate and C_{in} the concentration in the perfusate.

The absolute tissue concentrations, C_{tissue} , were obtained by correcting for RR using the following equation:

$$C_{tissue} = \frac{C_{out}}{RR}$$

Individual probe calibration was performed for all the probes at location. In the data analysis, the measured concentrations were attributed to the midpoint of the sampling intervals.

Animals, anaesthetic, and surgical procedures. Six female pigs were included in the study (Danish Landrace Breed, 72 to 81 kg). The anaesthetic consisted of a combination of propofol (450 to 500 mg/hour) and fentanyl (0.45 mg/hour). The pH and core temperature of the animals were monitored and regulated throughout the study and kept within the range of 7.40 to 7.48 and 36.9°C to 39.2°C, respectively.

Surgery was initiated immediately after induction of anaesthesia. With the pig in the supine position, the left proximal tibia was exposed via a medial incision. A 2 mm drill hole with a depth of 40 mm was made approximately 10 mm distal to the epiphyseal line in the cancellous bone of the tibial condyle. Next, a microdialysis probe (30 mm) was placed in the drill hole and fixed to the skin with a single suture. In addition, a microdialysis probe (30 mm) was placed in the SCT of the lateral thoracic wall, according to the guidelines of the manufacturer. Correct location of the bone probes was evaluated by necropsy.

Sampling procedures. Immediately after placement of the microdialysis probes, all probes were perfused with 0.9% sodium chloride (NaCl) holding 5 μ g/ml meropenem. Initially, a 30-minute tissue equilibration was allowed for. All probes were then calibrated by collecting three 20-minute samples. Following this, the perfusate was changed to blank 0.9% NaCl, and a 130-minute washout

Table I. Key pharmacokinetic parameters for plasma, subcutaneous adipose tissue, and cancellous bone

Pharmacokinetic parameter	Plasma	Subcutaneous adipose tissue	Cancellous bone	Overall comparison*
Mean AUC _{0–last} , min µg/ml (95% CI)	3347 (2689 to 4005)	4944 (4286 to 5602) [†]	1874 (1216 to 2532) [†]	p < 0.001
Mean C _{max} , µg/ml (95% CI)	74.3 (60.0 to 88.7)	81.6 (67.2 to 96.0)	21.4 (7.0 to 35.8) [‡]	p < 0.001
Mean T _{max} , mins (SD)	13.3 (8.2)	22.5 (0.0)	37.5 (0.0)	N/A
Mean T _{1/2} , mins (95% CI)	38.2 (32.8 to 43.5)	43.5 (38.2 to 48.9)	51.4 (46.0 to 56.7) [§]	p < 0.002
Mean AUC _{tissue} /AUC _{plasma} (95% CI)	N/A	1.50 (1.14 to 1.87)	0.56 (0.49 to 0.64)	N/A

*Overall comparison using F test for plasma, subcutaneous adipose tissue, and cancellous bone

[†]p < 0.005 for comparison with the corresponding plasma value

[‡]p < 0.001 for comparison with both plasma and subcutaneous adipose tissue

[§]p < 0.05 for comparison with both plasma and subcutaneous adipose tissue

AUC_{0–last}, area under the concentration–time curve from 0 to the last measured value; CI, confidence interval; C_{max}, peak drug concentration; T_{max}, time to C_{max}; N/A, not applicable; T_{1/2}, half-life at β-phase; AUC_{tissue}/AUC_{plasma}, tissue penetration expressed as the ratio of AUC_{tissue}/AUC_{plasma}

was performed. At time zero, a dialysate was collected to evaluate the efficacy of the washout and 1000 mg of meropenem was administered intravenously over five minutes. From time 0 to 60 minutes, dialysates were collected during 15-minute intervals, from time 60 to 120 minutes during 30-minute intervals, and from time 120 to 480 minutes during 60-minute intervals, giving a total of 13 samples over eight hours. Venous blood samples were drawn from a central venous catheter at time 10, 20, 30, 45, 60, 120, 180, 240, and 480 minutes.

Handling of samplings. The dialysates were instantly frozen and stored at -80°C until analysis. Serum blood samples were kept at room temperature for a minimum of 30 minutes and a maximum of 90 minutes before being centrifuged at 3000 g for 10 minutes. Serum aliquots were then frozen and stored at -80°C until analysis.

Ultra-high-performance liquid chromatography analysis of meropenem. Meropenem in dialysates and serum was analyzed using ultra-high-performance liquid chromatography (UHPLC). The UHPLC system consisted of an eluent pump, autosampler, column compartment, and a UV detector (Agilent 1290 Infinity; Agilent Technologies, Santa Clara, California) equipped with a 1.7 µm 100 by 2.1 mm C₁₈ column (Kinetex; Phenomenex, Torrance, California). For analysis of meropenem in dialysates, 15 µl of dialysate was mixed with 20 µl of phosphate buffer at pH 3 (10 nM NaH₂PO₄, adjusted with hydrogen chloride (HCl)). After mixing, 5 µl was injected into the UHPLC system. Standards of 0.25, 0.5, 1.0, and 2.0 µg/ml meropenem in 15 µl 0.9% NaCl were prepared and analyzed to prepare calibration curves for meropenem. For this purpose, the Chemstation Software (Agilent Technologies) was used. Before analysis of meropenem in the serum, 300 µl of serum was placed in an ultrafilter 96-well plate with a 30 kilodalton molecular mass cut-off (AcroPrep 30K Omega; Pall Corporation, New York, New York) and centrifuged for 30 minutes at 1000 g. A total of 15 µl of the filtrate was prepared for analysis as was done for the dialysates. The UHPLC system (Agilent 1290 Infinity) was equipped with a 1.7 µm 100 by 2.1 mm C₁₈ column (Kinetex), and chromatography was performed with a gradient of acetonitrile in phosphate buffer

(10 nM NaH₂PO₄, adjusted with HCl). The concentration of acetonitrile was increased from 0% to 30% over a time span of 2.5 minutes, and the total analysis time was four minutes with a post-run time of one minute. Meropenem was detected at 304 nm. The lower limits of quantification (LLOQ) was found to be 0.5 µg/ml. Interrun imprecision (percent coefficients of variation (%CV)) was 3.0% at 2.0 µg/ml for quantification of meropenem. The assays showed linearity in the measurement response, which was within the range of 0.5 µg/ml to 105 µg/ml. The accuracy of the quantification of meropenem was found to be between -4.3% and 4.8%.

Pharmacokinetics analysis and statistics. The standard pharmacokinetic parameters: area under the concentration–time curves from zero to the last measured value (AUC_{0–last}), peak drug concentration (C_{max}), time to C_{max} (T_{max}), and half-life (T_{1/2}) were determined separately for each compartment for each animal by non-compartmental analysis using the pharmacokinetic series of commands in Stata v. 14.1 (StataCorp LLC, College Station, Texas). The AUC_{0–last} was calculated using the trapezoidal rule. C_{max} was calculated as the maximum of all the recorded concentrations, and T_{max} as the time to C_{max}. T_{1/2} was calculated as ln(2)/λ_{eq}, where λ_{eq} is the terminal elimination rate constant estimated by linear regression of the log concentration on time. All the variables were analyzed using a mixed model taking the variance between pigs into account. Consequently, means and 95% confidence intervals (CIs) of AUC_{0–last}, C_{max}, T_{max}, and T_{1/2} are given in Table I. The model assumptions were tested by visual diagnosis of residuals, fitted values, and estimates of random effects. A correction for degrees of freedom due to small sample size was handled using the Kenward–Roger approximation method. Overall, comparisons between the compartments were conducted using the F test and pairwise comparisons with the *t*-test. A p-value < 0.05 was considered significant. No correction for multiple comparisons was applied. The tissue AUC_{0–last} to plasma AUC_{0–last} ratio (AUC_{tissue}/AUC_{plasma}) was calculated as a measure of the tissue penetration. Statistical analyses were also performed using Stata.

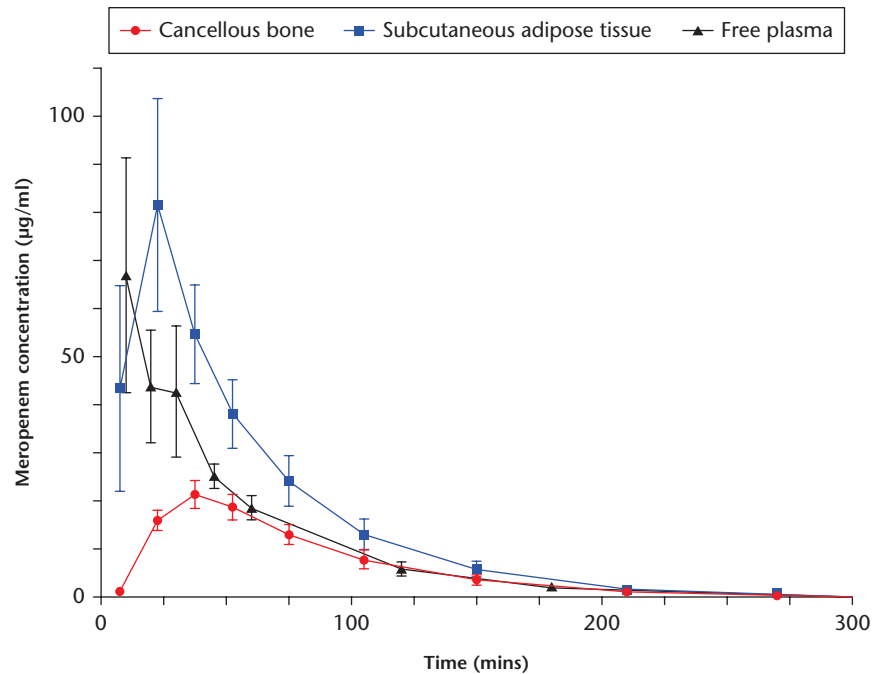


Fig. 1

Mean concentration-time profiles. Due to low concentrations, the x axis is cut at 300 minutes. Bars represent 95% confidence intervals.

By use of Microsoft Excel V. 16.16.11 (Microsoft Corporation, Redmond, Washington), the $T_{>MIC}$ was estimated using linear interpolation. The number of animals attaining the target of 40% $T_{>MIC}$ was counted manually. One-way analysis of variance with random animal effect was used for pairwise comparisons of the $T_{>MIC}$.

Results

All six pigs completed the study and data were obtained from all probes. The mean RRs (SD) were 35.5% (8.5) and 20.9% (5.0) for cancellous bone and SCT, respectively. As the mean concentration of meropenem at time zero was less than 0.1 µg/ml for both compartments, these results were not included in the analysis.

Mean meropenem concentration-time profiles for the different compartments are presented in Figure 1. Corresponding pharmacokinetic parameters are provided in Table I. For cancellous bone, the tissue penetration (95% CI) was incomplete 0.54 (0.47 to 0.61) and delayed. A lower AUC_{0-last} and C_{max} was found in cancellous bone in comparison with that of plasma. Furthermore, a prolonged elimination rate was found in cancellous bone compared with plasma and SCT. For SCT, the AUC_{0-last} was higher than that of plasma with a corresponding higher tissue penetration ratio (95% CI) of 1.46 (1.10 to 1.81).

The relationship between the $T_{>MIC}$ and different MICs for all compartments are shown in Figure 2. For MICs up to 0.5 µg/ml, the $T_{>MIC}$ was shorter for cancellous bone compared with both plasma and SCT ($p < 0.003$). For

MICs above 0.5 µg/ml, $T_{>MIC}$ in cancellous bone was only shorter than SCT ($p < 0.03$).

Table II shows the proportion of animals achieving the target of 40% $T_{>MIC}$ for a range of different MICs. For an MIC of 0.5 µg/ml, all animals achieved the target of 40% $T_{>MIC}$ in all compartments. However, for MICs above 0.5 µg/ml, the proportion of animals achieving 40% $T_{>MIC}$ decreased rapidly for both plasma and cancellous bone. Considering the SCT, all animals achieved the target of 40% $T_{>MIC}$ for MIC values up to 2 µg/ml. However, at higher MIC values, none or fewer than 20% of the animals achieved the target of 40% $T_{>MIC}$.

Discussion

To the best of our knowledge, this is the first study to report meropenem bone pharmacokinetics using microdialysis. The main finding was short $T_{>MIC}$ in cancellous bone. For meropenem, a probability of target attainment of 90% for the target of 40% $T_{>MIC}$ has been associated with bactericidal effect.¹⁹ In cancellous bone, this probability of target attainment was only achieved for an MIC of 0.5 µg/ml, while a proportional decrease was found with increasing MICs. Furthermore, we found an incomplete and delayed tissue penetration of meropenem from plasma into the cancellous bone. The most common pathogens in open tibial fractures and chronic osteomyelitis are *Staphylococcus aureus*, Coagulase-negative staphylococci, and *Enterococcus spp.* For meropenem, these pathogens exhibit MICs in the range of 0.125 µg/ml to 4 µg/ml.²⁰ For MICs of 4 µg/ml no animals achieved

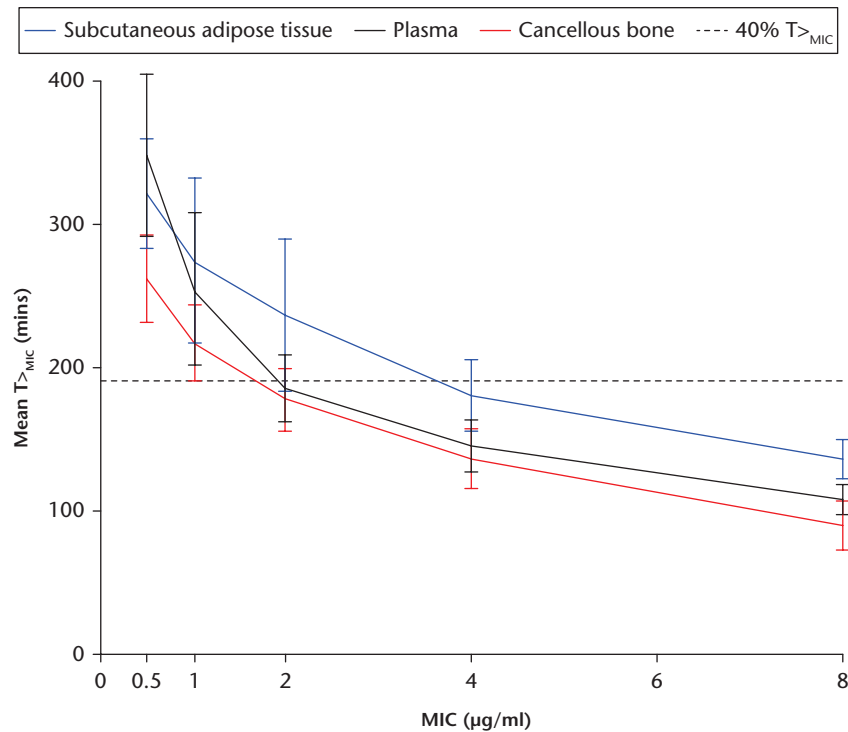


Fig. 2

Time with concentration above the minimal inhibitory concentration ($T_{>MIC}$) versus minimal inhibitory concentrations. Bars represent 95% confidence intervals.

Table II. The probability of target attainment (PTA) for different minimal inhibitory concentration (MIC) values in plasma, subcutaneous adipose tissue, and cancellous bone

MIC, µg/ml	Plasma PTA, % (40% $T_{>MIC}$)	Subcutaneous adipose tissue PTA, % (40% $T_{>MIC}$)	Cancellous bone PTA, % (40% $T_{>MIC}$)
0.5	100	100	100
1.0	83	100	83
2.0	33	100	17
4.0	0	17	0
8.0	0	0	0

$T_{>MIC}$, time with concentration above the minimal inhibitory concentration

Table III. Healthy porcine cancellous bone pharmacokinetic parameters of different antimicrobials obtained by microdialysis

Pharmacokinetic parameter	Meropenem	Cefuroxime ⁷	Vancomycin ²
Mean $AUC_{tissue}/AUC_{plasma}$ (95% CI)	0.56 (0.49 to 0.64)	0.79 (0.67 to 0.91)	0.59 (0.42 to 0.75)
C_{max} , µg/ml (95% CI)	Mean: 21.4 (7.0 to 35.8)	Mean: 22.9 (16.3 to 29.5)	Median: 15.7 (10.9 to 20.4)
T_{max} , mins	Mean: 37.5 (sd 0)	Median: 45 (range: 1.5 to 45)	Median: 191 (95% CI: 149 to 233)
$T_{1/2}$, mins (95% CI)	Mean: 51.4 (46.0 to 56.7)	Median: 36.7 (32.0 to 41.3)	Median: 255 (206 to 305)

$AUC_{tissue}/AUC_{plasma}$, tissue penetration expressed as the ratio of $AUC_{tissue}/AUC_{plasma}$; CI, confidence interval; C_{max} , peak drug concentration; T_{max} , time to C_{max} ; $T_{1/2}$, half-life at β -phase

the target of 40% $T_{>MIC}$ in plasma and cancellous bone, and fewer than 20% of the animals achieved it in the SCT. Accordingly, our findings suggest that 1000 mg of meropenem will not provide sufficient bone and tissue concentrations. Recently, higher targets up to 100% $T_{>MIC}$ have been proposed in several clinical studies.²¹⁻²³ If higher targets are applied to our data, the probability of target attainment will proportionally decrease with an increasing target. Consequently, in order to achieve sufficient tissue concentration in the cases of open tibial

fractures and chronic osteomyelitis, supplemental application of meropenem may be necessary.

On a more basic pharmacokinetic level, it is noteworthy that the highest AUC_{0-last} and tissue penetration was found in the SCT. Accordingly, the SCT displayed the best $T_{>MIC}$ results. This may be explained by a fast penetration and high C_{max} in the SCT combined with an elimination rate equal to that of plasma. These findings are important, as sufficient antimicrobial exposure should be reached in all relevant target tissues. When evaluating

antimicrobial tissue concentrations, it is therefore important to evaluate the pharmacokinetics in all relevant compartments and under different conditions.

Recent studies, with comparable setups, have indicated that the antimicrobial penetration may differ between drugs.^{2,7} Table III summarizes important antimicrobial cancellous bone pharmacokinetic differences between cefuroxime, vancomycin, and meropenem.^{2,7} When looking at these results, the cefuroxime cancellous bone penetration seems superior to both meropenem and vancomycin. This may indicate that the selection of antimicrobials for treatment and prevention of bone infections should not only be based on the sensitivity to the pathogen but also on the pharmacokinetic profile in relevant tissues. However, it is important to recognize the differences in the antimicrobial specific targets.

Open tibial fractures and chronic osteomyelitis are conditions that are difficult to treat. Recently, the use of meropenem and vancomycin in combination as first-line therapy has been recommended for these conditions.^{10,13} Previous studies have found both incomplete and delayed vancomycin tissue penetration into both bone and SCT.^{2,4} The present study contributes new knowledge regarding meropenem cancellous bone and SCT pharmacokinetics. However, both the vancomycin studies and the present study only evaluate single-dose monotherapy administration and do not assess the potential effects of dual-antimicrobial therapy and achievement of steady state. Therefore, future studies investigating these matters are warranted.

To some extent, pigs resemble humans in terms of physiology and anatomy.²⁴ Nonetheless, the interspecies and age-related differences are important to consider before uncritically extrapolating these results to a clinical setting. Applying the Cierny–Mader classification, these pigs may represent an A-host with both a good immune response and local delivery of the antimicrobial.²⁵ Furthermore, the animals in the present study did not suffer from either open tibial fracture or chronic osteomyelitis. Previous studies have, however, shown that bone infections lower the antimicrobial penetration by inducing intratrabecular suppuration resulting in ischaemic osseous sequestration and reduced vascularization.^{1,2,7,25,26} Chronic osteomyelitis may therefore lead to shorter $T_{>MIC}$ values and lower penetration ratios in bone, than found in this study. Moreover, the presence of bacterial biofilm will increase the pathogens tolerance towards the antimicrobial due to increased MIC values.²⁷ In contrast, fractures may lead to higher antimicrobial bone concentrations. Fractures are typically associated with an acute decrease in the bone blood flow. However, during the first day, the blood flow in the bone rapidly increases three to six times due to angiogenic mechanisms.^{28,29} This may, in contrast with bone infections, lead to longer $T_{>MIC}$ and higher bone penetration ratios.

Nonetheless, this remains speculative, and future studies investigating antimicrobial bone concentration after open tibial fractures and in chronic osteomyelitis are warranted.

In recent decades, microdialysis has emerged as a promising tool for antimicrobial pharmacokinetic studies in bone. This method is advantaged by serial sampling of the free and thus active fraction of drug from the interstitial space.¹⁸ However, when performing microdialysis studies, it is important to realise that the mandatory correction for RR is associated with a magnification of the variations associated with preanalytical sample handling and the chemical assay. Consequently, RR should exceed 20% to minimize the variations, which increases with decreasing RR.³⁰ Our setup resulted in mean RRs in the range of 20.9% to 35.5%, which seems acceptable. Furthermore, the variation of the dialysate concentrations was comparable with those found in plasma. It should also be noted that the LLOQ for meropenem was 0.5 µg/ml. As such, we were not able to assess $T_{>MIC}$ for MICs below 0.5 µg/ml. However, this LLOQ was found to be acceptable, as clinical treatment targets below MICs of 0.5 µg/ml seem insufficient.

In conclusion, microdialysis was successfully applied for the assessment of meropenem concentrations in cancellous bone and SCT. The main finding of this study was short $T_{>MIC}$ in cancellous bone after intravenous administration of 1000 mg meropenem, where no animals achieved the target of 40% $T_{>MIC}$ for MICs of 4 µg/ml. Consequently, in order to achieve sufficient tissue concentration in the cases of open tibial fractures and chronic osteomyelitis, supplemental application of meropenem may be required.

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Author contributions

- P. Hanberg: Conceptualized the study, Collected and analyzed the data, Wrote and edited the manuscript.
- A. Lund: Conceptualized the study, Collected and analyzed the data, edited the manuscript.
- K. Søballe: Conceptualized the study, Analyzed the data, edited the manuscript.
- M. Bue: Conceptualized the study, Collected and analyzed the data, edited the manuscript.

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Ethical review statement

- This study was approved by the Danish Animal Experiments Inspectorate and was carried out according to existing laws (License No. 2017/15-0201-01184).

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