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INFECTION

Tibial bone and soft-tissue concentrations following combination therapy with vancomycin and meropenem – evaluated by microdialysis in a porcine model

SHOULD PATIENTS WITH OPEN FRACTURES HAVE HIGHER DOSES OF ANTIBIOTICS?

S. Ø. Vittrup,
P. Hanberg,
M. B. Knudsen,
S. K. Tøstesen,
J. O. Kipp,
J. Hansen,
N. P. Jørgensen,
M. Stilling,
M. Bue

From Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

Aims

Prompt and sufficient broad-spectrum empirical antibiotic treatment is key to preventing infection following open tibial fractures. Succeeding co-administration, we dynamically assessed the time for which vancomycin and meropenem concentrations were above relevant epidemiological cut-off (ECOFF) minimal inhibitory concentrations (T > MIC) in tibial compartments for the bacteria most frequently encountered in open fractures. Low and high MIC targets were applied: 1 and 4 µg/ml for vancomycin, and 0.125 and 2 µg/ml for meropenem.

Methods

Eight pigs received a single dose of 1,000 mg vancomycin and 1,000 mg meropenem simultaneously over 100 minutes and 10 minutes, respectively. Microdialysis catheters were placed for sampling over eight hours in tibial cancellous bone, cortical bone, and adjacent subcutaneous adipose tissue. Venous blood samples were collected as references.

Results

Across the targeted ECOFF values, vancomycin displayed longer T > MIC in all the investigated compartments in comparison to meropenem. For both drugs, cortical bone exhibited the shortest T > MIC. For the low MIC targets and across compartments, mean T > MIC ranged between 208 and 449 minutes (46% to 100%) for vancomycin and between 189 and 406 minutes (42% to 90%) for meropenem. For the high MIC targets, mean T > MIC ranged between 30 and 446 minutes (7% to 99%) for vancomycin and between 45 and 181 minutes (10% to 40%) for meropenem.

Conclusion

The differences in the T > MIC between the low and high targets illustrate how the interpretation of these results is highly susceptible to the defined MIC target. To encompass any trauma, contamination, or individual tissue differences, a more aggressive dosing approach may be considered to achieve longer T > MIC in all the exposed tissues, and thereby lower the risk of acquiring an infection after open tibial fractures.

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Keywords: Microdialysis, Vancomycin, Meropenem, Open fracture, Antibiotic prophylaxis

Correspondence should be sent to Sofus Ørbæk Vittrup; email: sofusvittrup@clin.au.dk

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Article focus

- This study aimed to describe pharmacokinetics, with a special focus on time with concentration above relevant minimal inhibitory concentration (T > MIC) of co-administered single-dose vancomycin

and meropenem in orthopaedic compartments most relevant to open tibial fractures.

Key messages

- The main finding was short T > MIC, especially in cortical bone, for both vancomycin and meropenem across all MIC-values evaluated.
- Vancomycin demonstrated longer T > MIC values than meropenem across all compartments and MIC values evaluated.
- To achieve sufficient antibiotic prophylaxis in open fractures, alternative dosing regimens seem necessary.

Strengths and limitations

- This is the first study to evaluate co-administered vancomycin and meropenem by microdialysis, providing dynamic concentration-time profiles from tissue targets.
- The main limitation is that the experiment was conducted on young, healthy pigs with no open tibial fracture or soft-tissue damage.

Introduction

The nature of open fractures introduces an obligate bacterial contamination of the wound, inevitably resulting in higher infection rates compared with closed fractures.[1-3] The reported infection rates following open fractures vary from 0% to 50%,[3-6] reflecting the heterogeneity in contamination grade, anatomical location, soft-tissue damage and laceration, and presence of vascular compromise,[7] e.g. open tibial fractures are associated with infection rates twice those for open fractures in other locations.[6] This is also exemplified by the Gustilo-Anderson classification where higher grades are associated with both a higher risk of infection and a more diverse contamination.[3,4,8] Infection following open fractures is associated with multiple surgical revisions, prolonged antibiotic treatment, and increased morbidity.[9] To lower the risk of infection development, revision surgery should be accompanied by prompt antibiotic treatment with sufficient coverage of the bacteria that are most frequently causing infections after open fractures.[1,2] In cases of open tibial fractures, recent studies have found that the combination of vancomycin and meropenem as first-line antibiotic therapy covers up to 93% to 96% of the encountered bacteria causing infection, most frequently being *Staphylococcus aureus, Enterococcus spp., Pseudomonas aeruginosa, Enterobacter* species, and coagulase-negative staphylococci (CoNS).[1,2,10] Supported by the current European Bone and Joint Infection Society (EBJIS) guidelines on prevention of fracture-related infections,[11] the diverse contamination profile demands a broad antibiotic prophylactic spectrum encompassing both Gram-positive and Gram-negative bacteria.[12]

When administering antibiotics to prevent infection of a contaminated open fracture, it is essential that antibiotic target site concentrations, as a minimum, reach and remain above relevant bacteria's minimal inhibitory concentrations (MICs) for a sufficient amount of time. While it remains difficult to obtain sound evidence of the time during which the bacteria must be exposed to concentrations above MIC to ensure antibiotic prophylaxis, dynamic assessment of target concentrations in bone and soft-tissues of various antibiotics by use of microdialysis (MD) has gained increased interest during the last few years.[13-15] A dynamic assessment of target bone and soft-tissue concentrations for the combination of vancomycin and meropenem has the potential to create important pharmacokinetic knowledge, which can improve the antibiotic regimens and lower the risk of developing an infection following open tibial fractures. Open tibial fractures were chosen as a well-defined disease entity for the purpose of defining target MICs, although the results can be applicable to all open fractures, as they share similar microbiology.[16]

Therefore, we dynamically assessed single-dose vancomycin and meropenem concentrations following co-administration in tibial cancellous bone, cortical bone, and subcutaneous adipose tissue using MD in a porcine model. The primary endpoint was to evaluate the time with vancomycin and meropenem concentrations above relevant epidemiological cut-offs (ECOFF) (T > MIC) for bacteria most frequently encountered in infections following open tibial fractures.[1,2]

Methods

This study was conducted at the Institute of Clinical Medicine, Aarhus University. The study was approved by the Danish Animal Experiments Inspectorate and was carried out in agreement with existing laws (license no. 2017/15-0201-01184). The study adhered to the ARRIVE guidelines, as shown by the ARRIVE checklist included in the Supplementary Material. All chemical analyses were performed at the Department of Forensic Medicine, Aarhus University Hospital.

Microdialysis. A more elaborate description of MD can be found elsewhere.[17] In brief, the method allows for dynamic collection of samples simultaneously from different relevant tissues. MD is a catheter-based sampling method, allowing for extracellular water-soluble molecules to diffuse across a semipermeable membrane at the tip of the MD catheter along the concentration gradient. Due to continuous perfusion of the MD system, equilibrium across the membrane cannot occur. Accordingly, the sampled analyte concentration found in the dialysate will only represent a fraction of the absolute tissue concentration. This fraction is referred to as relative recovery (RR). In antibiotic pharmacokinetic studies, individual catheter determination of RR is mandatory to calculate absolute tissue concentrations. In the present study, retrodialysis

by drug was used to calculate RR using the following equation:[18]

$$RR\,(\%) = 100 \times \left(1 - \frac{C_{dialysate}}{C_{perfusate}}\right)$$

where $C_{dialysate}$ is the concentration of vancomycin or meropenem in the dialysate, and $C_{perfusate}$ is the concentration of vancomycin or meropenem in the perfusate. In the data analysis, the measured concentrations were attributed to the midpoint of the sampling interval resulting in a 450-minute dosing interval. The absolute tissue concentrations (C_{tissue}) were obtained by individual correction of the membrane-specific RR of each sample, for each drug, using the following equation:

$$C_{tissue} = 100 \times \frac{C_{dialysate}}{RR\,(\%)}$$

The MD equipment used was acquired from M Dialysis AB (Sweden). Specifically, the catheters used were CMA 70 (membrane lengths 10 and 20 mm with a 20 kDA molecule cut-off) and CMA 107 precision pumps producing at a flow rate of 1 µl/min.

Animals, anaesthetic, and surgical procedures. Eight female pigs (Danish Landrace breed; weight 78 to 82 kg) were included in the study. A combination of fentanyl (0.6 to 0.7 mg/hour) and propofol (550 to 600 mg/hour) was used to keep the pigs under general anaesthesia throughout the surgery. pH and body temperature were monitored and kept within a range of 7.40 to 7.55 and 36.5°C to 39°C, respectively. pH was regulated through ventilation and body temperature with blankets or icepacks.

With the pig in a supine position, the proximal part of the right tibia was exposed by a medial incision. Hereafter, a 25 mm drillhole was made into cancellous bone approximately 10 mm distal to the epiphysial line of the tibial condyle. Next, a 15 mm drillhole was made into the cortical bone by assessing the anterior margin of the tibial diaphysis with an anterolateral approach. Precautionary measures were taken when drilling all holes by only drilling for short periods of time and by continuously applying cold sodium chloride (NaCl) upon the drill site to prevent bone necrosis. A 20 mm and a 10 mm MD catheter were introduced into the drill holes of the cancellous bone and cortical bone, respectively, and fastened to the skin with single sutures. Lastly, a 20 mm MD catheter was placed in the subcutaneous adipose tissue of the right leg, lateral to the knee joint using an introducer. Correct location of all bone catheters was verified by fluoroscopy, and post-mortem CT was applied to verify that the cortical drill holes had not penetrated to the bone marrow and remained intracortical throughout their extent.

Sampling procedures. Immediately after placement of all MD catheters, the catheters were perfused with 0.9% NaCl, followed by a 20-minute tissue equilibrium period. Next, vancomycin and meropenem were administered simultaneously through different venous catheters; 1,000 mg of vancomycin over 100 minutes, and 1,000 mg of meropenem over 10 minutes. Time of administration defined time zero. Following this, dialysates were collected with 30-minute intervals from time 0 to 4 hrs and with 60-minute intervals from four to eight hours, resulting in a total of 12 samples over eight hours. Venous blood samples were collected at the midpoint of each sampling interval. After eight hours, all perfusates were changed to a 0.9% NaCl solution containing 100 µg/ml meropenem and 300 µg/ml vancomycin, allowing for calibration of the catheters with the retrodialysis by drug method. After a catheter equilibrium period of 30 minutes, one 40-minute recovery sample was collected. All dialysates were instantly frozen and stored at -80°C until analysis. Blood samples were stored at 5°C for no longer than six hours before being centrifuged at 3,000× g for ten minutes. Plasma aliquots were then frozen and stored at -80°C until analysis.

Minimal inhibitory concentration. Given the high infection rates following open tibial fractures, and diversity of the encountered bacteria, we opted to investigate a range of prophylactic MIC targets for both vancomycin and meropenem. We considered vancomycin as the relevant drug of choice for coverage against Gram-positive organisms, while meropenem would provide Gram-negative coverage.

ECOFF for planktonic *S. aureus, Enterococcus spp.,* and CoNS, defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), were used to evaluate the pharmacokinetic/pharmacodynamic target T > MIC for vancomycin, while ECOFF values for planktonic *Pseudomonas aeruginosa* and various members of the Enterobacterales order were used for meropenem targets. For vancomycin, these specific MICs have been reported in the range of 1 to 4 µg/ml and consequently we chose 1 and 4 µg/ml as low and high targets, respectively. For meropenem, MICs have been reported in the range of 0.125 to 2 µg/ml, and therefore we chose 0.125 and 2 µg/ml as low and high targets, respectively.[19]

Quantification of vancomycin and meropenem concentrations. The free concentrations of vancomycin in plasma were quantified with a clinical standard homogeneous enzyme immunoassay technique (Chemistry XPT, Advia Chemistry, Germany). The intra-run (total) imprecisions for this assay were ± 1.2 µg/ml (standard deviation (SD) 2) at 6.6 µg/ml and ± 3.7 µg/ml (SD 2) at 29.1 µg/ml.[20] For meropenem, free plasma concentrations were quantified using ultra-high performance liquid chromatography. The lower limits of quantification were found to be 0.5 µg/ml. Interrun imprecision (percentage coefficients of variation (%CV)) was 3.0% at 2.0 µg/ml. The accuracy of meropenem quantification was found to be between -4.3% and 4.8%, within a linearity range of 0.5 µg/ml to 105 µg/ml.[21]

Quantification of free vancomycin and meropenem concentrations in the microdialysate samples was done using ultra-high performance liquid chromatography and tandem mass spectrometry. Microdialysate samples were prepared by mixing 300 µl internal standard solution with 5 µl microdialysate sample in a 96-well

Table I. Time above minimal inhibitory concentration values for vancomycin and meropenem in cortical bone, cancellous bone, and subcutaneous adipose tissue, expressed as minutes and percentages of the dosing interval.

Parameter	Cortical bone	Cancellous bone	Subcutaneous adipose tissue	Plasma
Mean T > MIC, mins (95% CI)				
Vancomycin, µg/ml				
1.0 (low target)	208 (158 to 259)*	403 (352 to 453)	441 (390 to 491)	449 (399 to 499)
2.0	139 (79 to 200)*	379 (318 to 439)	433 (373 to 494)	448 (388 to 508)
4.0 (high target)	30 (-26 to 86)*	296 (240 to 352)	416 (360 to 473)	446 (390 to 502)
Meropenem, µg/ml				
0.125 (low target)	189 (137 to 241)†	278 (226 to 329)	331 (280 to 383)	406 (353 to 458)
0.25	177 (130 to 225)†	256 (208 to 303)	311 (264 to 359)	372 (324 to 419)
2.0 (high target)	45 (17 to 73)†	144 (116 to 172)	180 (152 to 208)	181 (153 to 209)
Mean %T > MIC (95% CI)				
Vancomycin, µg/ml				
1.0	46 (35 to 58)	89 (78 to 101)	98 (87 to 109)	100 (89 to 111)
2.0	31 (18 to 44)	84 (71 to 98)	96 (83 to 110)	100 (86 to 113)
4.0	7 (-6 to 19)	66 (53 to 78)	93 (80 to 105)	99 (87 to 112)
Meropenem, µg/ml				
0.125	42 (30 to 54)	62 (50 to 73)	74 (62 to 85)	90 (78 to 102)
0.25	39 (29 to 50)	57 (46 to 67)	69 (59 to 80)	83 (72 to 93)
2.0	10 (4 to 16)	32 (26 to 38)	40 (34 to 46)	40 (34 to 46)

$p < 0.001$ for overall comparison using repeated measurements analysis of variance (F test) for both vancomycin and meropenem T > MIC.
*$p < 0.001$ using analysis of variance (paired *t*-test) for comparison with all other compartments.
†$p \leq 0.025$ using analysis of variance (paired *t*-test) for comparison with all other compartments.
CI, confidence interval; MIC, minimal inhibitory concentration; %T > MIC, percentage of dosing interval of 450 minutes with concentration above MIC.; T > MIC, time with concentration above MIC

microplate. The internal standard solution was 0.1 µg/ml of Norvancomycin (Santa Cruz Biotechnology, USA) and Meropenem-D6 (Cayman Chemical, USA) in solvent water:methanol (85:15). Separate samples for calibration were prepared using reference compounds (Meropenem trihydrate Vetranal analytical standard and Vancomycin Hydrochloride EDQM Reference Standard CRS batch 3, both supplied from Sigma-Aldrich, Germany). A 3 µl sample volume was injected into an ultra-high performance liquid chromatography system (Waters Acquity UPLC, USA) with a C18 column (Waters UPLC HSS-C18) and analyzed with a mass spectrometer (Waters Xevo TQS) with conditions described previously.[13] Compounds were detected with positive electrospray ionization in the multiple reaction monitoring mode with the following m/z transitions: vancomycin (725.2→144.1); norvancomycin (718.5→144.1); meropenem (384.1→68); and meropenem-D6 (m/z: 390.2→147.1). Calibration curves were constructed by linear regression of the peak area ratio (analyte/internal standard) versus the nominal analyte concentrations and based on seven points (including the blank). Norvancomycin was used as internal standard for vancomycin and meropenem-D6 for meropenem quantification. The method showed acceptable levels of precision (CV < 15%) in the quantification ranges of 0.1 to 20 µg/ml.

Pharmacokinetic analysis and statistics. The standard pharmacokinetic parameters: area under the concentration–time curve from zero to the last measured value ($AUC_{0\text{-last}}$), peak drug concentration (C_{max}), and time to C_{max} (T_{max}) were determined separately for each compartment for each animal for both meropenem and vancomycin by noncompartmental analysis. The AUC was calculated using the linear-up/log-down method. C_{max} was calculated as the maximum of all the recorded concentrations and T_{max} as the time to reach C_{max}. The tissue AUC to plasma AUC ratio ($AUC_{tissue}/AUC_{plasma}$) was calculated as a measure of the tissue penetration. Microsoft Excel (v. 16.47.1, Microsoft, USA) was used to estimate the T > MIC for MIC 0.125, 0.25, and 2 µg/ml for meropenem and for MIC 1, 2, and 4 µg/ml for vancomycin using linear interpolation for each compartment and each animal. A general comparison of the pharmacokinetic parameters and T > MIC was conducted using repeated measurements analysis of variance (F test), followed by pairwise comparisons made by linear regression (paired *t*-test). A correction for degrees of freedom by the Kenward–Roger approximation method was used due to small sample size. The model assumptions were tested by visual diagnosis of residuals, fitted values, and estimates of random effects. A p-value less than 0.05 was considered statistically significant. The pharmacokinetic parameters and statistical analyses were performed using Stata (v. 16.1, StataCorp, USA)

Results

All eight pigs completed the study. Data were obtained from all pigs except for two catheters: one in cancellous bone and one in cortical bone. For four cortical bone catheters and two subcutaneous adipose tissue catheters, RR could not be reliably determined. However,

Table II. Key pharmacokinetic parameters for vancomycin and meropenem in cortical bone, cancellous bone, and subcutaneous adipose tissue.

Pharmacokinetic parameter	Cortical bone	Cancellous bone	Subcutaneous adipose tissue	Plasma
Vancomycin				
Mean $AUC_{0\text{-last}}$, min µg/ml (95% CI)	1,359 (-282 to 2,999)*	3,593 (1,953 to 5,233)	5,210 (3,570 to 6,851)	7,288 (5,648 to 8,928)
Mean C_{max}, µg/ml (95% CI)	7 (-2 to 16)	14 (5 to 23)	22 (13 to 31)	35 (26 to 44)
Mean T_{max}, mins (95% CI)	330 (276 to 384)	218 (164 to 271)	173 (119 to 226)	92 (38 to 146)
Meropenem				
Mean $AUC_{0\text{-last}}$, min µg/ml (95% CI)	320 (-3 to 644)†	1,157 (833 to 1,480)†	1,872 (1,548 to 2,196)	3,111 (2,787 to 3,434)
Mean C_{max}, µg/ml (95% CI)	3 (-6 to 11)*	14 (5 to 22)	22 (13 to 30)	67 (58 to 75)
Mean T_{max}, mins (95% CI)	94 (76 to 112)	41 (23 to 59)	26 (8 to 44)	15 (-3 to 33)

$AUC_{0\text{-last}}$, area under the concentration-time curve from 0 to the last measured value; C_{max}, peak drug concentration; T_{max}, time to C_{max}.
$p < 0.001$ using repeated measurements analysis of variance (F test) for overall comparison for both vancomycin and meropenem pharmacokinetic parameters.
*$p < 0.05$ using analysis of variance (paired *t*-test) for comparison with all other compartments.
†$p < 0.001$ using analysis of variance (paired *t*-test) for comparison with all other compartments.
AUC, area under the curve; CI, confidence interval.

dialysate concentrations from these catheters resembled the dialysate concentrations of the remaining catheters from the same locations, wherefore the mean RR values of the remaining catheters from the same locations were applied. For vancomycin, the mean RR was 27% to 32% across compartments, while for meropenem mean RR was 30% to 55%.

Vancomycin. T > MIC (1 to 4 µg/ml) in minutes and percentages (%T > MIC) of the eight-hour dosing interval are reported in Table I. For all the investigated MICs, T > MIC was shorter in cortical bone compared with cancellous bone, subcutaneous adipose tissue, and plasma. For the low MIC-target of 1 µg/ml, the mean T > MIC was 208 minutes (46%) for cortical bone, 403 minutes (89%) for cancellous bone, 441 minutes (98%) for subcutaneous adipose tissue, and 449 minutes (100%) for plasma. For the high MIC target of 4 µg/ml, the mean T > MIC was 30 minutes (7%) for cortical bone, 296 minutes (66%) for cancellous bone, 416 minutes (93%) for subcutaneous adipose tissue, and 446 minutes (99%) for plasma.

Key pharmacokinetic parameters are listed in Table II. Lower $AUC_{0\text{-last}}$ was found in cortical bone in comparison with all other investigated compartments, while plasma demonstrated the highest values.

Meropenem. T > MIC (0.125 to 2 µg/ml) in minutes and percentages (%T > MIC) of the eight-hour dosing interval are reported in Table I. For all MICs, T > MIC was shorter in cortical bone compared with cancellous bone, subcutaneous adipose tissue, and plasma. For the low MIC target of 0.125 µg/ml, the mean T > MIC was 189 minutes (42%) for cortical bone, 278 minutes (62%) for cancellous bone, 331 minutes (74%) for subcutaneous adipose tissue, and 406 minutes (90%) for plasma. For the high MIC target of 2 µg/ml, mean T > MIC was 45 minutes (10%) for cortical bone, 144 minutes (32%) for cancellous bone, 180 minutes (40%) for subcutaneous adipose tissue, and 181 minutes (40%) for plasma. Key pharmacokinetic parameters are listed in Table II. Lower $AUC_{0\text{-last}}$ and C_{max} were found in cortical bone in comparison with all other investigated compartments, while plasma demonstrated the highest values.

Discussion

We investigated tibial bone and adjacent soft-tissue concentrations of co-administered standard doses of vancomycin and meropenem during an eight-hour sampling interval. Across the targeted ECOFF values, vancomycin displayed longer T > MIC in all the investigated compartments in comparison to meropenem. For both drugs, cortical bone exhibited the shortest T > MIC (Figures 1 and 2). For the low MIC targets and across compartments, T > MIC ranged between 208 and 449 minutes (46% to 100%) for vancomycin and between 189 and 406 minutes (42% to 90%) for meropenem. For the high MIC targets, T > MIC ranged between 30 and 446 minutes (7% to 99%) for vancomycin and between 45 and 181 minutes (10% to 40%) for meropenem.

Treatment of open tibial fractures includes appropriate and prompt antibiotic treatment (within three hours),[22,23] surgical debridement, irrigation, bone stabilization, and fast and sufficient soft-tissue coverage.[24] When considering the high infection rates, the diversity of the encountered bacteria and the devastating consequences, e.g. risk of amputation, for patients suffering from open fracture-related infections, sufficient broad-spectrum antibiotic prophylaxis is important. Vancomycin and meropenem have been proposed as such a prophylaxis for open tibial fractures, as they provide excellent coverage against the majority of the encountered bacteria causing infection.[1,2] To keep the administration period as short as possible, an initial target of 100%T > MIC may be considered to ensure that the most resistant bacterial subpopulation is targeted.[4,25] Prudently, achievement of this target is determined by the MIC evaluated. In the present setup, mean 100%T > MIC was only reached for vancomycin in plasma. To achieve longer target tissue T > MIC for both drugs, alternative dosing regimens seem necessary,[26] e.g. weight-based dosing, additional local application, repeated dosing, or continuous infusion. For meropenem in particular, it has been shown that continuous infusion is superior to intermittent administration in terms of T > MIC in critically ill patients.[27,28] Although appropriate and prompt antibiotic treatment is considered imperative, no

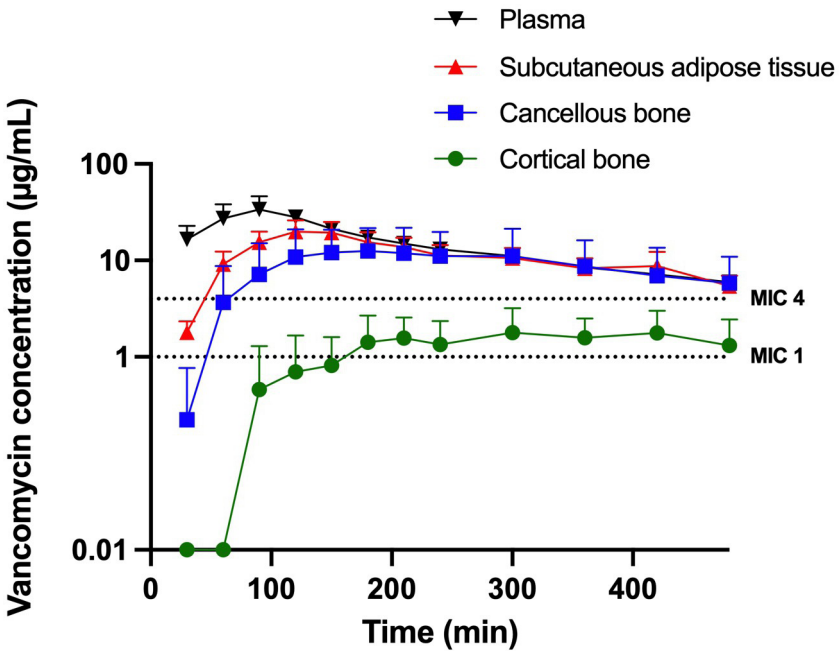

Fig. 1

Mean concentration time profiles of vancomycin in plasma, subcutaneous adipose tissue, cancellous bone, and cortical bone. Minimal inhibitory concentrations (MICs) of 1 and 4 µg/ml are indicated by the horizontal dotted lines. Y-axis is log-scaled. The error bars represent upper 95% confidence intervals.

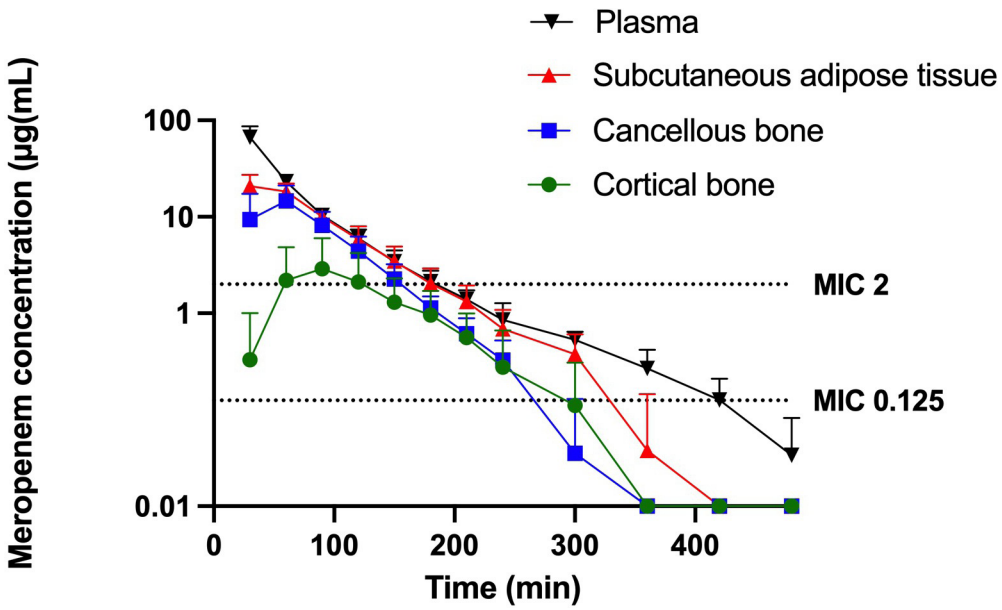

Fig. 2

Mean concentration time profiles for meropenem in plasma, subcutaneous adipose tissue, cancellous bone, and cortical bone. Minimal inhibitory concentrations (MICs) of 0.125 and 2 µg/ml are indicated by the horizontal dotted lines. Y-axis is log-scaled. The error bars represent upper 95% confidence intervals.

sound correlation between antibiotic target tissue exposure and prevention of infection development following open tibial fractures exists. This makes the interpretation and comparison of our results to fixed pharmacokinetic/pharmacodynamic targets theoretical rather than clinical. In this context, a recent study found no differences in infection rates in open fractures between one and three days of systemic antibiotic prophylactic treatment.[12] Further, local susceptibility patterns will likely also affect choice of antibiotic prophylaxis; for example, studies conducted on open tibial fractures sustained by military personnel in the battlefield found that *Acinetobacter*

species were among the most common contaminating bacteria in that specific setting, requiring an ECOFF-target for meropenem of 4.0 µg/ml.[29]

It is well recognized that sufficient and early soft-tissue coverage plays a significant role in open tibial fracture management,[12] and antibiotic treatment should be maintained until attainment of sufficient soft-tissue coverage. The significance of soft-tissue coverage is, presumably, due to the physical barrier preventing further contamination, as well as providing nutrients, antibiotic supply, and facilitating local immune response to the fracture site.[30] We found adjacent subcutaneous adipose tissue to provide vancomycin T > MIC ranging from 416 to 441 minutes (93% to 98%) across the investigated MICs, while the corresponding meropenem T > MIC values were 180 to 331 minutes (40% to 74%). However, the pigs in the present study were only exposed to incision-related tissue damage with neither tibial fracture nor contamination. It remains unknown to what extent soft-tissue damage and bone fracture shift the distribution of antibiotics, but it seems judicious that sufficient antibiotic concentrations should be reached in the entire soft-tissue envelope. A fracture induces activation of an acute phase response via increase in local bone concentrations of proinflammatory cytokines, such as interleukin-6, which increases perfusion in the fracture site and the permeability of the blood vessels.[31] Thus, in the current experimental setting, the measured concentrations may be underestimated compared to an open fracture setting. Similar alterations in soft-tissue following a fracture have not been investigated. Bacterial contamination has not been found to affect blood flow in musculocutaneous tissue.[32] Accordingly, the impact of soft-tissue and bone damage on the antibiotic distribution in open tibial fracture is extremely heterogenous and is difficult to predict upon initial assessment, which may advocate for an aggressive antibiotic prophylactic approach. Interestingly, a recent systematic review concludes that additional application of local antibiotics reduces the risk of subsequent fracture-related infection and may therefore be considered as an important supplement to the systemic treatment.[33]

Demonstrated by the $AUC_{0\text{-last}}$ values, vancomycin displayed an incomplete penetration into both bone tissue compartments (Figure 1). Also, meropenem $AUC_{0\text{-last}}$ exhibited noteworthy intercompartmental differences (Figure 2), with significant differences between all measured compartments. Differences in molecular size (meropenem, 383 Da, and vancomycin, 1450 Da) and antibiotic classes between the two drugs may explain the differences in tissue concentrations and penetration ratios. For both drugs, cortical bone presented with the lowest concentrations. In recent years, colonization of *S. aureus* within canaliculi of the cortical bone has been shown both in experimental and clinical studies.[34,35] Canaliculi diameters range from 80 to 710 nm,[36] making host immune cells too large to enter the canaliculi. This makes antibiotic penetration to cortical bone of utmost importance since failure of bacterial eradication could lead to chronic osteomyelitis. Although our results are largely in accordance with previously attained results, mean meropenem $AUC_{0\text{-last}}$ and C_{max} in subcutaneous adipose tissue were significantly lower than previously reported, while cancellous bone demonstrated a tendency of lower concentrations.[14,21] These previous studies evaluated vancomycin and meropenem administered as monotherapy, hence these differences may suggest an interaction between the drugs influencing tissue penetration for meropenem when co-administered. This is new knowledge, since previously only lack of interaction or synergistic interactions between the two drugs had been described.[37] To our knowledge, no studies have thoroughly evaluated the interaction of the two drugs and its effect on the tissue concentrations, particularly not in the case of a fracture. Future studies assessing this matter more thoroughly are warranted.

Our study has several limitations. Neither fracture nor contamination were introduced, and sampling was conducted on healthy young (aged five months) porcine tissue. Although pigs generally are considered a good experimental model for bone and soft-tissue research,[38] the lack of interventions may limit the translational potential and should be included in future studies. To introduce a fracture in an experimental study it is essential to have an established fracture model, which ensures a uniform fracture and local response each time. Furthermore, drilling in bone is obligatory when applying MD in bone tissue, which may induce thermal bone necrosis affecting the bone concentrations. However, as drilling is an integrated part of open fracture management, this likely reflects the clinical setting. Lastly, it is important to recognize that sampling was performed in an eight-hour sampling interval. While intermittent meropenem administration is normally given every eight hours, vancomycin is generally administered every 12 hours. Our vancomycin results can therefore not be attributed to a full dosing interval.

In conclusion, we found longer T > MIC for vancomycin than meropenem across all investigated compartments and ECOFFs. Cortical bone in particular exhibited short T > MIC for both vancomycin and meropenem. The differences in the T > MIC between the low and high targets illustrate how the interpretation of these results, and potentially the effect of the prophylaxis, is highly susceptible to the defined MIC target. Accordingly, treatment should be guided by local susceptibility patterns, but future studies are needed to evaluate the prophylactic efficacy in open tibial fractures with different antibiotic targets. To encompass any trauma, contamination, or individual tissue differences, a more aggressive dosing approach than just choosing a broad-spectrum combination to achieve longer T > MIC in all the exposed tissues, including application of local antibiotics, may be considered to lower the risk of acquiring an infection after open tibial fractures.

Supplementary material

An ARRIVE checklist is included to show that the ARRIVE guidelines were adhered to in this study.

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Author information:

- S. Ø. Vittrup, BSc.med, Research Year Student
- P. Hanberg, MD, PhD, Medical Doctor
- S. K. Tøstesen, BSc.med, Medical Student
- J. O. Kipp, MD, PhD Student
  Aarhus Denmark Microdialysis Research (ADMIRE), Orthopaedic Research Laboratory, Aarhus University Hospital, Aarhus, Denmark; Department of Clinical Medicine, Aarhus University, Aarhus, Denmark.
- M. B. Knudsen, BSc.med, Medical Student, Aarhus Denmark Microdialysis Research (ADMIRE), Orthopaedic Research Laboratory, Aarhus University Hospital, Aarhus, Denmark.
- J. Hansen, MSc, Molecular Biologist, Department of Forensic Medicine, Aarhus University Hospital, Aarhus, Denmark.
- N. P. Jørgensen, MD, PhD, Postdoctoral Researcher, Medical Doctor, Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark.
- M. Stilling, MD, PhD, Orthopaedic Surgeon, Professor
- M. Bue, MD, PhD, Assistant Professor
  Aarhus Denmark Microdialysis Research (ADMIRE), Orthopaedic Research Laboratory, Aarhus University Hospital, Aarhus, Denmark; Department of Clinical Medicine, Aarhus University, Aarhus, Denmark; Department of Orthopaedic Surgery, Aarhus University Hospital, Aarhus, Denmark.

Author contributions:

- S. Ø. Vittrup: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Visualization, Writing – original draft.
- P. Hanberg: Conceptualization, Formal analysis, Investigation, Supervision, Visualization, Writing – review & editing.
- M. B. Knudsen: Investigation, Writing – review & editing.
- S. K. Tøstesen: Investigation, Writing – review & editing.
- J. O. Kipp: Investigation, Writing – review & editing.
- J. Hansen: Data curation, Writing – review & editing.
- N. P. Jørgensen: Conceptualization, Writing – review & editing.
- M. Stilling: Conceptualization, Supervision, Writing – review & editing.
- M. Bue: Conceptualization, Formal analysis, Supervision, Writing – review & editing.

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