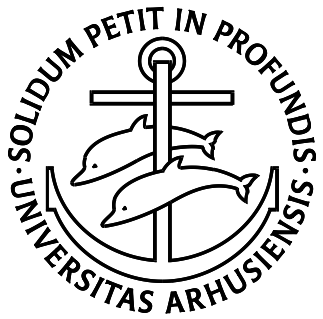
Bone, intervertebral disc and subcutaneous adipose tissue pharmacokinetics of vancomycin obtained by microdialysis

PhD thesis

Mats Høy Bue



Faculty of Health Sciences
University of Aarhus
2019

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Preface

In 2013, I was captured to this research group by Professor Kjeld Søballe and Mikkel Tøttrup (at that time a PhD student) and given the chance to conduct a research year. The field of orthopaedic research excited me, and I was fortunate enough to get the opportunity to perform an integrated PhD after finishing my research year. This period of time as an integrated PhD student, part time as a PhD student, and part time as a medical student, was in all honesty not the most pleasant, as having two jobs often results in priority difficulties. However, after graduating from medical school, I have had a most enjoyable time as a PhD student, and I genuinely hope to continue my scientific endeavours.

Research and learning is hard work: "*If I had only known then what I know now.*"

With humble respect for everything these past years have taught me.

Mats Høy Bue
Aarhus, January 2019

This PhD thesis is based on the scientific work conducted during my enrolment as a PhD student at the Faculty of Health Aarhus University from 2014-2019. This thesis consists of three papers assessing vancomycin concentrations in bone and the intervertebral disc in different orthopaedically relevant settings. In order to make this thesis comprehensible, a review of the scientific fields describing the existing knowledge, the clinical relevance, strengths and limitations of the applied methods, and a discussion of the findings will be presented.

In this thesis re-use and copy of my own work could occur (Study I-III).

The three studies were conducted at the following locations:

Study I: Department of Orthopaedic Surgery, Horsens Regional Hospital, Denmark.

Study II: Department of Veterinary Disease Biology, University of Copenhagen, Denmark.

Study III: Institute of Clinical Medicine, Aarhus University Hospital, Denmark.

All chemical analyses were performed at the Department of Clinical Biochemistry, Aarhus University Hospital, Denmark.

Acknowledgements

First of all, I would like to thank Gerhardt Teichert and Karsten Krøner and my main supervisor Professor Kjeld Søballe for providing me with the opportunity to conduct this PhD in a collaboration between the Orthopaedic Research Unit, Aarhus University Hospital and the Department of Orthopaedic Surgery, Horsens Regional Hospital. This PhD could not have been completed without your fantastic support.

I also would like to thank my co-supervisors Mikkel Tøttrup, Hanne B. Sørensen and Theis M. Thillemann for their great supervision, constructive criticism and helpfulness throughout the studies. You have all been very supportive, and you have inspired and encouraged me to become a better scientist.

A special thanks to Louise Kruse from the Department of Veterinary Disease Biology, University of Copenhagen for a professional and instructive collaboration. I would like to thank Tore F. Hardlei, Mette Vium, Ole H. Larsen and Torben L. Andersson for their great expertise and help in the chemical analyses. I am thankful to Aparna Udupi from the Biostatistical Advisory Service for her invaluable statistical advice and service. My gratitude goes to the patients who generously offered their time and made the clinical study possible. I am grateful for the help and guidance offered by Otto Langhoff, who conducted the surgeries in the clinical study.

Also, my gratitude goes to the research staff at the Orthopaedic Research Unit, Aarhus University Hospital, Anette Baatrup, Anna Bay, Maj Haubuf, Natasja Jørgensen, Kris Hede, Morten Lykke Olesen, Dang Le and Rasmus Cleemann for offering me a research environment with space and room for interesting discussions and great company.

A special thanks to our small orthopaedic microdialysis group in which Maja Thomassen, Martin Lundorff, Janne Koch and Pelle Hanberg offered invaluable assistance in conducting the studies.
I would like to extend my appreciation to my co-worker Pelle Hanberg for his friendship, assistance and support. I could not have asked for a better co-worker to help me through all the studies. This PhD could not have been completed without his help.

Finally, I am very grateful for the much-needed support from my family during these 4.5 years of research. Most of all, I wish to thank my wife, Tine, for her unconditional support and for making it possible for me to achieve my goals.

I wish to acknowledge and thank Bevica Fonden, Familien Hede Nielsens Fond, Korning Fonden, Fonden for Læger på Regionshospital Horsens, Aase og Ejnar Danielsens Fond, Elisabeth of Karl Ejnar Nis-Hanssens Mindelegat, Lippmann Fonden, Augustinus Fonden, Knud og Edith Eriksens Mindefond, Søster og Verner Lipperts Fond and Region Midtjyllands Sundhedsvidenskabelige Forskningsfond for the financial support of this PhD project.

List of papers

This PhD thesis is based on the following papers:

Paper I:
Bue M, Tøttrup M, Hanberg P, Langhoff O, Birke-Sørensen H, Thillemann TM, Andersson TL, Søballe K.
Bone and Subcutaneous Adipose Tissue Pharmacokinetics of Vancomycin in Total Knee Replacement Patients(1). *Acta Orthopaedica, 2017.*

Paper II:
Bue M, Hanberg P, Koch J, Kruse Jensen L, Lundorff M, Aalbæk B, Elvang Jensens H, Søballe K, Tøttrup M.
Single-Dose Bone Pharmacokinetics of Vancomycin in a Porcine Implant-Associated Osteomyelitis Model(2). *Journal of Orthopaedic Research, 2017.*

Paper III:
Bue M, Hanberg P, Tøttrup M, Thomassen MB, Birke-Sørensen H, Thillemann TM, Andersson TL, Søballe K.
Vancomycin Concentrations in the Cervical Spine after Intravenous Administration – Results from an Experimental Pig Study(3). *Acta Orthopaedica, 2018.*

The papers are referred to in the text by their Roman numerals (I-III).

Abbreviations

AUC	Area under the concentration-time curve
C_{max}	Peak drug concentration
MIC	Minimal inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
PK	Pharmacokinetics
PD	Pharmacodynamics
T_{max}	Time to C_{max}
$T_{1/2}$	Half-life
UHPLC	Ultra-high performance liquid chromatography

Table of contents

1. English summary

The prevention and treatment of bone, intervertebral disc and implant-associated bone infections remain a major challenge for clinicians. Treatment failure can have devastating complications for both the patients and the healthcare system. Treatment failure rates remain high, which may be a consequence of incomplete antimicrobial bone and intervertebral disc penetration.

The majority of orthopaedic infections are caused by *Staphylococcus aureus,* with an increasing incidence of *methicillin-resistant Staphylococcus aureus* (MRSA) infections. Vancomycin is effective against these bacteria and may become an important drug in some orthopaedic settings.

The evaluation of vancomycin bone and intervertebral disc pharmacokinetics is, however, a challenging task. At present, bone and intervertebral disc pharmacokinetics of vancomycin have mainly been investigated using bone and disc tissue samples and discectomy. These methods suffer from methodological limitations, which makes it hard to assess the pharmacokinetic profile of the investigated drugs. The pharmacokinetic tool, microdialysis, has shown promising evolvement for sampling of various antimicrobials in bone and the intervertebral disc. The advantages of serially sampling the extracellular and unbound fraction of drug make the method attractive compared to the existing methods.

The objective of this PhD project was to apply microdialysis for sampling of vancomycin in different orthopaedically relevant settings. The project consisted of three studies, one of which was a clinical study and two of which were experimental studies: two mimicking perioperative settings and one evaluating the infectious situation. Vancomycin was administered and sampled in the same way in all three studies: a single dose of 1,000 mg being given intravenously over 100 min, and concentrations were sampled over 8 hours. The quantification of drug was performed with ultra-high performance liquid chromatography with ultraviolet detection. Data were analysed by non-compartmental analysis.

In the clinical study, Study I, the penetration of vancomycin to bone and subcutaneous adipose tissue was found to be incomplete and delayed in male patients undergoing total knee replacement surgery. To evaluate the effect of infection on vancomycin bone penetration, Study II found that the *Staphylococcus aureus* implant-associated osteomyelitis reduced vancomycin bone penetration, especially to the implant cavity. In Study III, the vancomycin penetration to the vertebral cancellous bone and the intervertebral disc were also found to be incomplete and delayed.

In conclusion, microdialysis was successfully applied for the assessment of vancomycin concentrations in healthy and infected bone and in the intervertebral disc. In all three studies, clinical as well as experimental, an incomplete and delayed penetration of vancomycin to bone and the intervertebral disc was found. The lowest penetration ratios were found in cortical bone, the implant cavity, and the intervertebral disc. Overall, these results suggest that a single dose of 1,000 mg of vancomycin may not penetrate adequately to healthy bone, infected bone or the intervertebral disc.

2. Danish summary

Forebyggelse og behandling af infektioner i knogle, discus, og protesenær knogle, er fortsat en stor behandlingsudfordring for den behandlende kliniker. Behandlingsvigt kan have fatale konsekvenser for både patienten såvel som for vores sundhedssystem. Antallet af behandlingssvigt er relativt høj, hvilket kan være en konsekvens af insufficient antibiotika penetration til knogle og discus.

Størstedelen af de ortopædkirurgiske infektioner er forårsaget af *staphylococcus aureus* med en stigende forekomst af *methicillin-resistente staphylococcus aureus* (MRSA)-bakterier. Vancomycin er et af få stoffer effektivt mod disse bakterier, hvorfor det potentielt kan blive et vigtigt antibakterielt middel i forskellige ortopædkirurgiske situationer.

Historisk set, har det været en udfordring at beskrive de farmakokinetiske parametre for vancomycin i knoglevæv og discus. Eksisterende viden, beror hovedsageligt på knogle- og vævsbiopsier og udtagelse af discus. Disse metoder udviser imidlertid en række begrænsninger som gør det vanskeligt at evaluere den famakokinetiske profil af det undersøgte stof. Det velkendte farmakologiske redskab, mikrodialyse, er en attraktiv metode til opsamling af antibiotikakoncentrationer i knogle og discus. Metoden har potentiale til at generere bedre og mere anvendelige farmakokinetiske data idet tillader seriel opsamling af den ekstracellulære og frie fraktion af antibiotika.

Det overordnede formål med dette PhD-projekt var at anvende mikrodialyse til opsamling af vancomycinkoncentrationer i forskellige ortopædkirurgiske relevante situationer. Projektet bestod af tre studier, hvoraf et var et klinisk studie og to var eksperimentelle studier. To af studierne forsøgte at efterligne den perioperative situation mens det sidste studie forsøgte at efterligne den infektiøse situation. Vancomycin blev indgivet og opsamlet ens i alle tre studier; en enkeltdosis på 1.000 mg over 100 minutter, og koncentrationer blev opsamlet over 8 timer. Kvantificering af vancomycinkoncentrationerne blev udført på ultra-high performance liquid chromatography apparat med ultraviolet detektion. Data blev analyseret ved non-kompartmental analyse.

I det kliniske studie, studie I, fandt vi nedsat og forsinket penetration af vancomycin til knoglevæv og fedtvæv hos mandlige patienter som skulle have isat en total knæprotese. I studie II, var det vores mål at vurdere en infektions indflydelse på vancomycinpenetrationen til knoglevæv. Vi fandt at den *Staphylococcus aureus* implantat-associerede osteomyelit reducerede knoglepenetrationen af vancomycin, særligt til implantatkaviteten. I studie III fandt vi også nedsat og forsinket penetration af vancomycin til vertebral knogle og discus.

Sammenfattende har mikrodialyse været en god metode til at vurdere vancomycinkoncentrationer i rask og inficeret knoglevæv og i discus. I alle tre studier, klinisk såvel som eksperimentelt, blev der fundet en nedsat og forsinket penetration af vancomycin til knoglevæv og discus. De laveste penetrationsratioer blev fundet i hhv. kortikal knogle, implantatkaviteten og discus. Vores resultater tyder derfor på at et en enkelt dosis af 1.000 mg vancomycin ikke penetrerer sufficient til hverken rask knoglevæv, inficeret knoglevæv eller discus.

3. Background

3.1 Antimicrobial pharmacokinetics and pharmacodynamics

Pharmacokinetics (PK) is the sub-branch of pharmacology dedicated to describing the fate (i.e. absorption, distribution, metabolism and excretion) of a drug administered to a living organism, i.e. “what the organism does to the drug”(4, 5). Pharmacodynamics (PD), on the other hand, is the study of the pharmacologic and toxicologic effects of drugs, i.e. “what the drug does to the organism”(4, 5).

In the specific case of antimicrobial PDs, the correlation between the exposure and the microbiological and clinical effect is relatively simple(5). Whereas other drug PD settings are challenged by inter-individual differences in drug-receptor affinity, antimicrobial PDs allows for the isolation of the receptor (i.e. the bacteria) and thus for the quantification of the antimicrobial efficacy(5, 6). The antimicrobial efficacy is mostly evaluated *in vitro* on planktonic bacteria in relation to the minimum inhibitory concentrations (MIC), defined as the lowest antimicrobial concentration that prevents visible growth, and the minimum bactericidal concentration, defined as the lowest antimicrobial concentration required to kill a particular bacteria(5). *In vivo,* the antimicrobial effect relies on sufficient concentrations above the MIC for the invading bacteria. The antimicrobial effect can furthermore be divided into three categories, two of which are bactericidal and one is bacteriostatic(7, 8):

1) *Concentration-dependent killing*: a clear correlation is exhibited between the concentration of the drug and bacterial killing. Examples of drugs: aminoglycosides and fluoroquinolones.

2) *Time-dependent killing*: only limited concentration-dependent killing is displayed. The killing effect is determined by the time of exposure. Examples of drugs: beta-lactams.

3) *Bacteriostatic*: bacteria are not killed but the bacteria growth is inhibited. Examples of drugs: macrolides, tetracycline and linezolid.

In general, bactericidal drugs have not been found to display higher clinical efficacy than bacteriostatic drugs(8).

The quantitative correlation between a PK parameter, such as peak drug concentration (C_{max}) or area under the concentration-time curve (AUC), and a microbiological parameter, such as MIC, are referred to as PK/PD indices(9). Moreover, it has been emphasised that PK/PD indices should only be based on the unbound, and thus active, concentrations of antimicrobials and, unless stated otherwise, refer to steady state situations(9). The PK/PD targets that best predict efficacy for *concentration-dependent* antimicrobials are C_{max}/MIC or AUC/MIC, depending on the specific antimicrobial(5-7, 10). Specific PK/PD targets have been determined for a number of combinations of *concentration-dependent* antimicrobials and bacteria(11-13). For *time-dependent* antimicrobials, the time above the MIC (T>MIC) is the best predictor of efficacy. Depending on the drug, it is generally recommended that T>MIC should be reached in 30-70% of a dosing interval(14). However, a trend of more aggressive targets of 100%T>MIC, or even 100%T>X·MIC, has been applied and described for some specific combinations of bacteria and patient populations, although these approaches remain controversial(15, 16). Most of the existing antimicrobial PK/PD targets are based on plasma PKs rather than specific tissue

PKs(17) Indeed, understanding the different characteristics of the antimicrobials and their corresponding specific tissue targets plays a key role in selecting the optimal dosing regimen.

3.2 Antimicrobial tissue penetration

An optimal antimicrobial treatment is characterized by the achievement of therapeutic concentrations at the target site, i.e. at the site of infection or where infection is to be avoided, while keeping concentrations in other tissues as low as possible to avoid side effects. For the majority of bacterial infections, the target site for the bacteria is in the interstitial space of solid tissues(5). As explained above, antimicrobial dosing has traditionally been based on plasma indices rather than those of the tissues(17, 18). Recently, however, a number of studies have found incomplete and heterogeneous tissue distribution for a diverse combination of drugs and tissues under both physiological and pathological conditions(19-28). Antimicrobial tissue concentrations exceeding plasma concentrations have also been reported(29, 30). These findings contrast with the traditional conception that antimicrobial plasma concentrations reflect tissue concentrations. Accordingly, it can be speculated that insufficient antimicrobial tissue concentrations may account for some treatment failures. Therefore, it seems reasonable to assess specific antimicrobial tissue concentrations under different conditions in order to optimise the existing antimicrobial treatment regimens. This is also emphasised in the recommendations from the US Food and Drug Administration: tissue distribution studies ought to be a part of antimicrobial drug development(31, 32).

A number of methods have been applied in order to evaluate antimicrobial tissue concentrations, including the skin blister method, concentration measurements in wound exudates and drains, tissue biopsies and fibrin clots(33-39). These methods share notable methodological limitations; for example, concentrations are often given by mass and not by volume and the temporal resolution is usually poor(40). The tissue biopsy method, which predominantly has been the method of choice for bone and the intervertebral disc, also suffers from a number of limitations(41, 42). For example, antimicrobial concentrations are measured in a homogenate that may include lingering blood, and do not appreciate the different tissue compartments. Furthermore, it is difficult to distinguish between the intra- and extracellular elements, and the bound and unbound protein fractions. Due to the inherent invasiveness of the procedure, it is only possible to harvest one or at most a few biopsies, resulting in poor temporal resolution. Since it is the unbound antimicrobial concentrations in the interstitial space that are considered to be active against most bacteria, the tissue biopsy method may lead to an over- or underestimation of the antimicrobial concentrations, depending on whether the antimicrobial accumulates intra- or extracellularly(5). Consequently, it is difficult to relate the findings based on tissue biopsies to relevant PK/PD targets, and at worst, the use of this method could be harmful to the patients(43, 44).

3.3 Antimicrobials in orthopaedics

Orthopaedic-related infections can have devastating complications for both the patients as well as the healthcare system. A sufficient antimicrobial treatment plays a key role in lowering the risk of acquiring surgical site infections and in treating orthopaedic-related infections. It is important to recognise that a sufficient

antimicrobial effect relies not only on the antimicrobial's sensitivity against the invading bacteria, but also on adequate target site penetration so that optimal PK/PD indices can be achieved at the target site.

Bone is a less vascularised and more compact organ in comparison to, for example, the liver or skin(41). As such, it seems rational to acquire knowledge of antimicrobial bone PK/PD before administering the drugs in orthopaedic settings. In the case of bone infections, the infection induces intra-trabecular suppuration, which results in ischaemic osseous sequestration and therefore even more reduced vascularisation(45). Accordingly, it seems reasonable to hypothesise that antimicrobial bone penetration decreases with progression of an infection. This may partly explain the rather high relapse rates in the treatment of bone infections and implant-associated bone infections, even though surgical debridement and prolonged antimicrobial therapy have been applied(46, 47). This also emphasises the importance of differentiating the antimicrobial treatment in orthopaedics in perioperative prophylactic and infectious settings. Healthy and infected bone may exhibit very different antimicrobial bone penetration. Consequently, there is a need for increasing the limited knowledge of antimicrobial bone PKs.

The majority of studies assessing antimicrobial bone and intervertebral disc penetration have done so by measuring the concentrations in homogenised bone and intervertebral disc biopsies(41, 42). The bone and the intervertebral disc biopsy method suffer from the same limitations as those described above(41, 43). As such, heterogeneous data have been reported between drugs and between studies of the same drug(41). Nevertheless, for most of the investigated antimicrobials, an incomplete bone penetration has been described(41). An alternative or supplement to the bone biopsy approach to determine antimicrobial bone concentrations may be the method of monitoring radiolabelled antimicrobials in bone using positron emission tomography(PET)(41, 48-50). However, this method also does not allow for a differentiation between intra- and extracellular concentrations of antimicrobials, and the evaluation of concentration changes over time may be limited for ethical reasons. In contrast, the well-known probe-based PK technique, microdialysis, has the potential to overcome some of the inherent limitations associated with the bone biopsy and radiolabelled-PET approach. Significantly, microdialysis is advantaged by serial sampling of the unbound extracellular concentrations of drug. To date, microdialysis has been applied for sampling of bone concentrations of gentamycin, linezolid, daptomycin, fosfomycin and cefuroxime in both experimental and clinical studies(26-28, 30, 51-53). Considerations regarding antimicrobial sampling in bone using microdialysis can be found in section 5.1.2.

Irrespective of approach, it is however appreciated that the lack of a gold standard remains a significant challenge in validating the existing findings on antimicrobial bone concentrations.

3.4 Vancomycin

Vancomycin has been available on the Danish market for over 30 years. It is a glycopeptide antimicrobial exerting its bacterial effect by binding to and sequestering a lipid at the bacterial cell

surface, thus preventing cell-wall synthesis(54). Terminal elimination half-life and protein binding are generally considered to be in the range of 3-9 hours and 30-60%, respectively(55, 56).

For intravenous use, vancomycin is manufactured and delivered as vancomycin hydrochloride. Before administration, vancomycin hydrochloride must be dissolved in a relevant solution(57). Dissolved vancomycin has shown long stability at room temperature for at least 48 hours(58). The drug also has long stability in plasma after it has been frozen and its stability has been shown to persist over several freeze-and-thaw cycles(59). In Denmark, the recommended dosage is 1,000 mg every 12 hours, or 30-40 mg/kg/day, and may be correlated to the patients creatinine clearance(57). It is furthermore recommended that the minimal plasma concentration should aim to be in the range of 5-10 µg/mL before the administration of the following dose(57). Due to toxicity concerns related to the infusion, vancomycin should be administered slowly, not more than 10 mg/min in a sufficiently diluted solution(57). In patients suffering from hearing loss or decreased kidney function, vancomycin treatment should be performed with caution, or not at all, as it is known to cause potential nephro- and ototoxic side effects(57).

The quantification of vancomycin concentrations can be done through various methodological approaches, including microbiological assays, different immunoassays and high-performance liquid chromatography with ultraviolet detection(60-62). In PK studies, it is advantageous to use a very accurate and precise analytical assay to minimise the variances.

Vancomycin is used both prophylactically and in the treatment of infections caused by susceptible gram-positive bacteria. For vancomycin, the majority of orthopaedically relevant bacteria exhibit planktonic MICs in the range of 0.5-4 µg/mL, though higher MIC bacteria also may be encountered(63-66).

In the perioperative antimicrobial prophylactic situation, vancomycin tissue concentration targets for prevention of surgical site infections have not been established. The objective of antimicrobial prophylaxis in surgery is to lower the microbial load of intraoperative contamination to a level that host defences can overcome (67, 68). Accordingly, it seems prudent to attain tissue concentrations that, at least, exceeds relevant bacteria MICs throughout surgery(68, 69). Optimal prevention may, however, require target concentrations of vancomycin that may be multiples above the relevant MICs(69).

In the infectious situation, vancomycin tissue concentration targets have also not been established, but in plasma a 24-h (1,440 min) steady state target ratio of AUC/MIC greater than 24,000 (corresponding to a ratio of 400 when AUC is given in h·µg/mL) has been correlated with successful therapeutic outcomes in clinical settings(5, 70-72). Recently published *Vancomycin Therapeutic Guidelines* support these findings by recommending the achievement of a plasma AUC/MIC ratio above 24,000 when treating *Staphylococcus aureus* infections(69). However, it remains unclear if current plasma targets are applicable to the relevant tissues. Ambiguously, it has been shown that this target is unlikely to be reached for MICs ≥ 2 µg/mL(69, 70). If that

is the case, higher MICs may be equivalent to incomplete vancomycin tissue penetration, because the targets cannot be attained in the tissues. Moreover, high concentrations of vancomycin are also associated with toxicity, which has been observed at doses above 4 g/day or through serum concentrations above 15 µg/mL(70).

The predominant cause of orthopaedic infections is *Staphylococcus aureus,* with an increasing incidence of *methicillin-resistant Staphylococcus aureus* (MRSA) infections(65, 66). Vancomycin is effective against MRSA and is recommended as first-line choice in the treatment of orthopaedic MRSA infections(45, 73). In the years to come, vancomycin may therefore become increasingly important in some orthopaedic settings. However, routine use of vancomycin may not be indicated because of the risk of resistance development (74, 75).

Up to the present, vancomycin bone concentrations have predominantly been assessed using the bone biopsy approach (41, 76, 77). Bone/serum concentrations ratios have been found to vary up to 15-fold despite comparable doses. These rather large variations may be related to the inherent methodological limitations of the bone biopsy method, which makes the results difficult to interpret. Until now, no studies have assessed vancomycin bone PKs using microdialysis.

4. Aim of the thesis

The general purpose of this thesis was to assess vancomycin concentrations in bone, the intervertebral disc and subcutaneous adipose tissue by means of microdialysis in different orthopaedically relevant settings. Microdialysis allows for sampling of the unbound and extracellular concentrations from the tissue of interest, and it therefore has the potential to generate a valuable estimation of the key tissue PK parameters for vancomycin. The vancomycin concentrations were assessed in two perioperative settings and one infectious situation to evaluate different orthopaedically relevant settings. Vancomycin was administered and sampled in the same way in all three studies: a single dose of 1,000 mg being given intravenously over 100 min, and concentrations were sampled over 8 hours.

4.1 Hypotheses for Studies I-III

4.1.1 Study I

Hypothesis: In a clinical setting, the vancomycin penetration to bone and subcutaneous adipose tissue will be incomplete and delayed. A single dose of vancomycin will not provide adequate prophylactic bone and tissue concentrations in the perioperative setting.

4.1.2 Study II

Hypothesis: In a porcine setup, vancomycin bone penetration will decrease with the progression of infection and inflammation. Vancomycin treatment alone will not provide sufficient bone and tissue concentrations when treating acute osteomyelitis.

4.1.3 Study III

Hypothesis: Vancomycin penetration to the intervertebral disc will be incomplete and lower than that of vertebral cancellous bone. A single dose of vancomycin will not provide adequate prophylactic intervertebral disc and vertebral cancellous bone concentrations in perioperative spine settings.

5. Materials & methods

In all three studies, microdialysis was used as a PK tool to sample vancomycin concentrations in different orthopaedically relevant settings. The tissue concentrations of vancomycin were quantified using an ultra-high performance liquid chromatography (UHPLC) method with ultraviolet detection, while vancomycin plasma concentrations were measured with a homogeneous enzyme immunoassay technique. This chapter will outline the basic principles of these methods, and furthermore describe the clinical and porcine models and their attendant ethical and statistical considerations. A more detailed description of the application of these methods can be found in the separate papers in the appendix.

5.1 Microdialysis

Microdialysis is a probe-based method allowing for serial sampling of water-soluble molecules from the extracellular fluid in the tissue of interest by means of a semipermeable membrane at the tip of the microdialysis probe(19, 25, 26, 78). Microdialysis had its conception in the 1970s by Ungerstedt and Pyrock and was introduced into clinical practice in the early 1990s(79, 80). Since then, microdialysis has been used in a number of studies to sample the unbound and thus pharmaceutically active concentrations of antimicrobials in a variety of tissues(53, 78, 81-86). Since most bacteria reside extracellularly, microdialysis allows for serial sampling from the active site(5). Driven by a precision pump, the microdialysis probe is continuously perfused with a physiological solution, referred to as the perfusate. The delivery, or sampling, of molecules occurs across the semipermeable membrane as diffusion along the concentration gradient (see Figure 1). The solution that passes through the probe is referred to as the dialysate and can be collected in small vials for immediate analysis or stored for later use. Sampling can be done within the same subject without causing any pain or practical issues for the subject.

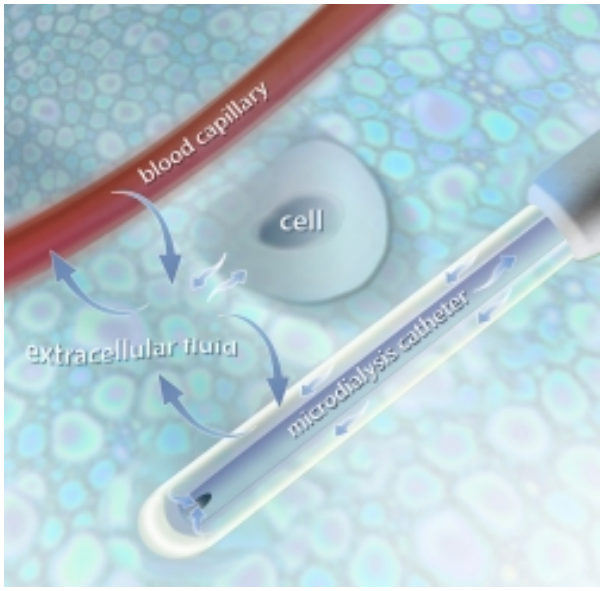

Figure 1. Schematic overview. There is an exchange of substances via the extracellular fluid. Courtesy of M Dialysis AB, Stockholm, Sweden.

Given that the probe is continuously perfused, a complete concentration equilibrium across the semipermeable membrane will never occur. Thus, the concentration in the dialysate represents only a fraction of the true tissue concentration. This is acceptable in studies investigating the changes and ratios of the concentrations, but in antimicrobial PK studies where the estimation of absolute tissue concentrations is the main objective, there is a need for correction. As a correction, relative recovery is used, which is the fraction of the true tissue concentration to be found in the dialysate. Relative recovery depends on factors such as the diffusion coefficient, perfusion rate, type of probe membrane, temperature, tissue structure and the chemical properties of the molecule being analysed, but is, importantly, independent of the

concentration gradient across the semipermeable membrane(24, 87, 88). Determining relative recovery is imperative in microdialysis antimicrobial PK studies.

Relative recovery can be determined through a variety of well-described methods. They all rely on the assumption that relative recovery by gain (RR_{gain}) equals relative recovery by loss (RR_{loss}). RR_{gain} can be calculated using Equation 1:

$$RR_{gain} = \frac{C_{dialysate}}{C_{media}} \quad (1)$$

where $C_{dialysate}$ is the concentration (µg/mL) in the dialysate and C_{media} is the concentration (µg/mL) in the media surrounding the probe. Equation 1 relies on the assumption that the concentration in the perfusate is 0. RR_{loss} can be calculated using Equation 2:

$$RR_{loss} = 1 - \frac{C_{dialysate}}{C_{perfusate}} \quad (2)$$

where $C_{perfusate}$ is the concentration (µg/mL) in the perfusate. Equation 2 relies on the assumption that the concentration in the media surrounding the probe is 0.

The absolute, extracellular concentrations (µg/mL), C_{tissue}, can be calculated by correcting for relative recovery using Equation 3:

$$C_{tissue} = \frac{C_{dialysate}}{RR} \quad (3)$$

The most common calibration methods are the no-net-flux method, the low-flow-rate method and retrodialysis by calibrator or by drug(24, 87, 88). In most antimicrobial PK studies, retrodialysis has been applied(19, 23, 26, 27, 53, 81, 89). This calibration method can be performed either at the beginning or at the end of the study. In practice, it is done by adding a known concentration of a drug to the perfusate. The concentration in the dialysate, during the calibration period, can be quantified, giving the opportunity to calculate the recovery by loss according to Equation 2. Originally, this method was proposed by Stahle et al. in 1991(90). In this PhD project, retrodialysis by drug was used to calibrate all the probes. Depending on the study design in the present PhD project, relative recovery was determined either before or after sampling of vancomycin concentrations. The most important advantages of the retrodialysis by drug method is that it is based on the drug of interest and that the procedure is simple and not very time-consuming. On the other hand, it is not possible to assess the possible changes in relative recovery over time. Furthermore, if the calibration is performed at the beginning of the study, a washout period is needed to prevent spillover of the drug into the investigated tissues; whereas if it is performed at the end of the study, residues of the study drug may still be present in the tissue, which violates the assumption that the concentrations in the media surrounding the probe are 0. However, if there is a considerable difference between the concentration in the perfusate and the concentration in the media surrounding the probe, the concentration in the media surrounding the probe may be neglected, resulting in only a minimal error(23, 91).

A microdialysis setup in antimicrobial PK studies typically consists of a number of probes, precision pumps and perfusion fluids. The probe designs may differ with respect to their dimensions (shaft, inlet and outlet, etc.), length, and the material and pore-size of the semipermeable

membranes. The precision pump provides a continuous and precise perfusate flow, typically in the range of 0.1-5 µL/min, through the system. The composition of the perfusate can be varied according to the objective but should generally closely mirror the ionic properties of the interstitial fluid surrounding the probe(87, 88). In the present studies, the microdialysis system consisted of CMA 107 precision pumps (M Dialysis AB, Stockholm, Sweden) and CMA 70 probes with membrane lengths of 10 mm, 20 mm and 30 mm and a molecular cut-off 20 kilo Daltons, depending on the design of the studies. The probes are manufactured for clinical use and are delivered in sterile packaging. In all three studies, the perfusate always consisted of 0.9% NaCl during the sampling intervals.

5.1.2 Microdialysis sampling in drill holes in bone

In bone, due to its compact nature, microdialysis probes must be introduced into drill holes in the bone(26, 27, 30, 51-53, 61, 78, 92, 93). The diameter of the drill hole must exceed that of the probe (0.6 mm), so that the probe can be placed without damaging the membrane. Given the compact composition of bone, there is a considerable risk of breaking the drill when drilling in bone. After thorough considerations, and with earlier experiences within the research group in mind, a diameter of 2 mm was chosen for all the bone drill holes involved in this PhD project. The difference in diameter between the probes and drill holes raised a concern regarding the dead space surrounding the probe. The questions were whether the microdialysis sampling of antimicrobials actually would reflect bone concentrations, a mixture of concentrations originating from the bone and the adjacent tissues or a blood clot filling the dead space around the probe. In cases of higher (or lower) adjacent tissue concentrations, this could lead to an overestimation (or underestimation) of the actual bone concentrations. However, the basic law of diffusion states that the diffusion time increases proportionally with the square of the distance. This law makes a significant contribution from the adjacent tissues unlikely because the diffusion distance from the adjacent tissues to the semipermeable membrane are much longer in comparison to the distance from the bone to the semipermeable membrane. Bøgehøj et al. applied microdialysis for the comparison of metabolite concentrations in a blood clot with metabolite concentrations from the femoral head of a minipig(94). A clear wash out pattern was demonstrated in the blood clot, whereas this was not found in the measurements from the femoral head. Stolle et al. found no difference in gentamicin measurements obtained from bone tissue samples and microdialysis(92). To address the issue of potential influence of adjacent tissue concentrations, Tøttrup et. al compared cefuroxime concentrations in two symmetric cortical drill holes using microdialysis: one was sealed at the top with bone wax and one was left unsealed(93). No significant differences between key PK parameters were found, and the concentration-time profiles were alike. Moreover, sealing the drill holes with bone wax has been associated with an increased infection risk(95). In summary, these studies indirectly suggest that microdialysis sampling in drill holes in bone does reflect the actual bone concentrations(92-94).

In this PhD project, sampling has been conducted in both cancellous and cortical bone. It was a challenge to generate and evaluate whether the cortical drill holes were purely intra-cortical with no contact to the bone marrow and whether the probes

were displaced during the sampling period. In clinical settings where immobilisation may be unethical, the displacement of the probes represents a considerable risk. Therefore, in Study I, before removing the probes, a computed tomography (CT) scan was performed to evaluate the cortical drill hole and probe placement. Representative sectional views of the cortical drill from one patient can be found in Figure 4.

5.1.3 Strengths and limitations

In contrast to other methods quantifying antimicrobial tissue PKs, microdialysis allows for serial sampling of the unbound extracellular antimicrobial concentrations and provides dynamic concentration-time profiles. Therefore, the PK parameters obtained by microdialysis are useful for evaluating PK/PD targets. This provides more solid data, with the ability to reduce the number of needed subjects for a given study.

The insertion of a microdialysis probe into the tissue of interest inevitably traumatises the tissue, which may influence the subsequent analysis. Therefore, the elevation of tissue trauma markers and changes in local blood flow have been investigated and described for a variety of tissues(24, 87, 88). The biochemical trauma-related changes have been reported to typically return to baseline within 30-60 min. However, the biochemical trauma-related changes have not been described for the insertion of microdialysis probes in bone tissue. This issue has not been investigated in this PhD project.

As dialysates are serially sampled, the dialysate concentrations represent the average tissue concentration during the sampling interval. The measured concentration is commonly ascribed to the midpoint of each sampling interval; however, this remains a simplification.

In microdialysis studies, it is generally recognised that an acceptable trade-off between the ideal setup and the experimental requirements is unavoidable. One of the most important factors in this context that may be compromised by experimental needs is relative recovery. The correction of the measured concentrations for relative recovery leads to a magnification of the variations associated with the pre-analytical sample handling and chemical assay. These variations will increase exponentially as the relative recovery decreases. It is therefore recommended that relative recovery should exceed 20%(87). It is possible to adjust some experimental factors to complement the experimental needs. An increase of relative recovery can, for example, be achieved by using a long membrane length and a low perfusion flow. However, the membrane length may be defined by anatomical factors, and the perfusion flow rate by the investigated drug: for example, in the case of short half-lived drugs, the flow rate has to be relatively high to provide an appropriate temporal resolution and at the same time produce sufficient volume of the dialysate for the subsequent chemical analysis. Lastly, it is important to acknowledge that changes in the peri-probe environment and the physiochemical properties of the analyte may alter the diffusion coefficient in the interstitial space and thus lead to unwanted changes in the relative recovery(24, 87, 88, 96, 97).

For the most part, microdialysis is limited to sampling only small water-soluble molecules. Lipophilic molecules tend to stick to the tubing and the probe and are incompatible with aqueous perfusates. Large pore-size membranes suited for

macromolecules and proteins may cause an unwanted fluid shift(24, 87, 88).

Microdialysis remains a sampling technique that must be linked to an appropriate analytical assay in order to determine the antimicrobial concentrations. The inherent magnification of the variations associated with the pre-analytical sample handling and the chemical assay calls for a very accurate and precise analytical assay, and the assay is required to meet the challenges of low concentrations and low volumes. In the present PhD project, vancomycin concentrations in the dialysates were quantified using an UHPLC method with UV detection. It is important to integrate information about the quality of the analytical assay into the adjustment of the experimental study design and into the evaluation of the resulting findings to achieve the most feasible methodological microdialysis setup(24). In the following section, the UHPLC method and the considerations about the analytical aspects of antimicrobial microdialysis studies will be presented along with a short description of the homogeneous enzyme immunoassay technique used to determine vancomycin plasma concentrations.

5.2 Ultra-high performance liquid chromatography (UHPLC)

An UHPLC (Agilent 1290 Infinity; Agilent Technologies, USA) with UV detection at 280 nm was used to quantify the vancomycin concentrations in the dialysates. This method was validated according to the Clinical and Laboratory Standards Institute (CLSI) recommendations(98). Briefly, the method validation was performed with respect to the selectivity, linearity, precision, accuracy, lower limit of quantification, stability and recovery (Andersson, TL – unpublished data). The practical procedures and setup for analysis of vancomycin concentration in a dialysate can be found in detail elsewhere(61). In brief, the standard volume needed is 15 µL and the overall chromatographic run time is approximately 2-4 min. Based on the peak areas of vancomycin, calculation of the vancomycin concentrations was conducted with ChemStation software (Agilent Technologies). An example of an UHPLC-apparatus is illustrated in Figure 2.

Figure 2. An example of an UHPLC apparatus.

A representative chromatogram for the measurement of vancomycin concentration in a dialysate is depicted in Figure 3.

The intra-run (total) imprecisions (percent coefficient of variation (%CV)) for the quantification of the vancomycin concentrations in the dialysates were evaluated at three different concentrations: 3.7% (5.7%) at 0.7 g/mL, 3.0% (3.5%) at 3.7 g/mL and 0.9% (2.2%) at 5.2 g/mL. The limit of the quantification was defined as the lowest concentration with an intra-run CV of <20% and was found to be 0.05 g/mL.

The stability of dissolved vancomycin (see section 3.4) is adequate for the present assay(58).

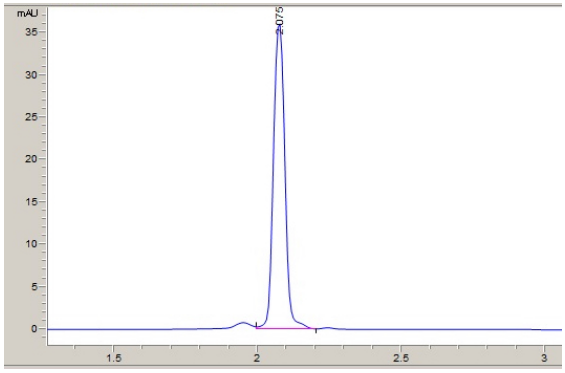

Figure 3. A representative chromatogram for the quantification of the vancomycin concentration in a dialysate.

In summary, applying the UHPLC-UV to quantify vancomycin is a reliable choice in terms of specificity, sensitivity and accuracy. As previously described, the majority of orthopaedically relevant bacteria exhibit MICs in the range of 0.5-4 µg/mL. Thus, the UHPLC gives the opportunity to measure considerably lower concentrations than those which are clinically relevant.

5.2.1 Limitations

Depending on the study design, dialysate volumes in microdialysis antimicrobial PK studies are generally less than 120 µL. In the present studies, the dialysate volumes ranged from 40 to 60 µL. With a standard dialysate volume demand of 15 µL, and given that not all of the dialysate could be pipetted from the microvials, only a limited number of analyses could be performed from each dialysate. It was challenging to pipette volumes at these levels and, as such, even small fluctuations could affect the resulting findings.

5.2.2 Plasma concentrations

The free concentration of vancomycin in plasma was quantified with the clinical standard homogeneous enzyme immunoassay technique. This method was chosen as it has proven to be reliable and precise and it is less time-consuming than the UHPLC method. In Studies I and II, the plasma analysis was done on the Cobas c501 platform (Roche, Basel, Switzerland). The intra-run (total) imprecisions (%CV) for this assay were 2.5% (3.0%) at 16.7 g/mL and 3.7% (4.4%) at 61.0 g/mL. In Study III, the platform was changed to the Siemens Chemistry XPT (Advia Chemistry, Erlangen, Germany) due to changes in the clinical standard at Aarhus University Hospital. The intra-run (total) imprecisions for this assay were ±1.2 µg/mL (2SD) at 6.6 µg/mL and ±3.7 µg/mL (2SD) at 29.1 µg/mL.

The stability of vancomycin in plasma (see section 3.4) is also adequate for the present assay(59).

In the following three sections (5.3-5.5) the clinical and porcine models used in this PhD project will be described along with their attendant ethical and statistical considerations.

5.3 The clinical total knee replacement model

In a recent porcine study, an incomplete and delayed penetration of vancomycin to bone has been demonstrated(61). Furthermore, differences between cortical and cancellous bone were found, with the lowest penetration to cortical bone. In order to evaluate if these findings could be translated into a clinical setting, it was decided to try to evaluate these findings in the best possible clinical setup. A patient category had to be identified in which a cancellous drill hole could be made within the surgical incision and a thick cortex was easily accessible. It was also important to choose a feasible study population that would allow the inclusion of enough patients within a foreseeable period of time. The fulfilment of all of these criteria and earlier experiences within the research group formed the basis for making the decision to use male patients undergoing total knee replacement surgery. Male patients were chosen to ensure safe intra-cortical placement of the drill holes, even though it would limit subsequent generalisability. The study was conducted at the Department of Orthopaedic Surgery, Horsens Regional Hospital. At this department, approximately 60 to 90 male patients undergo total knee replacement surgery each year. It was therefore expected that all the patients needed for this study (n=10) could be included within 1 year.

5.3.1 Ethical considerations

Study I: The study was approved by the Ethics Committee of the Central Denmark Region (registration number 1-16-02-472-14) and the Danish Health and Medicines Authority (EudraCT number 2014-000258-12). The study was conducted in accordance with the Declaration of Helsinki and the ICH Harmonised Tripartite Guideline for Good Clinical Practice (GCP). The GCP unit at Aalborg and Aarhus University Hospitals conducted the mandatory monitoring procedures. Written informed consent was obtained from all patients.

5.3.2 Overview

Ten competent male patients with knee osteoarthritis, who were scheduled for primary total knee replacement surgery were included in this study: the mean (SD) body mass index was 29.7 (4.5) kg and the mean (SD) creatinine level on surgery day was 82 (14) µmol/L. The one conducting surgeon (OL) identified the patients in the outpatient clinic. The exclusion criteria were an allergy to vancomycin; on-going treatment with vancomycin, warfarin or other new anticoagulants; and clinically reduced renal function. The microdialysis probes were placed at the end of the total knee replacement surgery in drill holes in cancellous bone in the medial tibial condyle and in cortical bone in the anterior margin approximately at the midpoint of the tibial diaphysis. The anatomical placement of the cortical drill hole was chosen to ensure an optimal intra-cortical placement. A reference probe was also placed in the subcutaneous adipose tissue of the medial part of the thigh. Vancomycin concentrations were sampled from the respective locations over 8 hours. The primary endpoints were tissue penetration ratios and time to relevant MICs (1-8 µg/mL). Secondary endpoints were the standard PK parameters, $AUC_{0\text{-last}}$, C_{max} and time to C_{max} (T_{max}).

5.3.3 Surgery

When drilling in the cortical bone, the drilling was paused every few seconds and saline was continuously applied to prevent heat necrosis of the bone tissue. For

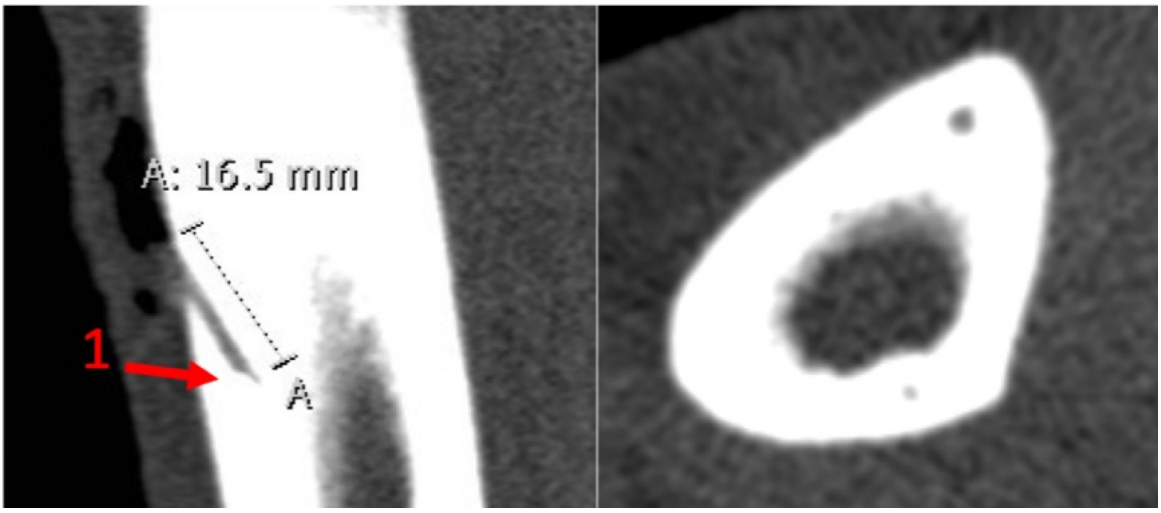

Figure 4. (1) Representative sectional views of the cortical drill hole showing the position of the drill holes and the location of the cortical microdialysis probe; 1: The gold thread within the microdialysis probe membrane tip.

practical reasons, the cancellous drill hole was made inside the knee capsule. Therefore, the cancellous bone probe had to enter the bone via the knee joint. Careful consideration prior to the study was made as to whether the placement of the cancellous bone probe would increase the risk of a prosthetic joint infection. A prosthetic joint infection is a serious complication to total knee replacement surgery, with a reported incidence of approximately 1-2%(99, 100). However, intra-articular drains, with a much larger diameter than the microdialysis probes, are routinely used in total knee replacement surgery. It has previously been found that no bacteria could be cultured from the tip of intra-articular drains removed after 24 hours(101). Moreover, infections related to microdialysis are not reported in clinical microdialysis studies(23, 26, 30, 53, 81, 85, 102). All probes were tunnelated a minimum of 3 cm to reduce the risk of infection. As such, the risk of inducing a microdialysis-related infection was considered to be minimal.

At the end of the total knee replacement surgery, a standard mixture of 150 mL ropivacaine (2 mg/mL), 1.5 mL toradol (30 mg/mL), and 0.75 mL adrenaline (1 mg/mL) was injected locally into the soft tissues surrounding the knee, intraarticularly, and in the posterior joint capsule of the knee as a routine part of pain management.

The patients were allowed to be mobilised. Therefore, before removing the probes, a CT scan of the drill hole in the anterior aspect of the tibia was conducted to verify that that drill had not penetrated to the bone marrow and that the probe had not been displaced. Representative sectional views of the cortical drill hole are illustrated in Figure 4.

5.3.4 Limitations

The results from the present study can be safely regarded only as representative for the specific study population and maybe also only for these specific anatomical regions. The measurements were conducted postoperatively in an anatomical area that had been subjected to a substantial surgical trauma. Moreover, adrenaline and ropivacaine were injected at the end of surgery. All of these matters may have affected the tissue PKs to some extent. While these factors seem to reflect the true perioperative situation for this specific study population, they may not be accurate in other orthopaedic settings.

5.4 The implant-associated acute osteomyelitis porcine model

Pigs have been shown to resemble humans in terms of physiology and anatomy, and when it comes to bone, the composition, density and quality of porcine bone have been found to be comparable with that of humans(103, 104). After assessing the vancomycin penetration into healthy bone in Study I, it seemed obvious to investigate the effect of an infection on vancomycin bone penetration. A novel collaboration with the Department of Veterinary Disease Biology, University of Copenhagen, made it possible to perform this study. The research group from Copenhagen has previously established an acute osteomyelitis porcine model and therefore has great experience in handling and performing orthopaedically related infected pig experiments in excellent local facilities(105-107). It was compelling to unite the research area of acute osteomyelitis and antimicrobial bone penetration and conduct the study at their facilities at the Department of Veterinary Disease Biology, University of Copenhagen.

A t1333 *Staphylococcus aureus* strain was used to induce the infection. This strain was chosen because the acute osteomyelitis porcine model was established with this strain. The strain has been completely characterised by whole genome sequencing, and recently it was demonstrated to be a biofilm forming strain(108). From previous studies and experience, it has histologically been demonstrated that an acute osteomyelitis response will be present already by day 5 in juvenile pigs (5 months), thus meaning that the pig is ill for only 5 days(106). Based on this work, we chose day 5 as the optimal day for antimicrobial measurements in the present porcine model.

5.4.1 Ethical considerations

Study II: The animal experiments were approved by the Danish Working Environment Authority and the Danish Animal Experiments Inspectorate and were carried out in accordance with existing laws (license No. 2013/15-2934-00946).

5.4.2 Overview

Eight female pigs were included in the study (Danish Landrace Breed; weight 75-86 kg), and all went through two surgeries. On day 0, a traumatically induced implant-associated *Staphylococcus aureus* osteomyelitis was induced in the proximal metaphysis of the right tibia. On day 5, microdialysis was applied for sampling of the vancomycin concentrations over 8 hours in the implant bone cavity, in cancellous bone adjacent to the implant cavity, in subcutaneous adipose tissue adjacent to the implant cavity, and in healthy cancellous bone and healthy subcutaneous adipose tissue in the contralateral leg. Venous blood samples were obtained as a reference. The primary endpoint was tissue penetration ratios. Secondary endpoints were the standard PK parameters; $AUC_{0\text{-last}}$, C_{max} and T_{max}.

5.4.3 Verification of probe location and assessment of infection

The correct location of the bone probes was evaluated by fluoroscopy. The degree of infection was evaluated by C-reactive protein level in serum, cultures of blood, swabs from the implant cavity, adjacent cancellous bone and subcutaneous adipose tissue and post-mortem CT scans. The CT scans were used to evaluate the destruction of the bone surrounding the implant cavity by measuring the increase of

the diameter and volume of the cavity. By the time of the autopsy at the end of the experiment, it was assessed that the probes had not been displaced from their locations. Figure 5 shows representative intraoperative fluoroscopy images and post-mortem CT-sectional views from one pig illustrating the implant cavity, the drill hole in cancellous bone adjacent to the implant cavity, and the drill hole in healthy cancellous bone.

5.4.4 Limitations

Even though pigs resemble humans in their physiology, anatomy and the composition of bone, the interspecies differences remain the major limitation of this study(103, 104). When it comes to PK studies, even small interspecies pharmacokinetic differences can have a large impact on how the results can be interpreted. Young female pigs (75-86 kg) were chosen to resemble the weight of the average human. However, young female pigs and their bones are still growing, as opposed to adult humans. These factors may limit generalisability.

The pigs in this study had to undergo two anaesthetic procedures and were kept under general anaesthesia during the entire sampling period to avoid displacements of the microdialysis probes. Anaesthesia over several hours may cause physiological changes that can alter the drug's PK. The length of anaesthesia also limited the duration of the study. As such, our setup only allowed us to sample during an 8-hour sampling interval.

5.5 The porcine spine model

In the last study of this PhD project, we assessed the vancomycin concentrations in a third and different orthopaedically relevant setting. Like all orthopaedic subspecialties, spine surgery struggles with postoperative surgical site infections. The reported incidence of postoperative spondylodiscitis ranges between 1% and 4% and may be even higher when implants are inserted(47, 109-111). The following sites were therefore chosen to assess vancomycin concentrations in spine tissue: the intervertebral disc and vertebral cancellous bone. It seemed especially interesting to evaluate vancomycin penetration into the avascular intervertebral disc. A safe and ethically judicious clinical methodological setup for assessing vancomycin concentrations in the intervertebral disc and vertebral cancellous bone could not be identified. Therefore, a feasible and reproducible porcine spine model was chosen for this study.

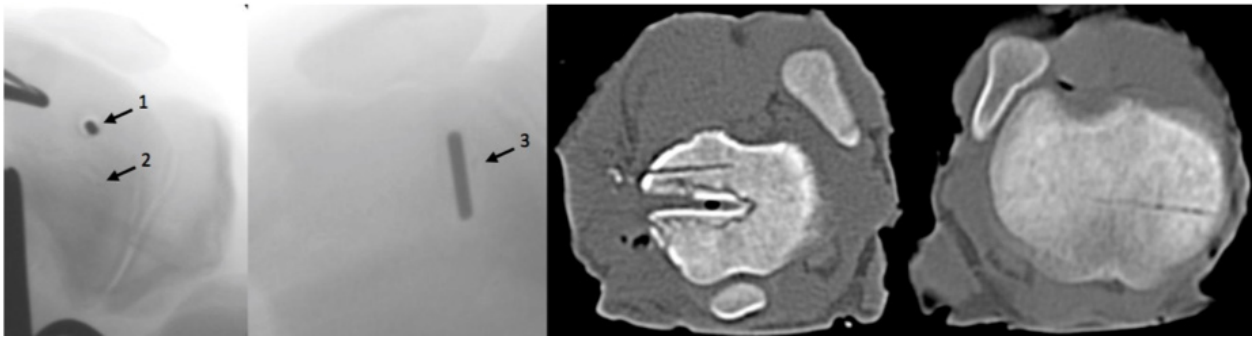

Figure 5. (2) Intraoperative fluoroscopic image (left panel) showing the location of the microdialysis probes in the infected bone. 1: Implant cavity with implant. 2: The gold thread within the microdialysis probe membrane tip in the adjacent drill hole in cancellous bone adjacent to the implant cavity 3: The gold thread in the implant cavity probe. Post-mortem CT sectional views of the drill hole in the implant cavity, cancellous bone adjacent to the implant cavity and healthy cancellous bone (right panel).

The experiment was conducted at the Institute for Clinical Medicine, Aarhus University Hospital, with the ideal facilities and equipment to conduct pig studies.

5.5.1 Ethical considerations

Study III: The animal experiments were approved by the Danish Working Environment Authority and the Danish Animal Experiments Inspectorate and were carried out in accordance with existing laws (license No. 2017 / 15-0201-01184).

5.5.2 Overview

Eight female pigs were included in the study (Danish Landrace Breed; weight 78-82 kg). Vancomycin concentrations were obtained over 8 hours, and microdialysis probes were placed in the C3-C4 intervertebral disc, the C3 vertebral cancellous bone, and subcutaneous adipose tissue. The primary endpoints were the tissue penetration ratios and the time to mean MICs of 2, 4 and 8 µg/mL. The secondary endpoints were the standard PK parameters: $AUC_{0\text{-last}}$, C_{max}, T_{max} and the half-life ($T_{1/2}$). The correct location of the probes in the C3 vertebral cancellous bone and the C3-C4 intervertebral disc was assessed by fluoroscopy. Figure 6 demonstrates a representative intraoperative fluoroscopy image from one pig, illustrating the placement of the microdialysis probe in the intervertebral disc and vertebral cancellous bone.

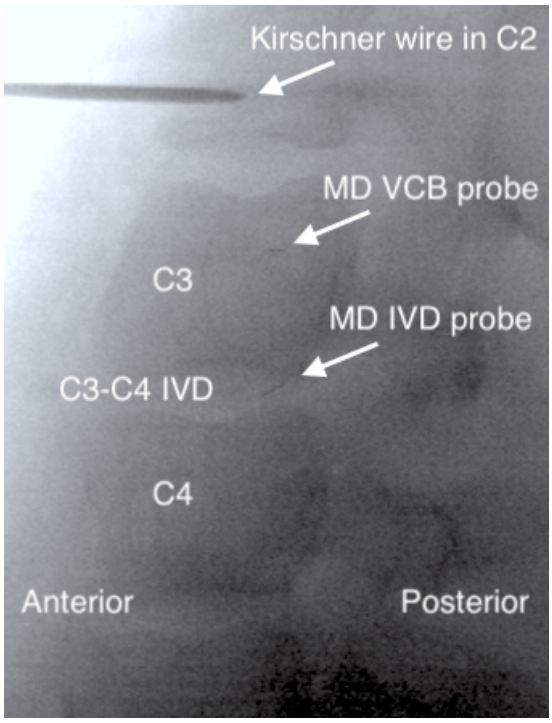

Figure 6. (3) Representative fluoroscopic image showing the location of the microdialysis (MD) probes in a sagittal view, the Kirschner wire with the fixating device in the C2 vertebral body, the C3 and C4 vertebral body, the C3-C4 intervertebral disc (IVD) and the gold thread.

5.5.3 Limitations

As was the case in Study II (see section 5.4.4), two of the major limitations of this study are the interspecies differences and the use of general anaesthesia. Moreover, the properties of the spine in juvenile pigs (aged 5 months) differ from adult humans in several ways. The annulus fibrosus in humans is only vascular during the first part of life. Hereafter, perfusion of the intervertebral disc relies only on diffusion from the endplates(64, 112). The porcine intervertebral disc is also thinner than in humans, indicating shorter diffusion distances(113). Finally, the body mass and the weight-bearing properties of the vertebral bodies and intervertebral disc differ between humans and pigs.

5.6 Statistical considerations

In 1959, Russell and Burch published the concept known as the "three R's": **R**eplacement, **R**eduction and **R**efinement(114). This concept constitutes the values of laboratory animal research. Although all three R's cannot be implemented in all types of research, e.g. it is difficult to do replacement studies in clinical research, researchers should strive to follow these general concepts. As such, every study design was meticulously considered in order to refine the studies, and sample size calculations were conducted for all studies to ensure that the studies were capable of answering the posed scientific questions and thereby justify the size of the study populations(115).

The current knowledge of vancomycin bone concentrations, based on the inherent single-point estimations from bone and disc tissue samples, made it difficult to estimate the vancomycin concentrations in bone and the intervertebral disc. Nevertheless, as shown by these biopsy studies, and outlined in our hypotheses, the vancomycin bone and intervertebral disc penetration was expected to be incomplete(41, 76, 77). The sample size calculations were calculated with respect to the recommended plasma target of AUC/MIC-ratio above 24,000 (corresponding to a ratio of 400 when AUC is given in h·µg/mL), given that this ratio will be reached in plasma with standard dosing of vancomycin. Estimates of the differences in plasma and tissue values and standard deviation were based on the transposition and visual inspection of plasma concentration-time profiles from previous studies. It was hypothesised that bone/plasma ratios would range from approximately 1/3 to 2/3 for vancomycin. These assumptions lead to a suggested AUC/MIC ratio of 16,000 (2/3 of 24,000) in bone, and a standard deviation of approximately 25%. The standard alpha and power were set to 0.05 and 0.9, respectively. Based on these estimates, a sample size of eight subjects was calculated to demonstrate a difference between bone and plasma concentrations (Stata, v. 14.1). Thus, it was decided to include eight pigs in the two animal studies, and in order to accommodate drop-out of patients and/or microdialysis probes, the sample size in the clinical study was increased to 10 patients. It has to be acknowledged that the bone/plasma ratio is probably not constant during a single dose of vancomycin and 8 hours of sampling. Furthermore, all measurements included in this PhD project were obtained before achievement of steady state. Consequently, this sample size calculation is limited by its simplified assumptions due to the sparse amount of existing data.

5.7 Statistical analysis

Different approaches can be used to analyse PK data. In the present PhD project, a non-compartmental analysis (NCA) has been applied. In a NCA, the key PK parameters are calculated from the individual concentration-time profiles for each compartment. Subsequently, descriptive and comparative statistics can be conducted, and different measures such as tissue penetration ratios can be calculated. A NCA is relatively simple to apply and requires fewer assumptions than model-based approaches(116). The main disadvantage of this approach is that it is restricted to the actual data, and can therefore not predict concentration-time profiles and PK parameters for other dosing regimens(116).

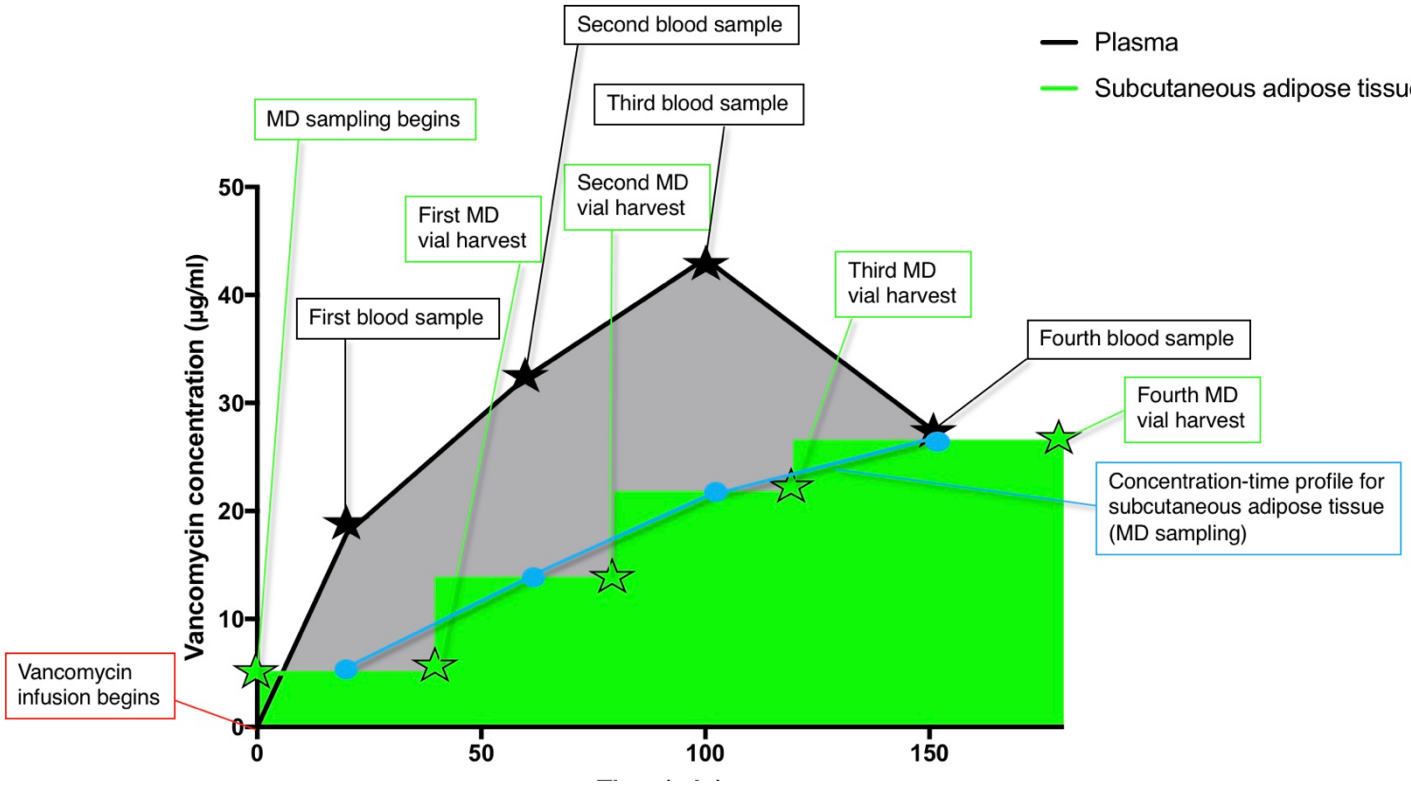

Figure 7. A schematic overview illustrating the simplified AUC calculation in microdialysis antimicrobial pharmacokinetic studies. By decreasing the sampling interval, the influence of this error decreases. The sampling intervals reflect that of the studies comprised in this PhD project. Illustration made by M. Bue.

The key PK parameters were computed in Stata (v. 14.1). The $AUC_{0\text{-last}}$ was calculated using the linear trapezoidal rule, determining the sum of each trapezoid. This methodology is not precise and thus is limited by the width of the sampling intervals (i.e. the trapezoids) and the form of the true concentration-time profile. If large sampling intervals are employed, there is an increased risk of either under- or overestimating the area. This applies to both the infusion phase and elimination phase. Accordingly, by decreasing the sampling interval, the potential error of under- or overestimating the area will be reduced. It is also important to remember that the measured concentrations were ascribed to the midpoint of the sampling interval. It appears that the correlation between the sampling interval and the drug specific half-life is defining the size of this estimation error. Figure 7 illustrates this simplified AUC calculation in a microdialysis study assessing antimicrobial PKs.

C_{max} was calculated as the maximum of all the recorded concentrations and T_{max} was calculated as the time to C_{max}. $T_{1/2}$ was calculated as $\ln(2)/\lambda_{eq}$, where λ_{eq} is the terminal elimination rate constant estimated by linear regression of the log concentration over time.

The PK parameters were determined in all compartments within the same subject. A mixed model for the repeated measurements was applied, taking the variance between pigs into account. The model assumptions were tested with a visual diagnosis of the residuals, fitted values and estimates of random effects. A correction for degrees of freedom due to the small sample size was handled using the Kenward-Roger approximation method. Overall comparisons between the

compartments were performed using Wald's test or the F-test.

Pairwise comparisons were conducted using the t-test. A p-value < 0.05 was considered significant. No correction for multiple comparisons was applied. The tissue $AUC_{0\text{-last}}$ to plasma $AUC_{0\text{-last}}$ ratio ($AUC_{tissue}/AUC_{plasma}$) was calculated as a measure of tissue penetration. These statistical analyses were also performed using Stata. Values below the lower limit of quantification were set to zero.

In Studies I and III, the time mean MICs of 1, 2, 4 and 8 µg/mL were estimated using linear interpolation in Microsoft Excel.

All three studies comprised in this PhD project evaluated the vancomycin concentrations in an 8-hour sampling interval. It was decided not to compute and calculate an extent of the curves to fit a full dosing interval of 12 hours. It was not considered that this would provide any additional information nor change the conclusions.

6. Summary of studies

6.1 Study I

Bone and Subcutaneous Adipose Tissue Pharmacokinetics of Vancomycin in Total Knee Replacement Patients (1)

Hypothesis: In a clinical setting, the vancomycin penetration to bone and subcutaneous adipose tissue will be incomplete and delayed. A single dose of vancomycin will not provide adequate prophylactic bone and tissue concentrations in the perioperative setting.

Hypothesis disproved: No. At least for this specific setting.

6.1.1 Comments

The main finding was that vancomycin penetration to bone and subcutaneous adipose tissue was found to be incomplete and delayed in this population of male patients undergoing total knee replacement surgery. The tissue penetration (95% confidence interval) was for cortical bone 0.17 (0.11-0.24), cancellous bone 0.45 (0.29-0.62) and subcutaneous adipose tissue 0.31 (0.16-0.46). The time to a mean MIC of 2 µg/mL was 3, 36, 27 and 110 min for plasma, subcutaneous adipose tissue, cancellous and cortical bone, respectively. For plasma, subcutaneous adipose tissue and cancellous bone a mean MIC of 4 µg/mL was reached after 6, 68 and 44 min, respectively, whereas a mean MIC of 4 µg/mL could not be reached in cortical bone. Furthermore, for cortical bone, $AUC_{0\text{-}last}$ and C_{max} were lower than those of cancellous bone, suggesting that bone should be considered as a heterogeneous compartment. The standard PK parameters are presented in Table 1 and the vancomycin tissue and plasma concentration-time profiles are shown in Figure 8. The mean (SD) relative recoveries were 19.9 (9.1) %, 35.3 (18.2) %, and 14.3 (5.7) % for subcutaneous adipose tissue, cancellous and cortical bone, respectively.

In summary, our findings suggest that in some combinations of individuals and pathogens, adequate vancomycin tissue concentrations may be reached with a substantial delay or not at all. Conclusively, it may be unsafe to rely only on a single dose of vancomycin antimicrobial prophylaxis for total knee replacement surgery in this population.

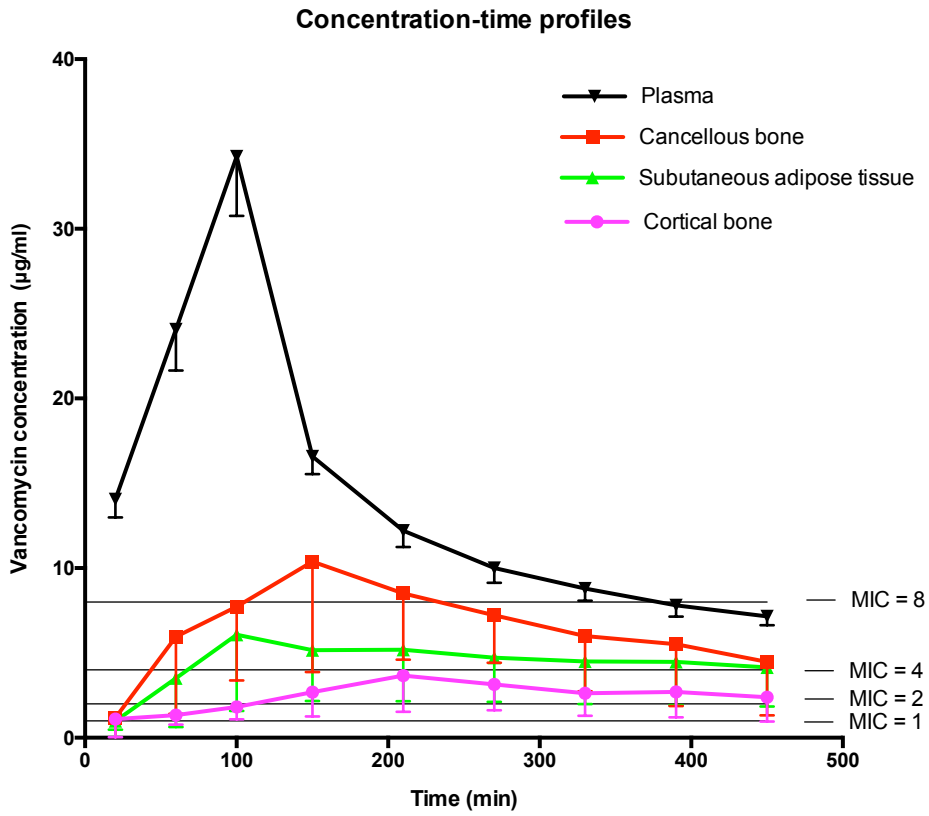

Figure 8. (1) Mean concentration-time profiles for plasma, subcutaneous adipose tissue, cancellous and cortical bone. Bars represent 95% confidence intervals. MICs of 1, 2, 4 and 8 µg/mL are also depicted.

Table 1. (1) Standard PK parameters for plasma, subcutaneous adipose tissue (SCT) and cancellous and cortical bone

	Pharmacokinetic parameter				
Tissue	$AUC_{0\text{-last}}$ (min µg/mL)	C_{max} (µg/mL)[a]	T_{max} (min)	$T_{1/2}$ (min)	$AUC_{tissue}/AUC_{plasma}$
Plasma (unbound)	6296 (5883-6709)	34.3 (31.3-37.2)	100 (64-136)	362 (311-414)	
SCT	1545 (698-2392)[1]	6.6 (3.4-9.8)[1]	200 (120-281)	583 (8-1158)	0.31 (0.16-0.46)
Cancellous bone	2636 (1527-3744)[1]	10.8 (6.3-15.3)[1]	148 (73-223)	360 (21-700)	0.45 (0.29-0.62)
Cortical bone	1016 (661-1371)[12]	4.0 (2.5-5.4)[12]	152 (81-223)	392 (67-716)	0.17 (0.11-0.24)
Overall comparison[b]	$p < 0.001$	$p < 0.001$	-	$p < 0.8$	($p = 0.008$[3])

Values are given as medians (95%-CI) unless stated otherwise.

AUC_{0-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}; $T_{1/2}$, half-life at β-phase; $AUC_{tissue}/AUC_{plasma}$, tissue penetration expressed as the ratio of $AUC_{tissue}/AUC_{plasma}$

[a] Values are given as means (95%-CI)

[b] Overall comparison using Wald's test for free plasma, subcutaneous adipose tissue, cancellous and cortical bone

[1] $p < 0.001$ for comparison with the corresponding free plasma value

[2] $p < 0.007$ for comparison with cancellous bone

[3] T-test comparison of cancellous and cortical bone

6.2 Study II

Single-Dose Bone Pharmacokinetics of Vancomycin in a Porcine Implant-Associated Osteomyelitis Model (2)

Hypothesis: In a porcine setup, vancomycin bone penetration will decrease with the progression of infection and inflammation. Vancomycin treatment alone will not provide sufficient bone and tissue concentrations when treating acute osteomyelitis.

Hypothesis disproved: No. At least for this specific setting.

6.2.1 Comments

The main finding was that vancomycin penetration to the implant cavity was found to be incomplete and lower than in all the other compartments. Accordingly, this *Staphylococcus aureus* implant-associated osteomyelitis was found to reduce vancomycin bone penetration. The tissue penetration (95% confidence interval) was for subcutaneous adipose tissue 0.87 (0.64-1.10), subcutaneous adipose tissue adjacent to the implant cavity 0.74 (0.52-0.96), cancellous bone 0.59 (0.42-0.75), cancellous bone adjacent to the implant cavity 0.41 (0.25-0.57), and implant cavity 0.20 (0.08-0.33). Thus, already 5 days after the induction of the infection in this porcine model, impaired vancomycin penetration to all compartments in the infected leg was found. A trend towards lower $AUC_{0\text{-last}}$ and C_{max} was found in the cancellous bone on the infected side in comparison to the healthy side. However, these differences were not statistically significant. The key PK parameters are provided in Table 2. The vancomycin plasma and tissue concentration-time profiles are shown in Figure 9. The mean (SD) relative recoveries were 21.3 (12.3) % (cancellous bone), 29.5 (11.0) % (cancellous bone adjacent to the implant cavity), 26.5 (6.0) % (subcutaneous adipose tissue), 36.7 (17.7) % (subcutaneous adipose tissue adjacent to the implant cavity) and 40.0 (6.4) % (implant cavity).

In summary, our findings suggest that vancomycin bone penetration may decrease with progression of infection and inflammation. It seems unlikely that sufficient vancomycin concentrations can be achieved in acute osteomyelitis complicated with metaphyseal cavities. Conclusively, it may be unsafe to rely on vancomycin treatment alone when treating acute osteomyelitis. It seems necessary to include surgical debridement in the treatment, especially when metaphyseal cavities are present.

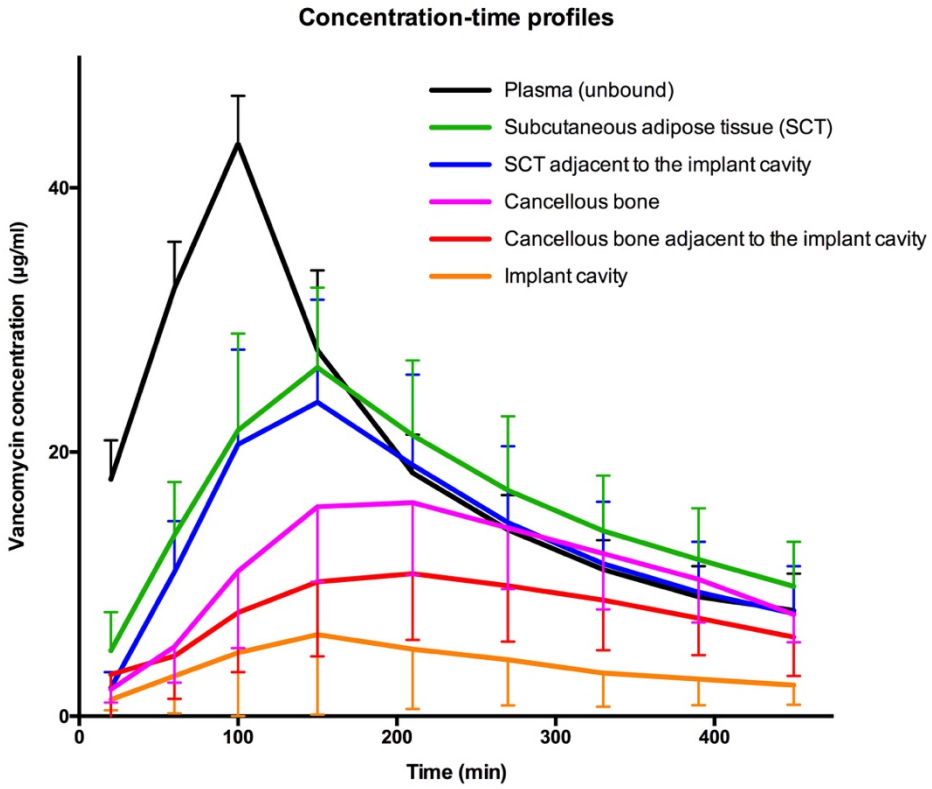

Figure 9. (2) Mean concentration-time profiles for plasma, subcutaneous adipose tissue (SCT), SCT adjacent to the implant cavity, cancellous bone, cancellous bone adjacent to the implant cavity and implant cavity. Bars represent 95% confidence intervals.

Table 2. (2) Standard PK parameters for plasma, subcutaneous adipose tissue (SCT), SCT adjacent to the implant cavity (ADJ-I SCT), cancellous bone, cancellous bone adjacent to the implant cavity (ADJ-I cancellous bone) and implant cavity

	Pharmacokinetic parameter				
Tissue	$AUC_{0\text{-last}}$ (min µg/mL)	C_{max} (µg/mL)	T_{max} (min)	$T_{1/2}$ (min)	$AUC_{tissue}/AUC_{plasma}$
Plasma (unbound)	8548 (7546-9551)[1]	43.2 (40.2-46.1)	100 (82-118)	216 (151-282)	
SCT	6997 (5426-8569)	25.4 (20.2-30.7)	136 (106-165)	227 (193-261)	0.87 (0.64-1.10)
ADJ-I SCT	5739 (3953-7524)	22.0 (15.3-28.7)	150 (123-177)	205 (169-242)	0.74 (0.52-0.96)
Cancellous bone	4640 (3415-5865)[4]	15.7 (10.9-20.4)[4]	191 (149-233)	255 (206-305)	0.59 (0.42-0.75)
ADJ-I cancellous bone	3011 (1758-4265)	9.9 (5.9-13.9)	222 (173-271)	290 (126-454)	0.41 (0.25-0.57)
Implant cavity	1103 (212-1995)[2]	4.1 (0.7-7.5)[3]	178 (136-220)	416 (15-817)	0.20 (0.08-0.33)
Overall comparison[a]	$P < 0.001$	$P < 0.001$	-	$P < 0.11$	

Values are given as medians (95%-CI).

[a] Overall comparison using F test for free plasma, SCT, ADJ-I SCT, ADJ-I cancellous bone, cancellous bone and implant cavity

[1] $P < 0.019$ for all comparisons between plasma and the other compartments. Except for plasma vs. SCT: P-value = 0.121.

[2] $P < 0.03$ for all comparisons between the implant cavity and the other compartments

[3] $P < 0.03$ for all comparisons between the implant cavity and the other compartments. Except for the implant cavity vs. ADJ-I cancellous bone: $P = 0.062$.

[4] $P < 0.089$ for comparison between cancellous bone and ADJ-I cancellous bone

6.3 Study III

Vancomycin Concentrations in the Cervical Spine after Intravenous Administration – Results from an Experimental Pig Study (3)

Hypothesis: Vancomycin penetration to the intervertebral disc will be incomplete and lower than that of vertebral cancellous bone. A single dose of vancomycin will not provide adequate prophylactic intervertebral disc and vertebral cancellous bone concentrations in perioperative spine settings.

Hypothesis disproved: Maybe.

6.3.1 Comments

The main finding was an incomplete and delayed intervertebral disc and vertebral cancellous bone penetration of vancomycin, with the lowest and most delayed penetration found in the intervertebral disc. However, when applying the standard recommendations for prevention of postoperative spondylodiscitis and planktonic MICs of relevant bacteria that may be encountered in spine surgery (0.5-4 µg/mL), adequate mean concentrations were achieved in all compartments. The tissue penetration (95% confidence interval) was for the intervertebral disc 0.24 (0.17-0.31), vertebral cancellous bone 0.46 (0.40-0.53) and subcutaneous adipose tissue 0.60 (0.48-0.72). Within 15 min, a mean MIC of 2 µg/mL was reached in all compartments. The time to a mean MIC of 4 µg/mL was 3, 17, 25 and 156 min for plasma, subcutaneous adipose tissue, vertebral cancellous bone and the intervertebral disc, respectively. A mean MIC of 8 µg/mL was reached after 7, 37 and 81 min in plasma, subcutaneous adipose tissue, and vertebral cancellous bone, respectively, whereas a mean MIC of 8 µg/mL could not be reached in the intervertebral disc. Interestingly, the vancomycin elimination rate was found to approximately three times longer in the intervertebral disc in comparison to the other compartments. This may enable vancomycin to maintain sufficient concentrations for a prolonged period. Lastly, it can be speculated that clinical spine tissue concentrations might be even lower than those found in the present porcine study, mainly due to the interspecies differences regarding the characteristics and properties of the intervertebral disc (described in section 5.5.3). The vancomycin tissue and plasma concentration-time profiles are shown in Figure 10. The corresponding PK parameters can be found in Table 3. The mean (SD) relative recoveries were 15.6 (5.8) %, 35.6 (3.9) %, and 32.7 (4.6) % for the intervertebral disc, vertebral cancellous bone and subcutaneous adipose tissue, respectively.

In summary, even though tissue penetration was incomplete, preoperative administration of 1,000 mg of vancomycin may provide adequate vancomycin tissue concentrations with a considerable delay. However, in order also to achieve adequate intervertebral disc concentrations in all individuals and accommodating a potentially higher MIC target, supplemental application of vancomycin (e.g. as perioperative powder) may be necessary.

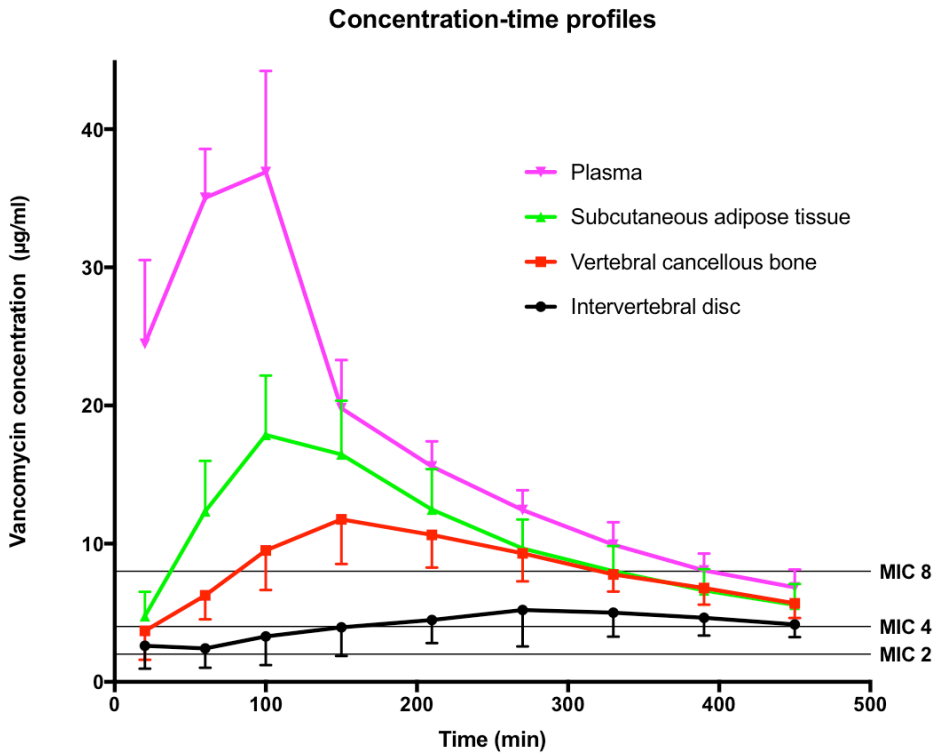

Figure 10. (3) Mean concentration-time profiles for plasma, subcutaneous adipose tissue, vertebral cancellous bone and the intervertebral disc. Bars represent 95% confidence intervals. MICs of 2, 4 and 8 µg/mL are also depicted.

Table 3. (3) Standard PK parameters for plasma, subcutaneous adipose tissue (SCT), vertebral cancellous bone and theintervertebral disc

	Pharmacokinetic parameter				
Tissue	$AUC_{0\text{-last}}$ (min µg/mL)	C_{max} (µg/mL)	T_{max} (min)	$T_{1/2}$ (min)	$AUC_{tissue}/AUC_{plasma}$
Plasma (unbound)	7880 (7164-8597)[1]	40.0 (35.7-44.3)[1]	75 (61-89)	325 (99-552)	
SCT	4719 (4002-5436)	18.0 (14.1-21.9)	113 (96-129)	224 (197-250)	0.60 (0.48-0.72)
Vertebral cancellous bone	3677 (2960-4393)[2]	12.3 (9.6-15.0)[2]	159 (134-184)	271 (227-315)	0.46 (0.40-0.53)
Intervertebral disc	1983 (1237-2729)[3]	6.6 (3.6-9.6)[3]	270 (187-353)	933 (527-1339)[4]	0.24 (0.17-0.31)
Overall comparison[a]	p<0.001	p<0.001	-	p<0.008	

Values are given as means (95%-CI).

AUC_{0-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}; $T_{1/2}$, half-life at β-phase; $AUC_{tissue}/AUC_{plasma}$, tissue penetration expressed as the ratio of $AUC_{tissue}/AUC_{plasma}$

[a] Overall comparison using F test for plasma (unbound), subcutaneous adipose tissue, vertebral cancellous bone, and intervertebral disc

[1] p<0.001 for all comparisons between plasma and the other compartments

[2] p<0.01 for comparison with subcutaneous adipose tissue

[3] p<0.01 for comparison between intervertebral disc and the other compartments

[4] p<0.001 for all comparisons between intervertebral disc and the other compartments

7. Discussion

Traditionally, antimicrobial plasma concentrations have been considered to reflect antimicrobial tissue concentrations. Recently, however, a number of studies have found that this may not always be true, under both physiological and pathological conditions(19-28). Since the majority of bacteria reside in solid tissues in the interstitial space, it is increasingly appreciated that adequate antimicrobial concentrations at the target site are a prerequisite for a successful therapeutic outcome. Even with more regular administration of antimicrobials in orthopaedic perioperative settings, the incidence of postoperative surgical site infections remains rather high. The average risk of prosthetic joint infection following total knee replacement surgery is reported in the range of 1% and 2%, while the incidence for postoperative spondylodiscitis is reported in the range of 1% and 4%(47, 99, 100, 109-111). Correspondingly, in the treatment of bone infections, treatment failure is common, despite prolonged antimicrobial therapy, often in combination with surgical intervention, being applied. This may partly be explained by the lack of knowledge regarding target site concentrations of antimicrobials(117, 118).

In the particular case of bone and the intervertebral disc, an assessment of the antimicrobial Pks has been limited by the paucity of ideal sampling methods. Until now, the predominant approach has been the bone and intervertebral disc biopsy methods(41). However, these methods suffer from important methodological limitations, e.g. the concentrations are given by mass and not by volume, the temporal resolution is poor, and it is impossible to distinguish between the intra- and extracellular fractions(41, 43). For example, the vancomycin bone/serum ratios have been found to vary up to 15-fold despite comparable doses using the bone biopsy method(41, 76, 77). In contrast, microdialysis has emerged as a promising technique for serial sampling of the unbound extracellular concentrations of antimicrobials from the compartment of interest (19, 25, 26, 78). Additionally, sampling can be continued after surgery(24). Consequently, microdialysis has the potential to generate more useful antimicrobial PK data from bone and the intervertebral disc, and it furthermore allows for high-resolution concentration-time profiles, which can be used to evaluate relevant PK/PD-targets. After the preliminary feasibility evaluation, microdialysis can be used to sample an array of antimicrobials from various compartments under different conditions. It is appreciated, however, that no reference method currently exists to validate the findings obtained with microdialysis.

To our knowledge, the studies included in this thesis, are the first of their kind to apply microdialysis for the assessment of vancomycin concentrations in bone, the intervertebral disc and subcutaneous adipose tissue in these orthopaedically relevant settings.

Table 4. $AUC_{0\text{-last}}$ and C_{max} for plasma and cancellous bone (vertebral and tibial) and tissue penetration ratios for Studies I-III.

	$AUC_{0\text{-last}}$ (8 hours)	C_{max}	Tissue penetration ratios	Weight
Plasma (Study I – man)	6296 (5883-6709)[1]	34.3 (31.3-37.2)	-	Mean: 97 kg
Plasma (Study II – infectious model)	8548 (7546-9551)[1]	43.2 (40.2-46.1)[1]	-	75-86 kg
Plasma (Study III – spine model)	7880 (7164-8597)	40.0 (35.7-44.3)	-	78-82 kg
Tibial cancellous bone (Study I – man)	2636 (1527-3744)[1]	10.8 (6.3-15.3)	0.45 (0.29-0.62)	-
Tibial cancellous bone (Study II – pig)	4640 (3415-5865)[1]	15.7 (10.9-20.4)[1]	0.59 (0.42;0.75)	-
Vertebral cancellous bone (Study III – pig)	3677 (2960-4393)	12.3 (9.6-15.0)	0.46 (0.40-0.53)	-

Values are given as means (95%-CI) unless stated otherwise.
[1] Values are given as medians and (95%-CI).

In the two studies mimicking perioperative situations (Studies I and III), a delayed and incomplete vancomycin penetration was found in bone and subcutaneous adipose tissue in the clinical setup, and correspondingly in porcine vertebral cancellous bone and the intervertebral disc. In the infectious setting, an incomplete and delayed vancomycin bone penetration was also demonstrated, especially to the infected implant cavity. In all of the studies, the patients as well as the pigs received the same dose of vancomycin, 1,000 mg over 100 min, and sampling was conducted over 8 hours. Accordingly, these results can only be safely regarded as representative for this dose, setting and timeframe for the specific patient and porcine populations included in these studies.

A rough comparison between the clinical and porcine PK data ($AUC_{0\text{-last}}$, C_{max}, and tissue penetration ratios) from plasma and cancellous bone (vertebral and healthy tibial) is depicted in Table 4. These results may indicate a limited interspecies difference regarding vancomycin plasma and bone PK. The clinical $AUC_{0\text{-last}}$ and C_{max} for plasma and cancellous bone are somewhat comparable to that of the porcine plasma values, healthy porcine tibial cancellous bone and porcine vertebral cancellous bone. The same translational trend is even more notable in the corresponding tissue penetration ratios.

Even though pigs resemble humans in terms of physiology, anatomy and bone composition, the significance of the interspecies differences remains unknown(103, 104). It is important to acknowledge that even small interspecies PK differences can have a large impact on the resulting findings. Although the data may indicate resemblances, more data are needed to firmly evaluate the translational potential of porcine vancomycin bone PKs, and thus the generalisability of the data.

All measurements included in this PhD project were obtained before the achievement of steady state. This makes it difficult to do a direct comparison to the recommended clinical steady state PK/PD targets. For treatment-of-an-infection, all PK/PD targets should refer to steady state

situations(9). As denoted in section 3.4, vancomycin tissue-specific targets for both the prevention and treatment of an infection in orthopaedics are currently not established. Prudently, antimicrobial therapy in orthopaedics should not be generalised; rather, it should be subdivided and reflect the actual situations and needs: for example, perioperative prophylaxis, treatment of acute (or chronic) osteomyelitis, involvement of implants, anatomical location (upper vs lower extremity) etc.

With use of current antiseptic skin techniques before surgery, it may be impossible to completely eradicate all skin bacteria. Small skin appendages have been shown to form protected microbial reservoirs into which the antiseptic substances are unable to penetrate(119). Thus, the orthopaedic surgeon may very well have to accept that bacteria will inevitably drop into the surgical field. The goal of antimicrobial prophylaxis in surgery must then be to lower the intraoperative contamination of the invading bacteria to a level that the host defences can effectively combat(67, 68). Consequently, in the perioperative antimicrobial prophylactic situation, it is recommended that the antimicrobial tissue concentrations should, at least, exceed relevant planktonic bacteria MICs throughout surgery in order to be effective(68, 69). However, these recommendations lack a sufficient scientific evaluation and should be critically approached: What is the optimal target concentration of an antimicrobial for the prevention of a surgical site infection? Is it enough only to exceed relevant planktonic MICs? How many multiples above relevant MICs are needed for an optimal treatment for all individuals? The results from Studies I and III raises some concerns regarding the use of vancomycin in the perioperative setting: the concentrations are low, especially in cortical bone and the intervertebral disc, and it takes more than 2 hours to reach C_{max}. If the standard recommendations for the prevention of surgical site infections and relevant planktonic MIC values are applied, adequate mean concentrations may be reached in some of the investigated compartments. However, our data also suggest a rather narrow or no margin at all in individuals with low tissue concentrations in some of the compartments for bacteria displaying high MICs. In the particular case of MRSA infections, an increase in vancomycin MICs has been demonstrated over recent years, which may argue for even more concerns regarding the use of vancomycin as a prophylactic agent(120). Further research is warranted to scientifically evaluate the perioperative prophylactic targets for vancomycin (and for other antimicrobials) and to assess perioperative vancomycin concentrations in other orthopaedically relevant settings, especially when considering the potentially devastating complications of postoperative infection in orthopaedic surgery.

Two other interesting and intriguing aspects to the peri- and postoperative situation and antimicrobial dosing are: 1) The ability of some of the invading bacteria to hibernate. Antimicrobials only exert their effect on active bacteria. This may necessitate several post-operative doses to lower the risk of growth from bacteria that hibernate immediately after inoculation of the surgical site. 2) The involvement of implants in surgery increases the bacterial virulence by over a 75,000-fold(121). This emphasises the crucial need for antimicrobial prophylaxis in surgery involving implants. Further discussion regarding the correlation between the duration of the antimicrobial prophylaxis and bacteria

hibernation and the aspects of bacteria virulence is, however, beyond the scope of this PhD thesis.

The only existing target for vancomycin in a treatment-of-an-infection setting is based on plasma indices and advocates for a target of AUC/MIC > 24,000 (corresponding to a ratio of 400 when AUC is given in h·µg/mL) when treating *Staphylococcus aureus* infections(69). If this target is applicable to infected bone tissue, and/or if bacteria with high MICs are encountered, it seems unlikely that adequate target site concentrations of vancomycin can be achieved when considering the 8-hour AUC values presented in Study II. Indisputably, the study design would have benefitted from steady state measurements in order to do a more precise comparison to the established target.

The findings from all three studies may have important clinical implications regarding the use of vancomycin and calls for some considerations concerning vancomycin dosing, timing and administration. In order to achieve higher and prolonged vancomycin tissue concentrations, other ways of administering vancomycin may be considered, because an increase in the intravenous dose is restricted due to toxicologic considerations(70). Different approaches have been applied, e.g. as perioperative powder, as local administration of diluted vancomycin in the surgical wound or intra-articularly before closure of the capsule, or as intraosseous regional administration(68, 122, 123). All these studies suggest that a local application of vancomycin improves the prophylactic effect. The increased effect is achieved through higher and prolonged target site concentrations, in comparison to the systemic administration. However, none of these studies were able to assess peak drug concentrations or evaluate the concentration-time profiles or the diffusion potential. It seems appealing to combine local application of vancomycin with serial microdialysis sampling in different distances from the local application.

For Studies II and III, two similar studies, assessing cefuroxime concentrations, have been performed(28, 124). This allowed us to compare the PK differences between cefuroxime and vancomycin in infected bone tissue and spine tissue. Both cefuroxime studies found higher cefuroxime tissue penetration ratios in general than those found in the studies that comprise this thesis. These findings suggest that cefuroxime may be a better choice than vancomycin in some orthopaedic settings than vancomycin when using standard dosing. Moreover, this emphasises that the selection of antimicrobials in orthopaedic settings should be based not only on the sensitivity of the antimicrobial against the invading bacteria, but also on its abilities to provide adequate target site concentrations. In this context, the respective PK/PD target for the different antimicrobials should also be considered. Overall, this stresses a need of studies assessing target site PKs for specific combinations of drugs and tissues in order to optimise the antimicrobial treatment in orthopaedics for both perioperative antimicrobial prophylaxis and the treatment of bone infections.

In the present PhD project, two of the three studies deal with vancomycin PKs in presumably healthy bone. Accordingly, the results are restricted to reflect similar perioperative settings. At present, only limited data on antimicrobial bone PKs in infected bone are available(28, 41, 125). Thus, the effect of infection and inflammation on antimicrobial bone PKs

remains somewhat unclear. Already 5 days after inoculation in the implant-associated osteomyelitis model, an acute, or at least sub-acute, osteomyelitis was histologically confirmed(126). At this point, an incomplete vancomycin penetration was found in relation to all the compartments in the infected leg. Furthermore, a trend towards lower vancomycin $AUC_{0\text{-last}}$ and C_{max} was found in cancellous bone on the infected side in comparison to the healthy side. These differences were not statistically significant, but may represent a trend that deserves more interest. In fact, a thorough histological evaluation assessing the peri-implant pathological bone area (PIBA) has been performed(Jensen LK, unpublished data). This evaluation showed that the PIBA included suppuration, which caused the destruction of the infected implant cavity relief, and a non-vascular zone of primarily necrotic bone tissue. A strong negative correlation between the PIBA width and the vancomycin $AUC_{0\text{-last}}$ and C_{max} was found. At a PIBA (or histo-pathology) width of 3 mm, the vancomycin $AUC_{0\text{-last}}$ and C_{max} decreased dramatically. Consequently, it can be speculated that vancomycin bone penetration decreases with the progression of infection and inflammation.

In chronic bone infections and implant-associated infections with biofilm formation and in which lesions of ischaemic osseous sequestration and reduced vascularisation may be more manifest, the antimicrobial bone exposure may be even more insufficient. In this context, it would be interesting to evaluate how vancomycin penetration presents in infected porcine (and clinical) bone tissue at both an earlier and later stage than presented in this specific setting. Altogether, the findings from Study II advocates for an early diagnosis and a rapid induction of antimicrobial therapy in the treatment of acute osteomyelitis. If metaphyseal cavities are present, surgical debridement with complete PIBA excision seems necessary.

In Study III, the vancomycin elimination rate was found to be approximately three times longer in the intervertebral disc in comparison to the other compartments. A prolonged elimination rate may be favourable in some compartments because vancomycin then continues to eradicate bacteria even throughout long surgical procedures and after surgery has ended. However, this is only relevant if adequate concentrations are reached. Accordingly, it is important to gain knowledge about tissue-specific elimination rates from all relevant tissues in order to evaluate optimal antimicrobial treatment regimens.

In summary, the findings in the present PhD project suggest that vancomycin exhibits a heterogeneous tissue distribution, depending on both the specific compartment and the pathophysiological condition of the tissue. Accordingly, if vancomycin dosing is only based on plasma PKs, there is an increased risk of achieving inadequate vancomycin concentrations at the target site. A single dose of 1,000 mg of vancomycin may not penetrate adequately to healthy bone, infected bone or the intervertebral disc.

7.1 Limitations

The limitations and weaknesses of the applied methods and of the individual study designs have been discussed on a general level in the methodology sections, 5.1-5.5. In the following section, the limitations will be considered in relation to the interpretation of the results presented in this thesis.

Pre-clinical research is a valuable tool to assess feasibility and recognise potential pitfalls before advancing to clinical trials. In orthopaedic research, the pig represents an excellent model since its size, composition, density and quality of bone have been shown to compare reasonably to that of humans(104). The age of the porcine bones and the intervertebral discs is however very different from that of adult humans. Even though the weight of the pigs was chosen in order to resemble that of the average human weight, a young female pig, weighing approximately 80 kg, is still juvenile (aged 5 months). The application of surgery and serial *in vivo* sampling from the pig, also necessitated the use of general anaesthesia during the entire sampling intervals. Thus, any differences in the antimicrobial PK data between humans and pigs can be attributed to a number of reasons. Nonetheless, we believe that the porcine model is a highly feasible model and may be the best pre-clinical model for antimicrobial bone experiments using microdialysis.

In the clinical study, Study I, the patient population was a very selected group, and for practical and ethical reasons, the sampling of vancomycin was performed after the end of the total knee replacement surgery. Thus, generalising to the actual perioperative situation is questionable. The influence of the surgical trauma and the local administration of adrenaline and ropivacaine may also have affected the tissue PKs. Therefore, the results are more likely to reflect the postoperative antimicrobial prophylaxis situation for this specific procedure. The absolute PK data obtained from this study seem to be restricted to the present study setting, whereas the tissue penetration ratios, on the other hand, may be more generalisable to other settings.

In total hip replacement surgery, lower infection rates have been correlated to an administration of antimicrobials four times on the day of surgery(127). It has therefore been proposed that antimicrobial prophylaxis following total joint replacement surgery should be continued for at least 24 hours(128). The sampling interval in the clinical study, Study I, was restricted to 8 hours due to practical and ethical reasons. Data based on sampling over 24 hours and subsequent doses of the drug would certainly have been superior to the present setup.

In the experimental studies, the sampling interval was also confined to an 8-hour sampling interval due to the need for general anaesthesia. Drug steady state is normally reached after 4 to 6 half-lives. As mentioned above, especially in Study II, a treatment-of-an-infection setting, sampling in steady state would have been interesting.

Microdialysis studies are dependent on a sensitive, accurate and precise chemical assay. The UHPLC method with UV detection fulfilled these demands. Given the low volumes and low concentrations, and rather low relative recoveries, (mean range 14-40% across all studies) it was imperative to have an adequate chemical assay. Lower levels of relative recovery are relatively more exposed to variations regarding the chemical analysis and pre-analytical handling. The resulting variations will increase exponentially with decreasing relative recovery. This explains the need for a suitable chemical assay and careful sample handling. In Study III, the variances in plasma and tissue PKs were comparable, indicating an acceptable precision of the measurements within the biological variation. In Studies I and II, the tissue variations exceeded those of plasma

to a small extent, which has also been found in other clinical and experimental antimicrobial microdialysis studies(52, 129). In addition to the well-known biological variation in antimicrobial PK studies, the surgical trauma, induction of infection, and local injection of adrenaline, which were presented differently across the studies, are all factors expected to have additional effects on the variations. Interestingly, the variations were lowest in the last study, which may indicate a learning curve for all involved parties. Thus, we believe that the variations found in these studies are unlikely to represent an inadequate precision of the methodological setup.

It is always difficult to define the acceptable trade-off between the ideal setup and the experimental needs in microdialysis studies. Higher relative recoveries are always attractive in order to determine more precise PK data. In the present study setups, relative recovery could have been increased by lowering the perfusion rate or increasing the membrane length. However, the membrane length was limited by anatomical factors, and a lower perfusion rate would have resulted in lower volume and thus a poorer temporal resolution. The trade-off was therefore a perfusion rate of 1 µL/min and membrane lengths varying from 10 to 30 mm, resulting in mean relative recoveries ranging from 14 to 40% throughout all studies. We found this to be acceptable due to the anatomical limitations and the restrictions to the respective study designs.

Relative recovery was determined by retrodialysis by drug either before or after sampling of vancomycin concentrations. It cannot be excluded that transient changes in the peri-probe environment may have affected relative recovery during the sampling interval. This limitation cannot be ignored especially in the clinical study, Study I, where no restrictions regarding mobilisation were applied. The introduction of an internal calibrator, giving continuous relative recovery values, would have overcome the concerns regarding this issue. For future research, such an approach seems desirable.

8. Conclusion

Microdialysis was successfully applied for the assessment of vancomycin concentrations in healthy and infected bone and in the intervertebral disc. The aim of two of the studies was to mimic a perioperative orthopaedic situation (Studies I and III) and one study assessed the effect of an infection on vancomycin bone penetration (Study II). In all three studies, clinical as well as experimental, an incomplete and delayed penetration of vancomycin into bone and the intervertebral disc was found. The lowest penetration ratios were found in cortical bone, the implant cavity and the intervertebral disc. Accordingly, for all of these investigated orthopaedic settings, it seems unlikely that a single dose of 1,000 mg of vancomycin can provide adequate target site concentrations in all individuals, especially in case of bacteria exhibiting high MICs. Therefore, when choosing vancomycin as the antimicrobial treatment in orthopaedic settings, focus should be not only on the characteristics of the infectious bacteria, but also on how to achieve sufficient vancomycin tissue concentrations. This may call for alternative ways of applying vancomycin in some orthopaedic settings.

9. Perspectives and future research

The assessment of antimicrobial bone concentrations by means of bone and disc tissue samples is associated with important limitations. Therefore, it has been argued that the data obtained from these methods may be misleading and potentially harmful to the patients if applied uncritically. The findings in the present thesis suggest that microdialysis may become a useful alternative to the existing methods to evaluate antimicrobial bone and intervertebral disc PKs. In bone, as well in other tissues, an increased knowledge regarding antimicrobial target site concentrations can be used to optimise dosing regimens, which may improve clinical outcomes. This is of great interest from a patient, a public health and a socio-economic point of view.

The present thesis evaluates vancomycin PKs of a single dose of 1,000 mg in some orthopaedically relevant settings, leaving many other and perhaps more relevant settings to be investigated. As discussed, future studies should also focus on evaluating steady state PKs of vancomycin and on assessing different ways of administering the drug, e.g. local application, continuous infusion, etc. Ultimately, more studies should be conducted in clinical settings with clear clinical endpoints, although the risk of heterogeneity, especially in infectious settings, will present a methodological challenge. Hopefully in the future, the dosing of antimicrobials will be based on evidence rather than tradition.

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Appendix

Acta Orthopaedica

ISSN: 1745-3674 (Print) 1745-3682 (Online) Journal homepage: http://www.tandfonline.com/loi/iort20

Bone and subcutaneous adipose tissue pharmacokinetics of vancomycin in total knee replacement patients

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To cite this article: Mats Bue, Mikkel Tøttrup, Pelle Hanberg, Otto Langhoff, Hanne Birke-Sørensen, Theis M Thillemann, Torben L Andersson & Kjeld Søballe (2017): Bone and subcutaneous adipose tissue pharmacokinetics of vancomycin in total knee replacement patients, Acta Orthopaedica

To link to this article: http://dx.doi.org/10.1080/17453674.2017.1373497

Bone and subcutaneous adipose tissue pharmacokinetics of vancomycin in total knee replacement patients

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Submitted 2017-04-05. Accepted 2017-08-14.

Background and purpose — The incidence of orthopedic methicillin-resistant Staphylococcus aureus (MRSA) infections is increasing. Vancomycin may therefore play an increasingly important role in orthopedic perioperative antimicrobial prophylaxis. Studies investigating perioperative bone and soft tissue concentrations of vancomycin are sparse and challenged by a lack of appropriate methods. We assessed single-dose plasma, subcutaneous adipose tissue (SCT) and bone concentrations of vancomycin using microdialysis in male patients undergoing total knee replacement.

Methods — 1,000 mg of vancomycin was administered postoperatively intravenously over 100 minutes to 10 male patients undergoing primary total knee replacement. Vancomycin concentrations in plasma, SCT, cancellous, and cortical bone were measured over the following 8 hours. Microdialysis was applied for sampling in solid tissues.

Results — For all solid tissues, tissue penetration of vancomycin was significantly impaired. The time to a mean clinically relevant minimal inhibitory concentration (MIC) of 2 mg/L was 3, 36, 27, and 110 min for plasma, SCT, cancellous, and cortical bone, respectively. As opposed to the other compartments, a mean MIC of 4 mg/L could not be reached in cortical bone. The area under the concentration-time curve from 0 to the last measured value and peak drug concentrations (C_{max}) for SCT, cancellous, and cortical bone was lower than that of free plasma. The time to C_{max} was higher for all tissues compared with free plasma.

Interpretation — Postoperative penetration of vancomycin to bone and SCT was impaired and delayed in male patients undergoing total knee replacement surgery. Adequate perioperative vancomycin concentrations may not be reached using standard prophylactic dosage.

The objective of antimicrobial prophylaxis in surgery is to lower the microbial load of intraoperative contamination to a level that host defenses can overcome (Mangram et al. 1999). Though specific antimicrobial target concentrations for this task are not established, it is advocated that not only plasma but also tissue concentrations should, at least, exceed minimal inhibitory concentrations (MIC) of potential antimicrobial pathogens throughout surgery (Whiteside 2016). Traditionally, plasma concentrations of antimicrobials have been considered to reflect tissue concentrations. Recently, however, a number of studies have indicated that this may not always be true (Joukhadar et al. 2001, Barbour et al. 2009). Consequently, surgical antimicrobial dosing regimens based on plasma pharmacokinetics, i.e. the fate of drug in plasma, may result in insufficient perioperative tissue concentrations.

Determination of antimicrobial tissue concentrations is challenging. Various methodological approaches have been employed, but all seem to suffer from important methodological limitations (Mouton et al. 2008, Landersdorfer et al. 2009, Pea 2009). Particularly for bone, no ideal method has been established. Recently, the pharmacokinetic tool microdialysis (MD) has been shown to be useful for sampling various antimicrobials in drill holes in bone (Stolle et al. 2004, Schintler et al. 2009, Traunmuller et al. 2010a, Tottrup et al. 2014, Bue et al. 2015, Tottrup et al. 2015, Hanberg et al. 2016). In the context of orthopedic antimicrobial surgical prophylaxis, measurements obtained by means of MD seem relevant, and reflective of the true perioperative situation.

The most frequent cause of orthopedic infections is *Staphylococcus aureus* and the incidence of methicillin-resistant

DOI 10.1080/17453674.2017.1373497

Staphylococcus aureus (MRSA) infections is increasing (Murillo et al. 2015, Benito et al. 2016). Vancomycin remains one of the few drugs effective against these bacteria and is recommended as first-line choice in the treatment of orthopedic MRSA infections (Lew and Waldvogel 2004, Trampuz and Widmer 2006). In the years to come, vancomycin may therefore become the first choice for perioperative antimicrobial prophylaxis in some orthopedic settings. So far, vancomycin bone concentrations have only been assessed using the bone biopsy approach (Kitzes-Cohen et al. 2000, Vuorisalo et al. 2000, Landersdorfer et al. 2009). The rather large variation in tissue concentrations in these studies may be related to methodological challenges. In a recent pig study, we have successfully employed MD for measurement of vancomycin in drill holes in healthy cancellous and cortical bone and found incomplete and delayed bone penetration (Bue et al. 2015). If this is also the case in the clinical setting, perioperative tissue concentrations may be inadequate for optimal prevention of infection. We therefore assessed single-dose plasma, SCT, and bone concentrations of vancomycin using MD in male patients undergoing total knee replacement (TKR).

Material and methods

This study was conducted at the Department of Orthopaedic Surgery, Horsens Regional Hospital, Denmark between March 2015 and January 2016. All chemical analyses were performed at the Department of Clinical Biochemistry, Aarhus University Hospital, Denmark.

Study design, patients, drug, and endpoints

Competent male patients with knee osteoarthritis were offered enrolment in the study if they were scheduled for a primary TKR. The patients were identified in the outpatient clinic by a single surgeon (OL) conducting the planned TKR. Exclusion criteria included allergy to vancomycin, on-going treatment with vancomycin, warfarin or other newer anticoagulants, and clinically reduced renal function.

10 patients were included in this study. All 10 patients completed the study. Mean (SD) weight of the patients was 97 (16) kg, giving a mean (SD) BMI of 30 (4.5), and they had a mean (SD) creatinine level on surgery day of 82 (14) µmol/L. As preoperative antimicrobial prophylaxis, all patients were given 1,500 mg of cefuroxime prior to surgery, which is the standard regimen in Denmark.

The MD probes were implanted at the respective locations during the TKR surgery. After the surgical procedures and calibration of the MD probes, 1,000 mg of vancomycin were administered intravenously in a peripheral catheter over 100 min. Sampling was conducted over 8 hours starting at the beginning of vancomycin infusion. Tissue penetration ratios and time to mean relevant minimal inhibitory concentrations (MICs) (1–8 mg/L) were the primary endpoints. Secondary endpoints were standard pharmacokinetic parameters; the area under the concentration-time curves ($AUC_{0–last}$), peak drug concentration (C_{max}), and time to C_{max} (T_{max}).

Surgery

At the end of TKR surgery, MD probes were placed in drill holes in cancellous bone in the medial tibial condyle and in cortical bone in the anterior margin approximately at the midpoint of the tibial diaphysis. While the medial tibial condyle was accessed via the TKR incision, a small 2 cm separate incision was used for the tibial diaphysis. The anatomical location of the cortical drill hole was chosen to ensure optimal intra cortical placement. The depths of the drill holes were aimed to be 25 mm for cancellous bone and 15 mm for cortical bone, and MD probes with membrane lengths of 20 and 10 mm were used. A new 2 mm drill was used for each patient. When drilling in cortical bone, saline was continuously applied, and drilling was ceased every few seconds to prevent heat necrosis of the bone. At both locations, the probes were tunneled approximately 2–3 cm under the skin before entering the drill holes. In addition to the bone probes, an SCT probe (20 mm membrane) was placed in the medial part of the thigh using the manufacturer's standard introducer. To prevent displacement, all probes were fixed to the skin with single sutures. At the end of TKR surgery, a mixture of 150 mL ropivacaine (2 mg/mL), 1.5 mL toradol (30 mg/mL), and 0.75 mL adrenaline (1 mg/mL) was injected locally in the soft tissues surrounding the knee, intra-articularly, and in the posterior joint capsule of the knee as a routine part of pain management.

Microdialysis and sampling procedures

In vivo microdialysis is a probe-based technique, allowing for continuous sampling of water-soluble molecules in the interstitial space of accessible tissues (Joukhadar et al. 2001, Stolle et al. 2004, Schintler et al. 2009, Shukla et al. 2009, Traunmuller et al. 2010b, Hutschala et al. 2013). A semipermeable membrane at the tip of the probe allows for diffusion of molecules following the concentration gradient. As the probe is continuously perfused, equilibrium will never occur. Accordingly, the concentration in the dialysate will represent only a fraction of the actual concentration in the tissue. This fraction is referred to as the relative recovery (RR) and must be determined in order to estimate absolute tissue concentrations. In this study, retrodialysis by drug was applied for individual calibration of all the MD probes (Scheller and Kolb 1991). The principle of the retrodialysis method relies on the assumption that the diffusion across the semipermeable membrane is quantitatively equal in both directions. Therefore, vancomycin was added to the perfusion medium and the disappearance rate trough the membrane was taken as the RR. The RR was calculated using the following equation:

$$RR(\%) = 100 \times (1 - C_{dialysate}/C_{perfusate})$$

where $C_{dialysate}$ is the concentration (µg/mL) in the dialysate, and $C_{perfusate}$ is the concentration (µg/mL) in the perfusate.

The absolute, extracellular concentrations (µg/mL), C_{tissue}, were obtained by correcting for RR using the following equation:

$$C_{tissue} = C_{dialysate} \times RR^{-1}$$

A detailed description of MD can be found elsewhere (Muller 2002, Joukhadar and Muller 2005).

In the present study, the MD system consisted of CMA 107 precision pumps (µ-Dialysis AB, Stockholm, Sweden) and CMA 70 probes (membrane length 20 mm and 10 mm, molecular cut-off 20 kilo Daltons). After surgery, the MD probes were perfused with 0.9% NaCl containing vancomycin at a concentration of 1.25 µg/mL at a perfusion rate of 1 µL/min. After a 30-min tissue equilibration period, all catheters were individually calibrated by collecting a 60-min sample. Following calibration, the perfusate was changed to isotonic saline, and a 120-min washout period was allowed for. Vancomycin was then administered to the patient as previously described. For the first 2 hours, dialysates were harvested at 40-min intervals, and thereafter at 60-min intervals for the following 6 hours, giving a total of 9 samples in a sampling period of 8 hours. Venous blood samples were drawn from a peripheral catheter (cubital vein) in the middle of every dialysate sampling interval. Dialysates were instantly frozen on dry ice for a maximum of 10 hours, before being transferred to a –80°C freezer until analysis. Dialysate concentrations were corrected for RR and ascribed to the midpoint of the sampling interval. Venous blood samples were stored at 5°C for a maximum of 24 hours before being centrifuged at 3,000 g for 10 minutes. Plasma aliquots were then frozen and stored at –80°C until analysis.

Before removal of the probes, a CT scan of the drill hole in the anterior aspect of the tibia was conducted to verify that the drill had not penetrated to the bone marrow and that the probe had not been displaced.

Quantification of vancomycin concentrations

Measurement of the free concentration of vancomycin in plasma was determined with a homogeneous enzyme immunoassay- technique on the Cobas c501 platform (Roche, Switzerland) (Bue et al. 2015). The dialysate concentrations of vancomycin were quantified with Ultra High Performance Liquid Chromatography as previously described (Bue et al. 2015). The lower limit of quantification was defined as the lowest concentration with intra-run CV < 20%, and was found to be 0.05 µg/mL.

Pharmacokinetic analysis and statistics

The pharmacokinetic (PK) parameters $AUC_{0–last}$, C_{max}, T_{max} and terminal half-life ($T_{1/2}$), were determined separately for each subject by non-compartmental analysis using the pharmacokinetic-series of commands in Stata (v. 14.1, StataCorp LLC, College Station, TX, USA). 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 $\ln(2)/\lambda_{eq}$, where λ_{eq},is the terminal elimination rate constant estimated by linear regression of the log concentration on time. These PK parameters were obtained in all 4 compartments from the same subject and hence a mixed model for repeated measurements was used with compartments as fixed effect and subject identification variable as a random effect. Furthermore, distinct residual variance was assumed within each compartment. The normality of the residuals was estimated using a Quantile–Quantile (QQ) plot for the residuals and the homogeneity of the residual variance was checked by plotting residuals vs. best linear unbiased prediction estimates. The normality of the estimated random effects was checked using a QQ-plot of the estimated random effects. Overall comparisons between the compartments were conducted using Wald's test and pairwise comparisons using a t-test. The variables $AUC_{0–last}$, T_{max}, and $T_{1/2}$ were analyzed using their log transformed data. A correction due to small sample size was handled using the Kenward–Roger approximation method. Consequently, $AUC_{0–last}$, T_{max}, and $T_{1/2}$ values are given as medians with 95% CIs, and the pairwise comparisons were conducted on the log scale. 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 for tissue penetration. Statistical analyses were also performed using Stata. Values below the lower limit of detection were set to zero. The washout concentrations were low, and therefore not included in the analysis. Using Microsoft Excel (Microsoft Corp, Redmond, WA, USA), the time to mean MICs of 1, 2, 4, and 8 mg/L was estimated using linear interpolation.

Ethics, funding, and potential conflicts of interest

The study was approved by the Ethics Committee of the Central Denmark Region (registration number 1-16-02-472-14) and the Danish Health and Medicines Authority (EudraCT number 2014-000258-12). Written informed consent was obtained from all patients.

The study was conducted in accordance with the Declaration of Helsinki and the ICH Harmonised Tripartite Guideline for Good Clinical Practice (GCP). The GCP unit at Aalborg and Aarhus University Hospitals conducted the mandatory monitoring procedures.

The work was supported by grants from the Korning Foundation, the Familien Hede Nielsen Foundation, the Scientific Foundation for Medical Doctors at Horsens Regional Hospital and the Bevica Foundation. No competing interests were declared.

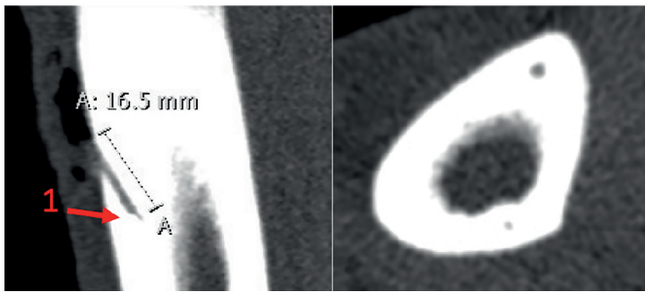

Figure 1. Representative sectional views of the cortical drill hole showing the position of the drill holes and the location of the cortical MD probe. 1: The gold thread within the MD probe membrane tip.

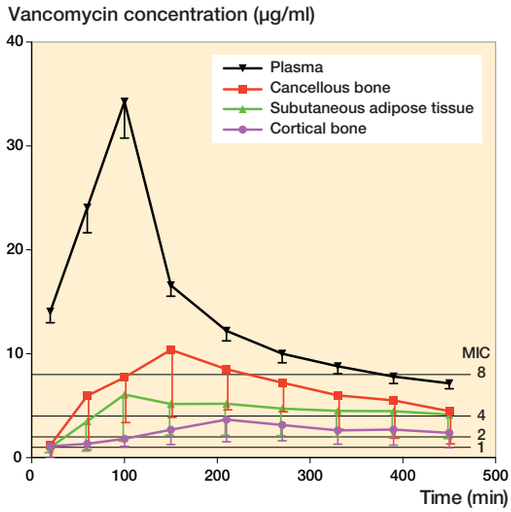

Figure 2. Mean concentration-time profiles for plasma, subcutaneous adipose tissue, cancellous, and cortical bone. Bars represent 95% confidence interval. MICs of 1, 2, 4, and 8 µg/mL are also depicted.

Table 1. Key pharmacokinetic parameters for plasma, subcutaneous adipose tissue, and cancellous and cortical bone. Values are medians (95% CI) unless otherwise stated.

Pharmacokinetic parameter	Plasma (unbound)	Subcutaneous adipose tissue	Cancellous bone	Cortical bone	Overall comparison [a]
$AUC_{0–last}$ (min µg/mL)	6,296 (5,883–6,709)	1,545 (698–2,392) [c]	2,636 (1,527–3,744) [c]	1,016 (661–1371) [c,d]	< 0.001
C_{max} (µg/mL) [b]	34.3 (31.3–37.2)	6.6 (3.4–9.8) [c]	10.8 (6.3–15.3) [c]	4.0 (2.5–5.4) [c,d]	< 0.001
T_{max} (min)	100 (64–136)	200 (120–281)	148 (73–223)	152 (81–223)	–
$T_{1/2}$ (min)	362 (311–414)	583 (8–1,158)	360 (21–700)	392 (67–716)	0.8
$AUC_{tissue}/AUC_{plasma}$		0.31 (0.16–0.46)	0.45 (0.29–0.62)	0.17 (0.11–0.24)	0.008 [e]

$AUC_{0–last}$ = area under the concentration-time curve from 0 to the last measured value;
C_{max} = peak drug concentration; T_{max} = time to Cmax; $T_{1/2}$ = half-life at -phase;
$AUC_{tissue}/AUC_{plasma}$ = tissue penetration expressed as the ratio of $AUC_{tissue}/AUC_{plasma}$
[a] Overall comparison using Wald's test for free plasma, subcutaneous adipose tissue, cancellous, and cortical bone.
[b] Values are means (95% CI).
[c] $p < 0.001$ for comparison with the corresponding free plasma value.
[d] $p < 0.007$ for comparison with cancellous bone.
[e] T-test comparison of cancellous and cortical bone.

Results

No serious adverse events or serious adverse reactions were observed. Dialysate concentrations could not be reliably determined in 2 patients, due to UHPLC apparatus failure, and their data were therefore excluded from the analysis. 2 cortical bone probes and 3 cancellous bone probes were malfunctioning, thus leaving data from 6 cortical bone locations, 5 cancellous bone locations, 8 SCT locations, and blood samples from all 10 patients. For 1 cancellous bone probe RR could not be reliably determined. Since the dialysate measurements for this probe resembled that of the other cancellous probes, RR for this probe was determined as the mean value of the remaining RR's of the cancellous bone probes. CT- scans confirmed that all drill holes were positioned in cortical bone without communication to the bone marrow, and that all cortical bone probes were located within the drill-holes. Representative sectional views of the cortical drill hole are illustrated in Figure 1. Mean (SD) RRs were 20 (9) %, 35 (18) %, and 14 (6) % for SCT, cancellous, and cortical bone, respectively. For SCT, cancellous, and cortical bone, the mean (SD) measured concentrations in the last washout samples were 0.06 (0.07), 0.12 (0.25), and 0.10 (0.13) µg/mL, respectively.

Vancomycin plasma and tissue concentration-time profiles are provided in Figure 2. Corresponding key pharmacokinetic parameters can be found in Table 1. Tissue penetration ratios ($AUC_{tissue}/AUC_{plasma}$) were below 0.5 for all solid tissues. Accordingly, $AUC_{0–last}$ for both bone compartments and SCT was also significantly lower than that of free plasma ($p < 0.001$). For the same 3 compartments, lower C_{max} ($p < 0.001$) and higher T_{max} compared with plasma were also found. Finally, $AUC_{0–last}$ and C_{max} were lower in cortical compared with cancellous bone ($p < 0.007$).

Table 2. Time (min) to mean concentrations of 1, 2, 4, and 8 mg/L for plasma, subcutaneous adipose tissue, cancellous, and cortical bone (MICs)

	MIC 1	MIC 2	MIC 4	MIC 8
Cortical bone	18	110	–	–
Cancellous bone	17	27	44	105
Subcutaneous adipose tissue	20	36	68	–
Plasma (unbound)	1	3	6	11

The time to mean concentrations of 1, 2, 4, and 8 mg/L (MICs) are depicted in Table 2. The time to a mean MIC of 2 mg/L was 3, 36, 27, and 110 min for plasma, SCT, cancellous, and cortical bone, respectively. A mean MIC of 4 mg/L was reached after 6, 68, and 44 min for plasma, SCT, and cancellous bone, respectively. For cortical bone, a mean MIC of 4 mg/L could not be reached.

Discussion

This is the first clinical study to investigate vancomycin bone concentrations using the MD technique. The main finding is that vancomycin bone and SCT penetration is incomplete and delayed. This contrasts with the traditional conception that antimicrobial plasma concentrations reflect tissue concentrations and calls for special considerations with respect to vancomycin surgical prophylaxis in the orthopedic setting. Although antimicrobial tissue targets for prevention of surgical site infections are unknown, attaining tissue concentrations that, at least, exceeds MICs of relevant pathogens throughout surgery seems prudent (Rybak et al. 2009, Whiteside 2016). The majority of orthopedic pathogens exhibit MIC values in the range of 0.5–2.0 mg/L for vancomycin, while pathogens with higher MICs are infrequently encountered (EUCAST 2017). Our data shows that in some combinations of individual and pathogen, adequate vancomycin tissue concentrations are reached with substantial delay or not at all, particularly in cortical bone. The obvious solution to overcome this problem would be a dose increase. However, due to the potential toxicity associated with higher doses, this approach may not be feasible. On the other hand, the rather long half-life of vancomycin is advantageous in order to maintain adequate concentrations for a prolonged period. Other ways of applying vancomycin may be considered, e.g. administering diluted vancomycin locally in the surgical wound or intra-articularly before closure of the capsule (Whiteside 2016). Despite the fact that vancomycin target tissue concentrations for prevention of orthopedic infections are unknown, our pharmacokinetic study suggest that 1,000 mg of vancomycin may not be a safe choice for single-drug antimicrobial prophylaxis for TKR in male patients with normal renal function

On a more basic pharmacokinetic level, it is noteworthy that both cortical bone $AUC_{0–last}$ and C_{max} were lower than those of cancellous bone. These findings are in accordance with previous findings for cefuroxime and vancomycin in pig studies and suggest that bone may not be considered as a homogenous compartment (Tottrup et al. 2014, Bue et al. 2015, Tottrup et al. 2015). Not surprisingly, the tissue penetration ratios, we found in male TKR patients are lower than those found for juvenile (aged 5 months) pigs (Bue et al. 2015). From a pharmacokinetic perspective, it is also interesting that inter-tissue differences exist with respect to tissue penetration ratios. Consequently, it seems reasonable that surgical prophylaxis PK studies assess pharmacokinetics in all relevant tissues.

For practical reasons, vancomycin was administered postoperatively. As such, the present study setup does not truly mimic the clinical situation where antimicrobial prophylaxis is administered preoperatively. The MD measurements in bone were conducted in drill holes. Currently no gold standard approach exists to determine antimicrobial bone concentrations, but MD measurements in small drill holes seem to reflect true orthopedic perioperative conditions: MD measurements appear to be clinically relevant with respect to the orthopaedic prophylactic situation. Another advantage of MD is that it allows not only for continuous sampling, but also for sampling after the end of surgery, which provides more useful pharmacokinetic data compared with alternative approaches like bone biopsies.

Some important limitations must be addressed. First, the study population consisted of a group of healthy but overweight males (as depicted by their BMI) undergoing TKR. The results can only safely be regarded as representative for this specific population and maybe also only for this specific anatomical region. It would be interesting to assess the effect of weight-based dosing, sex, and alternative anatomical regions. Second, the local injection of adrenaline and ropivacaine at the end of surgery may also have affected the tissue pharmacokinetics to some extent since both adrenaline and ropivacaine induces vasoconstriction. This may indeed explain the low concentrations found in SCT. Nonetheless, it reflects the true perioperative situation for this specific population. Finally, a number of bone probes were malfunctioning, possibly indicating poor reproducibility for MD bone measurements. Nevertheless, the variance in bone and SCT measurements was comparable. Thus, the malfunctioning bone probes are more likely to reflect that application of MD for clinical bone measurements is technically demanding rather than questioning the validity of measurements that are obtained.

In summary, the postoperative penetration of vancomycin to bone and SCT was found to be delayed and incomplete in this population of male patients undergoing TKR surgery. These findings suggest that in some combinations of individual and pathogen, adequate vancomycin tissue concentrations may be reached with substantial delay or not at all. Consequently, vancomycin may not be a safe choice for single-drug antimicrobial prophylaxis for TKR in this population.

MB, MT, HBS, KS, OL, and PH initiated and designed the study. OL conducted the surgery and MB assisted with the placement of the probes. MB and PH collected the data. TLA performed the chemical analyses. Statistical analysis and interpretation of data was done by MT, HBS, KS, TMT, and TLA. All authors drafted and revised the manuscript.

Acta thanks Henrik Husted and Alex Soriano for help with peer review of this study.

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Single-Dose Bone Pharmacokinetics of Vancomycin in a Porcine Implant-Associated Osteomyelitis Model

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Received 21 June 2017; accepted 13 October 2017
Published online in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.23776

ABSTRACT: The increasing incidence of orthopaedic *methicillin-resistant Staphylococcus aureus* (MRSA) infections represents a significant therapeutic challenge. Being effective against MRSA, the role of vancomycin may become more important in the orthopaedic setting in the years to come. Nonetheless, vancomycin bone and soft tissue penetration during infection remains unclear. In eight pigs, implant-associated osteomyelitis was induced on day 0, using a *Staphylococcus aureus* strain. Following administration of 1,000 mg of vancomycin on day 5, vancomycin concentrations were obtained with microdialysis for 8 h in the implant bone cavity, in cancellous bone adjacent to the implant cavity, in subcutaneous adipose tissue (SCT) adjacent to the implant cavity, and in healthy cancellous bone and healthy SCT in the contralateral leg. Venous blood samples were also obtained. The extent of infection and inflammation was evaluated by post-mortem computed tomography scans, C-reactive protein serum levels and cultures of blood and swabs. In relation to all the implant cavities, bone destruction was found. Ranging from 0.20 to 0.74, tissue penetration, expressed as the ratio of the area under the concentration–time curve from 0 to the last measured value, was incomplete for all compartments except for healthy SCT. The lowest penetration was found in the implant cavity. In conclusion, *Staphylococcus aureus* implant-associated osteomyelitis was found to reduce vancomycin bone penetration, especially in the implant cavity. These findings suggest that it may be unsafe to rely solely on vancomycin therapy when treating acute osteomyelitis. Particularly when metaphyseal cavities are present, surgical debridement seems necessary. © 2017 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 9999:1–6, 2017.

Keywords: tissue pharmacokinetics; microdialysis; MRSA; glycopeptide; bone penetration

Implant-associated osteomyelitis and periprosthetic joint infections are devastating complications of joint replacement surgery. It is projected that up to 4 million joint arthroplasties will be completed in the UK and USA by 2030, and up to 5% of these patients will develop an infection around the implant.[1–3] Treatment is a specialist-assignment and involves a combination of surgical debridement and prolonged antimicrobial therapy. Treatment failure is common and may partly be a consequence of lack of knowledge regarding target-site penetration of antimicrobials.[4,5]

Plasma pharmacokinetic/pharmacodynamic (PK/PD) targets have been established for most antimicrobials but specific tissue PK/PD targets are widely unknown.[6] Particularly, in tissues with infection and inflammation, PK/PD targets are often not existing. In cases with insufficient tissue penetration, dosing based on plasma concentrations may therefore lead to treatment failures. Bone infection induces intra-trabecular suppuration which results in ischemic osseous sequestration and reduced vascularization.[7] Accordingly, it seems rational to hypothesize that antimicrobial bone penetration to infected bone decreases with the progression of infection and inflammation.

Grant sponsor: Aase og Ejnar Danielsens Fond; Grant sponsor: The Elisabeth and Karl Ejnar Nis-Hanssens Memorial Trust; Grant sponsor: Health Research Fund of Central Denmark Region.
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The predominant pathogen in orthopaedics is *Staphylococcus aureus*. Over the last decade, the incidence of orthopaedic *methicillin-resistant Staphylococcus aureus* (MRSA) infections has increased markedly.[8,9] Being effective against MRSA, vancomycin is recommended as first-line choice for treatment of orthopaedic MRSA infections.[10–12]

Vancomycin bone pharmacokinetics has almost solely been investigated using bone biopsies, and only few studies have assessed the impact of infection and inflammation.[13–16] The bone biopsy approach suffers from methodological challenges, which may reduce the applicability of the findings. Indeed, large variations in tissue concentrations have been found.[13,17,18] In recent years, the pharmacokinetic tool microdialysis (MD) has successfully been applied for sampling of various antimicrobials in drill holes in bone.[19–26] The objective of this study was to evaluate the effect of a traumatically induced, implant-associated acute osteomyelitis on vancomycin bone penetration in a porcine model.[27]

MATERIALS AND METHODS

The study was approved by the Danish Working Environment Authority and The Danish Animal Experiments Inspectorate, and was carried out in accordance with existing laws (license No. 2013/15-2934-00946). The study was performed on a previously described osteomyelitis porcine model and analogous to a former study where cefuroxime was the study drug.[26,27]

Overview

Eight female pigs (Danish Landrace breed, weighing from 75 to 86 kg) were included in the study. They all went through

two surgeries (day 0 and 5). On day 0, traumatically induced implant-associated *S. aureus* osteomyelitis was induced in the proximal metaphysis of the right tibia. On day 5 sampling of vancomycin was performed using MD in the implant bone cavity, in cancellous bone adjacent to the implant cavity (ADJ-I cancellous bone), in subcutaneous adipose tissue adjacent to the implant cavity (ADJ-I SCT), and in healthy cancellous bone and healthy SCT in the contralateral leg. Venous blood samples were also collected from a central venous catheter. Following surgery and calibration of the MD probes, 1,000 mg of vancomycin ("Hospira," Sweden) was administered intravenously in a peripheral catheter over 100 min. Sampling was conducted over 8 h starting at the beginning of vancomycin infusion. Tissue penetration ratios were the primary endpoint. Secondary endpoints were standard PK parameters; the area under the concentration-time curves ($AUC_{0\text{-last}}$), peak drug concentration (C_{max}), and time to C_{max} (T_{max}). After the last samples were collected, the pigs were euthanized using pentobarbital.

Study Procedures

Bacterial Strain

A concentration of 10^4 colony-forming units of a beta-hemolytic *S. aureus* (non MRSA) strain S54F9 of spa type t1333 (spaserver.ridom.de) in a 10-μl saline solution was used for inoculation.[28]

Anesthesia

Induction of anesthesia on day 0 and 5 and postoperative care was effected as previously described.[26] A combination of fentanyl (0.35–0.5 mg/h, continuous infusion) and propofol (10–15 mg/kg/h, continuous infusion) maintained the anesthesia during surgery and the sampling period.

Induction of Implant-Associated Osteomyelitis, Surgery 1 on Day 0

In lateral position, under sterile conditions and fluoroscopic guidance, a cavity was created in cancellous bone of the anteromedial aspect of the right proximal tibia. The cavity was placed parallel to and approximately 10 mm distal to the epiphyseal line and created using a 4-mm Kirschner wire. The depth of the cavity was aimed to be 27 mm. 10 μl of inoculum was injected to the cavity and an implant of a 20×2 mm Kirschner wire was introduced. Periosteum, subcutaneous adipose tissue, and skin were closed in separate layers.

Implantation of Microdialysis Probes, Surgery 2 on Day 5

On day 5, all animals had developed subcutaneous abscesses adjacent to the implant cavity. Again, in the lateral position and under sterile conditions, the proximal tibia was exposed via the incision from surgery 1. This also led to drainage of the abscesses. Approximately 8 mm parallel to the implant cavity and 10 mm distal to the epiphyseal line, and under fluoroscopic guidance, another drill hole with a diameter of 2 mm and a depth of 27 mm was made, simulating adjacent infected cancellous bone. The pig was then placed in its opposite side, to drill an identical hole in contralateral, healthy, left tibia. Next, the pig was placed in supine position, and MD probes were placed in the SCT: parallel to and 10 mm distal to the skin incision on both the infected and healthy leg using the manufacturers standard introducer. MD probes were then placed in the drill holes bilaterally and in the implant cavity. The periosteum above the implant cavity was intact, and was perforated with a cannula before introduction of the MD probe. The perforation did not lead to drainage of the cavity. To prevent displacement, the skin was closed and all probes were fixed to the skin with single sutures. For each MD-location, the following membrane lengths were used: Implant cavity (20 mm), ADJ-I cancellous bone (20 mm), ADJ-I SCT (30 mm), cancellous bone (20 mm), and SCT (30 mm). Correct location of the bone probes was assessed by fluoroscopy.

Assessment of Infection

The extent of infection was evaluated by C-reactive protein (CRP) level in serum, cultures of blood, swabs from the implant cavity, ADJ-I cancellous bone, and ADJ-I SCT and post-mortem computed tomography (CT) scans. The CT scans were used to evaluate destruction of the bone surrounding the implant cavity by measuring the increase of diameter and volume of the cavity.

Microdialysis and Sampling Procedures

A thorough description of MD can be found elsewhere.[29,30] In brief, MD is a probe-based method enabling continuous sampling of water-soluble molecules from the tissue of interest.[19,21,31–34] Owing to the continuous perfusion and the semipermeable membrane at the tip of the probe, a non-equilibrium diffusion of molecules following the concentration gradient will occur. Thus, the dialysate-concentrations act only as a fraction of the actual concentration; expressed as relative recovery (RR). All MD probes were individually calibrated using retrodialysis.[35]

The MD system in the present study consisted of CMA 107 precision pumps (μ-Dialysis AB, Stockholm, Sweden) and CMA 70 probes (membrane length 30 and 20 mm, molecular cut-off 20 kilo Daltons). After surgery, all MD probes, except for the implant cavity-probes, were perfused with 0.9% NaCl containing vancomycin at a concentration of 2.5 μg/ml at a perfusion rate of 1 μL/min. The implant cavity-probes were perfused with pure 0.9% NaCl after surgery and throughout the sampling time. Following a 30-min tissue equilibration period, all probes, except for the implant cavity-probes, were individually calibrated by collecting a 60-min sample. After calibration, the perfusate was changed to blank isotonic saline, and a 180-min washout period was allowed for. The implant cavity-probes were calibrated with 0.9% NaCl containing vancomycin at a concentration of 100 μg/ml when the last dialysate was collected. This approach was applied to avoid falsy high levels of vancomycin in a presumably poorly perfused cavity. 1,000 mg of vancomycin was then administered intravenously in a peripheral catheter over 100 min. From time 0–120 min dialysates were collected every 40 min and thereafter every 60 min from 120 to 480 min, giving a total of nine samples over 8 h. Venous blood samples were collected from a central venous catheter in the middle of every dialysate sampling interval.

Dialysates were immediately frozen on dry ice and were then transferred to a −80°C freezer until analysis. Dialysate concentrations were corrected for RR and attributed to the midpoint of each sampling interval. Venous blood samples were stored at 5°C for a maximum of 24 h before being centrifuged at 3,000*g* for 10 min. Plasma aliquots were then frozen and stored at −80°C until analysis.

Quantification of Vancomycin Concentrations

Dialysate concentrations were determined using Ultra High Performance Liquid Chromatography (UHPLC) as previously

described.[24] The limit of quantification was defined as the lowest concentration with intra run CV <20%, and was found to be 0.05 μg/ml. The free concentration of vancomycin in plasma was measured on the Cobas c501 platform (Roche, Switzerland).[24]

Pharmacokinetic Analysis and Statistics

The pharmacokinetic (PK) parameters $AUC_{0\text{-last}}$, C_{max}, T_{max} and terminal half-life ($T_{1/2}$), were determined separately for each subject by non-compartmental analysis (NCA) using the pharmacokinetic-series of commands in Stata (v. 14.1). The area under the concentration-time curves (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 $\ln(2)/\lambda_{eq}$, where λ_{eq} is the terminal elimination rate constant estimated by linear regression of the log concentration on time. The variables $AUC_{0\text{-last}}$, C_{max}, T_{max} and $T_{1/2}$ were analyzed using their log transformed data in a mixed model taking the variance between the pigs into account. Consequently, after back-transforming to the original scale, medians and 95%CIs of $AUC_{0\text{-last}}$, C_{max}, T_{max} and $T_{1/2}$ are given. 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 F test and pairwise comparisons using *t*-test. A *p*-value <0.05 was considered significant. No correction for multiple comparisons was applied. The tissue $AUC_{0\text{-last}}$ to plasma $AUC_{0\text{-last}}$ ratio ($AUC_{tissue}/AUC_{plasma}$) was calculated as a measure for tissue penetration. Statistical analyses were also performed using Stata. Values below lower limit of quantification were set to zero. The washout concentrations were low, and therefore not included in the analysis.

RESULTS

All eight pigs completed the study. Dialysate concentrations could not be reliably determined for one implant cavity-probe, due to UHPLC apparatus failure, and this probe was therefore excluded from the analysis. For three cancellous bone probes, and one ADJ-I SCT probe, RR could not be reliably determined. The dialysate concentrations for these probes resembled the dialysate concentrations of the other probes from the same locations. For probes where RR could not be determined, mean RR from the same locations were therefore used to obtain absolute tissue concentrations. Flouroscopy confirmed correct placement of all probes.

S. aureus was re-isolated from seven of eight implant cavity swabs, six of eight ADJ-I SCT swabs and three of eight ADJ-I cancellous bone drill-holes. Five of eight implant cavity swabs were typed to the infecting strain. No bacteria were isolated from the blood. Mean CRP (±SD) increased from 20.9 ± 12.6 mg/l at day 0 (before bacterial inoculation) to 120.7 ± 99.1 mg/l at day 5.

Bone destruction was found in relation to all the implant cavities as shown by the increase in diameter of the implant cavity from 4 mm to a mean (±SD) of 5.6 ± 1.4 mm and an increase of volume (±SD) of $1.13 \pm 1.04\ cm^3$. No radiologic changes in the bone surrounding the ADJ-I cancellous drill hole were observed. A representative intraoperative fluoroscopy image and post-mortem CT sectional views of the implant cavity, drill hole in ADJ-I cancellous bone and drill hole in healthy cancellous bone from one pig are illustrated in Figure 1.

Mean (±SD) RRs were $21.3 \pm 12.3\%$ (cancellous bone), 29.5 ± 11.0 % (ADJ-I cancellous bone), 26.5 ± 6.0 (SCT), $36.7 \pm 17.7\%$ (ADJ-I SCT), and $40.0 \pm 6.3\%$ (implant cavity).

Vancomycin plasma and tissue concentration-time profiles are shown in Figure 2. Corresponding key pharmacokinetic parameters are provided in Table 1. Ranging from 0.20 to 0.74, tissue penetration (expressed as the ratio of $AUC_{tissue}/AUC_{plasma}$) was incomplete for all compartments except for SCT. The lowest penetration was found in the implant cavity. $AUC_{0\text{-last}}$ for the implant cavity was also significantly lower than all the other compartments ($p < 0.03$). Accordingly, C_{max} for the implant cavity differed from the remaining ($p < 0.03$), except for ADJ-I cancellous bone ($p = 0.062$). Finally, $AUC_{0\text{-last}}$ and C_{max} were lower in ADJ-I cancellous bone compared with healthy cancellous bone, although not significantly ($p < 0.089$).

DISCUSSION

Management of osteomyelitis traditionally involves a combination of surgical debridement and long-standing antimicrobial therapy, but recently there has been a trend toward antimicrobial therapy alone.[36,37] The success of antimicrobial treatment alone obviously relies upon sufficient target site penetration. Findings like metaphyseal cavities and subperiosteal abscesses are usually considered indicators of insufficient antimicrobial target site penetration. In this study, single-dose vancomycin tissue penetration was evaluated in a porcine model where implant associated osteomyelitis was induced in the proximal tibial metaphysis. Bone destruction was observed around the implant resembling a metaphyseal cavity. The main finding was that implant cavity vancomycin penetration was significantly incomplete and lower than all the other compartments. In fact, the vancomycin tissue distribution in general was rather heterogeneous, and for both cancellous bone and SCT, tissue penetration was lower on the infected side. Tissue specific targets for bone and soft tissue infections have not been established for vancomycin, but in plasma a 24-h (1,440 min) steady state target ratio of AUC/MIC ≥ 24,000 (corresponding to a ratio of 400 when AUC is given in h · μg/ml) for vancomycin has been associated with clinical success.[38] For vancomycin, most orthopaedic pathogens exhibit relevant MICs in the range of 0.5–2 mg/l.[39] As our measurements were obtained over 8-h (480 min) and before achievement of steady state, the present single-dose study setup does not allow for a direct comparison to established targets. Nonetheless, it

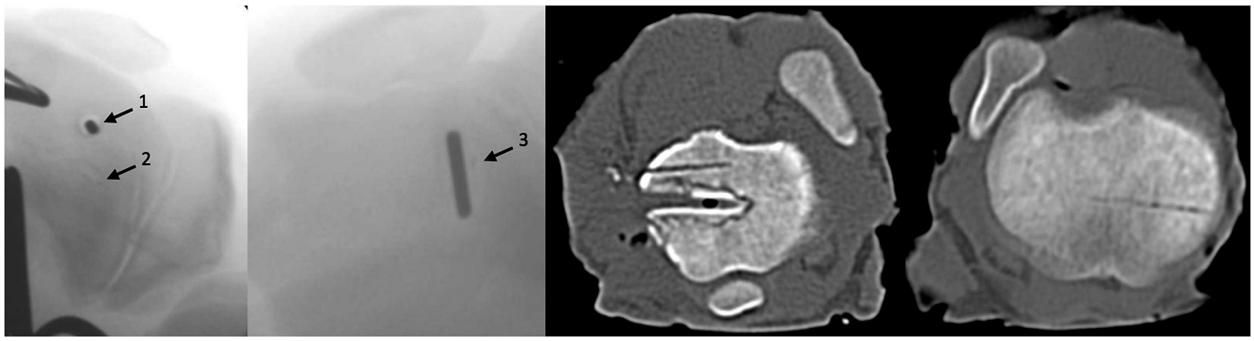

Figure 1. Intraoperative fluoroscopic image (left panel) showing the location of the MD probes in the infected bone. 1: Implant cavity with implant. 2: The gold thread within the MD probe membrane tip in the adjacent dill hole in ADJ-I cancellous bone. 3: The gold thread in the implant cavity probe. Post-mortem CT sectional views of the drill hole in the implant cavity and ADJ-I cancellous bone and healthy cancellous bone (right panel).

seems unlikely that sufficient target site concentrations can be achieved in osteomyelitis complicated with metaphyseal cavities. Appreciating the toxicity of vancomycin, merely increasing doses is hardly a safe solution.[40] Consequently, the findings in this study supports that the presence of metaphyseal cavities is an indication for surgical debridement in acute osteomyelitis.

Already 5 days after inoculation in this porcine model, significantly impaired penetration of vancomycin to all compartments in the infected leg was found. Interestingly, though not completely significant, a difference was also noted between cancellous bone in the infected and the healthy leg with the poorest penetration found in the infected leg. This finding may indicate that vancomycin bone penetration decreases with the progression of the infection. Though such a correlation would be better investigated with a different study setup, it supports the importance of early diagnosis and initiation of antimicrobial treatment in the management of acute osteomyelitis.

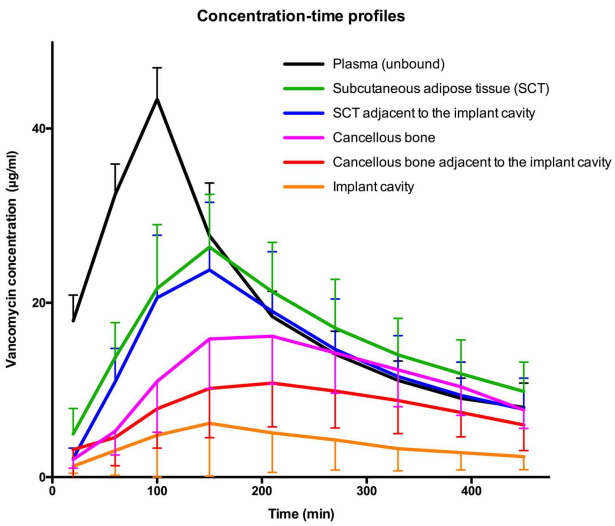

Figure 2. Mean concentration-time profiles for plasma, subcutaneous adipose tissue (SCT), ADJ-I SCT, cancellous bone, ADJ-I cancellous bone, and implant cavity. Bars represent 95%CI.

As it has been shown for other tissues, the antimicrobial penetration may differ significantly between drugs.[31,41–43] In an analogous study where cefuroxime was investigated, tissue penetration ratios were indeed higher for all tissues compared to the findings for vancomycin in the present study.[26] This indicate that selection of antimicrobials in the treatment of bone infections should not only be based on the antimicrobial sensitivity of the invading pathogen, but also on the specific bone pharmacokinetics of the drug. In turn, this stresses the importance of studies evaluating bone penetration of relevant antimicrobials. The findings in the present porcine osteomyelitis model suggest that cefuroxime may be more suitable for treatment of osteomyelitis compared to vancomycin when using standard dosing.

The assessment of antimicrobial bone pharmacokinetics during infection is challenged not only by the heterogeneous clinical presentation of osteomyelitis but also by the shortness of ideal sampling methods. Traditionally, bone pharmacokinetics of antimicrobials has been investigated using bone biopsies.[13] As opposed to this approach, MD allows for continuous sampling of the unbound extracellular fraction of drug, which, except for the rare cases where intracellular pathogens are present, is the compartment of interest.[44] Additionally, measurements can be continued after the end of surgery.[29] When conducting MD-studies, however, certain experimental factors must be considered and optimized. Of particular importance is the RR when evaluating absolute tissue concentrations. It is generally recommended that RR should exceed 20% as lower levels of RR are more exposed to a magnification of the variations associated with pre-analytical sample handling and the chemical assay.[45] The resulting variation will increase exponentially with a decreasing RR. In the present study, measures were taken to optimize the RR by using MD probes with membranes as long as permitted by anatomical factors and by using a low perfusion rate, while maintaining acceptable sampling intervals and sample volumes sufficient for chemical analysis. This resulted in acceptable mean RR values in the range of 21.3–40.0%. The present application of MD in a

Table 1. Key Pharmacokinetic Parameters for Plasma, Subcutaneous Adipose Tissue (SCT), SCT adjacent to the implant cavity (ADJ-I SCT), Cancellous Bone, Cancellous Bone adjacent to the implant cavity (ADJ-I cancellous bone), and Implant Cavity

	Pharmacokinetic Parameter				
Tissue	$AUC_{0\text{-last}}$ (min μg/ml)	C_{max} (μg/ml)	T_{max} (min)	$T_{1/2}$ (min)a	$AUC_{tissue}/AUC_{plasma}$
Plasma (unbound)	8548 (7546;9551)[b]	43.2 (40.2;46.1)	100 (82;118)	216 (151;282)	
SCT	6997 (5426;8569)	25.4 (20.2;30.7)	136 (106;165)	227 (193;261)	0.87 (0.64;1.10)
ADJ-I SCT	5739 (3953;7524)	22.0 (15.3;28.7)	150 (123;177)	205 (169;242)	0.74 (0.52;0.96)
Cancellous bone	4640 (3415;5865)[e]	15.7 (10.9;20.4)[e]	191 (149;233)	255 (206;305)	0.59 (0.42;0.75)
ADJ-I cancellous bone	3011 (1758;4265)	9.9 (5.9;13.9)	222 (173;271)	290 (126;454)	0.41 (0.25;0.57)
Implant cavity	1103 (212;1995)[c]	4.1 (0.7;7.5)[d]	178 (136;220)	416 (15;817)	0.20 (0.08;0.33)
Overall comparison[a]	$p < 0.001$	$p < 0.001$	–	$p < 0.11$	

Values are given as medians (95%CI).
AUC_{0-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}; $T_{1/2}$, half-life at β-phase; $AUC_{tissue}/AUC_{plasma}$, tissue penetration expressed as the ratio of $AUC_{tissue}/AUC_{plasma}$.
[a]Overall comparison using F test for free plasma, SCT, ADJ-I SCT, ADJ-I cancellous bone, cancellous bone, and implant cavity.
[b]$p < 0.019$ for all comparisons between plasma and the other compartments. Except for plasma versus SCT: p-value = 0.121.
[c]$p < 0.03$ for all comparisons between the implant cavity and the other compartments.
[d]$p < 0.03$ for all comparisons between the implant cavity and the other compartments. Except for the implant cavity versus ADJ-I cancellous bone: $p = 0.062$.
[e]$p < 0.089$ for comparison between cancellous bone and ADJ-I cancellous bone.

validated porcine osteomyelitis model therefore seems to be an important step toward understanding antimicrobial penetration in osteomyelitis.[27]

In conclusion, *Staphylococcus aureus* implant associated osteomyelitis was found to significantly reduce vancomycin bone penetration, especially in the implant cavity. Consequently, it may be unsafe to rely solely on vancomycin therapy when treating acute osteomyelitis. Particularly when metaphyseal cavities are present, surgical debridement seems necessary.

AUTHORS' CONTRIBUTIONS

MB, PH, JK, LKJ, BA, HEJ, KS and MT initiated and designed the study. MB, PH, JK, LKJ, and ML conducted the surgery and collected the data. Statistical analysis and interpretation of data was done by MB, PH, ML, HEJ, KS, and MT. MB drafted the manuscript. All authors read, revised and approved the manuscript.

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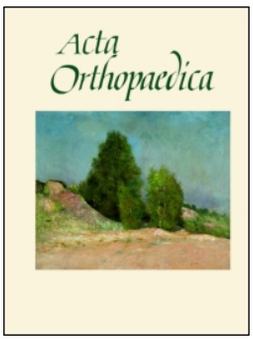

Acta Orthopaedica

ISSN: 1745-3674 (Print) 1745-3682 (Online) Journal homepage: http://www.tandfonline.com/loi/iort20

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To cite this article: Mats Bue, Pelle Hanberg, Mikkel Tøttrup, Maja B Thomassen, Hanne Birke-Sørensen, Theis M Thillemann, Torben L Andersson & Kjeld Søballe (2018): Vancomycin concentrations in the cervical spine after intravenous administration: results from an experimental pig study, Acta Orthopaedica, DOI: 10.1080/17453674.2018.1501548

To link to this article: https://doi.org/10.1080/17453674.2018.1501548

Published online: 06 Aug 2018.

Vancomycin concentrations in the cervical spine after intravenous administration: results from an experimental pig study

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Submitted 2018-04-17. Accepted 2018-07-03.

Background and purpose — Vancomycin may be an important drug for intravenous perioperative antimicrobial prophylaxis in spine surgery. We assessed single-dose vancomycin intervertebral disc, vertebral cancellous bone, and subcutaneous adipose tissue concentrations using microdialysis in a pig model.

Material and methods — 8 female pigs received 1,000 mg of vancomycin intravenously as a single dose over 100 minutes. Microdialysis probes were placed in the C3–C4 intervertebral disc, C3 vertebral cancellous bone, and subcutaneous adipose tissue, and vancomycin concentrations were obtained over 8 hours. Venous blood samples were obtained as reference.

Results — Ranging from 0.24 to 0.60, vancomycin tissue penetration, expressed as the ratio of tissue to plasma area under the concentration-time curve from 0 to the last measured value, was incomplete for all compartments. The lowest penetration was found in the intervertebral disc. The time to a mean clinically relevant minimal inhibitory concentration (MIC) of 4 µg/mL was 3, 17, 25, and 156 min for plasma, subcutaneous adipose tissue, vertebral cancellous bone, and the intervertebral disc, respectively. In contrast to the other compartments, a mean MIC of 8 µg/mL was not reached in the intervertebral disc. An approximately 3-times longer elimination rate was observed in the intervertebral disc in comparison with all the other compartments ($p < 0.001$), and the time to peak drug concentration was higher for all tissues compared with plasma

Interpretation — Preoperative administration of 1,000 mg of vancomycin may provide adequate vancomycin tissue concentrations with a considerable delay, though tissue penetration was incomplete. However, in order also to achieve adequate intervertebral disc concentrations in all individuals and accommodating a potentially higher MIC target, supplemental application of vancomycin may be necessary.

Postoperative surgical site infections following spine surgery can have devastating complications such as mechanical fixation failure, neurological injury, pseudoarthrosis, and sepsis (Fang et al. 2005). The reported incidence of postoperative spondylodiscitis ranges between 1% and 4% and may be even higher when instrumentation is applied (Gaynes et al. 2001, Deyo et al. 2004, Fang et al. 2005, Smith et al. 2011). Perioperative antimicrobial prophylaxis plays a key role in lowering the risk of postoperative spondylodiscitis. The antimicrobial effect relies not only on its sensitivity against the invading bacteria, but also on adequate target site concentrations. For vancomycin, tissue targets for prevention of postoperative spondylodiscitis have not been established. Nonetheless, it seems prudent to maintain tissue concentrations that, as a minimum, exceed minimal inhibitory concentrations (MIC) of relevant bacteria throughout surgery and until a few hours after the incision is closed (Mangram et al. 1999, Whiteside 2016). Vancomycin may become increasingly important as perioperative antimicrobial prophylaxis in spine surgery due to the increasing incidence of methicillin-resistant Staphylococcus aureus (MRSA) infections (Al-Nammari et al. 2007, Gouliouris et al. 2010). However, routine use of vancomycin is not indicated due to the risk of resistance development (Mangram et al. 1999, Bryson et al. 2016). Incomplete vancomycin penetration has recently been reported for a number of tissues (Bue et al. 2018a, b). Given the avascular nature of the intervertebral disc, vancomycin penetration may be inadequate for optimal prevention of postoperative spondylodiscitis.

It is challenging to determine antimicrobial spine tissue concentrations. Intervertebral disc and bone concentrations of vancomycin have almost only been evaluated using bone and disc tissue samples and discectomy (Scuderi et al. 1993, Conaughty et al. 2006, Landersdorfer et al. 2009, Komatsu et al. 2010). However, these methods suffer from methodological challenges, which makes it difficult to relate the results

DOI 10.1080/17453674.2018.1501548

to relevant pharmacokinetic/pharmacodynamic endpoints (Landersdorfer et al. 2009, Pea 2009). Recently, microdialysis has evolved as a promising method for sampling various antimicrobials in different types of tissues, including bone and the intervertebral disc (Stolle et al. 2004, Bue et al. 2015, Tottrup et al. 2015, Hanberg et al. 2016, Bue et al. 2018a, b).

We assessed single-dose vancomycin concentrations in the C3–C4 intervertebral disc, the C3 vertebral cancellous bone, and subcutaneous adipose tissue using microdialysis in a pig model mimicking a perioperative situation. Tissue penetration ratios, expressed as the ratio of tissue to plasma area under the concentration-time curve from 0 to the last measured value ($AUC_{tissue}/AUC_{plasma}$), and time to mean MICs of 2, 4, and 8 µg/mL were the primary endpoints. The secondary endpoints were pharmacokinetic parameters: the area under the concentration-time curves ($AUC_{0–last}$), peak drug concentration (C_{max}), time to C_{max} (T_{max}), and half-life ($T_{1/2}$).

Material and methods

This study was conducted at the Institute for Clinical Medicine, Aarhus University Hospital, Aarhus, Denmark. All chemical analyses were performed at the Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark.

Overview

8 female pigs were included in the study (Danish Landrace Breed; weight 78–82 kg). Vancomycin was administered intravenously as a single dose of 1,000 mg over 100 min, and sampling was conducted over 8 hours starting at the beginning of vancomycin infusion. Vancomycin concentrations were obtained using microdialysis in the C3–C4 intervertebral disc, the C3 vertebral cancellous bone, and subcutaneous adipose tissue.

Anesthesia and surgical procedures

The pigs were kept under general anesthesia using a combination of fentanyl (0.35–0.5 mg/h, continuous infusion) and propofol (500–600 mg/h, continuous infusion) during the surgery and the sampling period. Arterial pH was monitored throughout the study and kept in the range of 7.36–7.47 by regulating ventilation. Blankets were used to keep the core temperatures within the range of 36.2–39.1ºC.

Immediately after induction of anesthesia, the surgical procedures were initiated. With the pig in supine position, and under fluoroscopic guidance, the C2–C4 vertebrae were exposed via an anterolateral incision. At approximately 45º to the sagittal plane, a drill hole with a diameter of 2 mm and a depth of 25 mm was created in the middle of C3. Parallel to this drill hole, a Kirschner wire with a fixating device (PEBAX, M Dialysis AB, Stockholm, Sweden) was drilled into the caudal part of C2. A microdialysis probe (membrane length 20 mm) was inserted into a splittable introducer with the membrane protruding approximately 30 mm from the tip of the introducer. This probe-introducer setup was then placed in the drill hole in C3 and fixed to the fixating device; an endo clip was then attached to the introducer. These steps were taken to avoid subsequent displacement of the probe. At the same angle, a splittable introducer with a needle was introduced into the intervertebral disc between C3 and C4 parallel to, and in the middle of, the adjacent endplates. After the annulus fibrosus was penetrated, the needle was retracted, and the introducer was carefully advanced into the nucleus pulposus until resistance from the opposite wall of the annulus fibrosus was felt. A microdialysis probe (membrane length 10 mm) was then placed in the introducer, and the splittable introducer was retracted until the entire membrane of the probe was exposed in the intervertebral disc. The probe was attached to the introducer with endo clips. Fluoroscopy was used to assess correct location of the probes in the C3 vertebral body and the C3–C4 intervertebral disc. In addition to the bone and intervertebral disc probes, a subcutaneous adipose tissue probe (membrane length 20 mm) was placed in the lateral part of the right thigh, based on the manufacturer's guidelines.

Microdialysis and sampling procedures

A detailed description of microdialysis can be found elsewhere (Muller 2002, Joukhadar and Muller 2005). Briefly, microdialysis is a probe-based method that allows for serial sampling of water-soluble molecules from the extracellular fluid in the tissue of interest by means of a semipermeable membrane at the tip of the microdialysis probe (Joukhadar et al. 2001, Hutschala et al. 2013). Due to continuous perfusion of the probe, a non-equilibrium diffusion of molecules following the concentration gradient will occur. Consequently, the concentration in the dialysate represents only a fraction of the true tissue concentration, which is expressed as the relative recovery. Thus, relative recovery must be determined to calculate the absolute tissue concentrations. In the present study, all the microdialysis probes were individually calibrated at the end of the study using the retrodialysis by drug method (Scheller and Kolb 1991). The relative recovery was calculated using the following Equation 1:

$$\text{Relative recovery } (\%) = 100 \times (1 - C_{dialysate}/C_{perfusate}) \quad (1)$$

where $C_{dialysate}$ is the concentration (µg/mL) in the dialysate and $C_{perfusate}$ is the concentration (µg/mL) in the perfusate.

Absolute, extracellular concentrations (µg/mL), C_{tissue}, were calculated by correcting for relative recovery using the following Equation 2:

$$C_{tissue} = 100 \times C_{dialysate}/\text{Relative recovery } (\%) \quad (2)$$

The microdialysis system consisted of CMA 107 precision pumps (M Dialysis AB, Stockholm, Sweden) and CMA 70 probes (membrane length 20 mm and 10 mm, molecular cut-off 20 kilo Daltons). All the microdialysis probes were perfused with 0.9% NaCl at a perfusion rate of 1 µL/min throughout the sampling time. Following a 30-min tissue equilibration

Pharmacokinetic parameters for plasma, subcutaneous adipose tissue, vertebral cancellous bone, and intervertebral disc

Tissue	$AUC_{0–last}$ (min µg/mL)	C_{max} (µg/mL)	T_{max} (min)	$T_{1/2}$ (min)	$AUC_{tissue}/AUC_{plasma}$
Plasma (unbound)	7,880 (7164–8597) [b]	40.0 (35.7–44.3) [b]	75 (61–89)	325 (99–552)	
Subcutaneous adipose tissue	4,719 (4002–5436)	18.0 (14.1–21.9)	113 (96–129)	224 (197–250)	0.60 (0.48–0.72)
Vertebral cancellous bone	3,677 (2960–4393) [c]	12.3 (9.6–15.0) [c]	159 (134–184)	271 (227–315)	0.46 (0.40–0.53)
Intervertebral disc	1,983 (1237–2729) [d]	6.6 (3.6–9.6)4	270 (187–353)	933 (527–1,339) [e]	0.24 (0.17–0.31)
p-value [a]	< 0.001	< 0.001	–	< 0.008	

Values are given as means (95% confidence interval).
$AUC_{0–last}$, area under the concentration-time curve from 0 to the last measured value; C_{max}, peak drug concentration; T_{max}, time to C_{max}; T1/2, half-life at -phase; $AUC_{tissue}/AUC_{plasma}$, tissue penetration expressed as the ratio of $AUC_{tissue}/AUC_{plasma}$.
[a] Overall comparison using F test for plasma (unbound), subcutaneous adipose tissue, vertebral cancellous bone, and intervertebral disc.
[b] $p < 0.001$ for all comparisons between plasma and the other compartments.
[c] $p < 0.01$ for comparison with subcutaneous adipose tissue.
[d] $p < 0.01$ for comparison between intervertebral disc and the other compartments.
[e] $p < 0.001$ for all comparisons between intervertebral disc and the other compartments.

period after placement of the microdialysis probes, 1,000 mg of vancomycin was administered intravenously over 100 min starting at time zero. For the first 2 hours, dialysates were harvested at 40-min intervals and, thereafter, at 60-min intervals for the following 6 hours, resulting in a total of nine samples over 8 hours. The relative recovery-corrected dialysate concentrations were ascribed to the midpoint of each sampling interval. Venous blood samples were harvested from a central venous catheter at the midpoint of the same 9 sampling intervals. When the last dialysate was collected, all the probes were individually calibrated using 0.9% NaCl with vancomycin at a concentration of 300 µg/mL by collecting 60-min samples. This high calibration concentration of vancomycin was chosen to minimize the influence of the residual local tissue concentrations. After the last dialysates were collected, the pigs were killed using pentobarbital.

The dialysates were immediately placed in a –80°C freezer until analysis. The venous blood samples were stored at 5°C for a maximum of eight hours before being centrifuged at 3,000 g for 10 minutes. The plasma aliquots were then frozen and stored at –80°C until analysis.

Chemical analysis of vancomycin

The vancomycin concentrations in the dialysates were quantified using ultra-high-performance liquid chromatography as previously described (Bue et al. 2015). The quantification limit was defined as the lowest concentration with intra-run CV < 20%; it was found to be 0.05 µg/mL. The free concentration of vancomycin in plasma was measured with a homogeneous enzyme immunoassay technique using the Siemens Chemistry XPT platform (Advia Chemistry, Erlangen, Germany). Intra-run (total) imprecisions for the assay were ±1.2 µg/mL (2SD) at 6.6 µg/mL and ±3.7 µg/mL (2SD) at 29.1 µg/mL.

Pharmacokinetic analysis and statistics

Using Microsoft Excel (Microsoft Corp, Redmond, WA, USA), the time to mean clinically relevant MICs of 2, 4, and 8 µg/mL was estimated using linear interpolation. The pharmacokinetic parameters, $AUC_{0–last}$, C_{max}, T_{max}, and $T_{1/2}$, were determined separately for each compartment for each pig by non-compartmental analysis using the pharmacokinetic-series of commands in Stata (v. 14.1, StataCorp LLC, College Station, TX, USA). The $AUC_{0–last}$ was calculated using the trapezoidal rule. The tissue $AUC_{0–last}$ to plasma $AUC_{0–last}$ ratio ($AUC_{tissue}/AUC_{plasma}$) was calculated as a measure of tissue penetration. C_{max} was calculated as the maximum of all the recorded concentrations and T_{max} was calculated as the time to C_{max}. $T_{1/2}$ was calculated as $\ln(2)/\lambda_{eq}$, where λ_{eq} is the terminal elimination rate constant estimated by linear regression of the log concentration on time. These pharmacokinetic parameters were obtained in all 4 compartments from the same pig and a mixed model for repeated measurements had compartments as fixed effect and subject identification variable as a random effect was applied. Also, distinct residual variance was assumed within each compartment. The normality of the residuals was estimated using a quantile–quantile (QQ) plot for the residuals and the homogeneity of the residual variance was checked by plotting residuals vs. best linear unbiased prediction estimates. The normality of the estimated random effects was checked using a QQ plot of the estimated random effects. A correction for degrees of freedom due to small sample size was handled using the Kenward–Roger approximation method. The F-test was used to determine the overall comparisons between the compartments and the t-test was used to determine pairwise comparisons. A p-value < 0.05 was considered to be significant. Statistical analyses were also performed using Stata. Values below the lower limit of quantification were set to zero. The means and 95% CI of $AUC_{0–last}$, C_{max}, T_{max}, and $T_{1/2}$ are presented in the Table.

Ethics, funding, and potential conflicts of interest

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

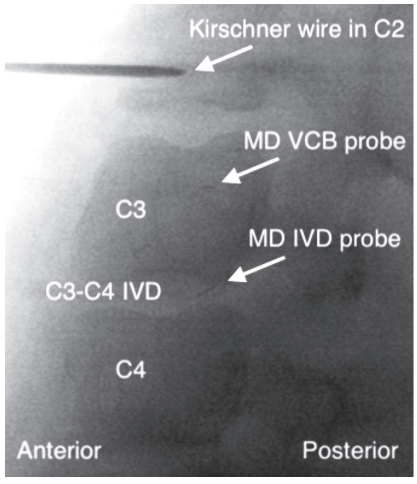

Figure 1. Representative fluoroscopic image showing the location of the microdialysis (MD) probes in a sagittal view: the Kirschner wire with the fixating device in the C2 vertebral body, C3 and C4 vertebral body, C3–C4 intervertebral disc (IVD), the gold thread within the microdialysis probe membrane tip in the vertebral cancellous bone (VCB) and intervertebral disc.

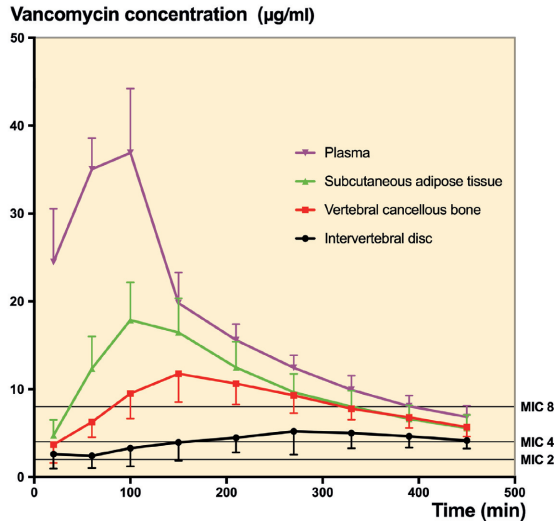

Figure 2. Mean concentration-time profiles for plasma, subcutaneous adipose tissue, vertebral cancellous bone, and the intervertebral disc. Bars represent 95% confidence intervals. MICs of 2, 4, and 8 µg/mL are also inserted.

This work was supported by unrestricted grants from the Augustinus Foundation, the Lippmann Foundation, the Knud and Edith Eriksens Memorial Foundation, the Søster and Verner Lipperts Foundation, and the Health Research Fund of Central Denmark Region. No competing interests were declared.

Results

All 8 pigs completed the study. Except for one malfunctioning intervertebral disc probe, data were obtained from all probes. Fluoroscopy confirmed correct placement of all of the probes (Figure 1). Mean (SD) relative recovery for the intervertebral disc, vertebral cancellous bone, and subcutaneous adipose tissue was 16% (6), 36% (4), and 33% (5), respectively.

Tissue penetration ($AUC_{tissue}/AUC_{plasma}$) of vancomycin (95% CI) was incomplete for the subcutaneous adipose tissue 0.60 (0.48–0.72), vertebral cancellous bone 0.46 (0.40–0.53), and intervertebral disc 0.24 (0.17–0.31). After 15 min, a mean concentration of 2 µg/mL (MIC) was reached in all compartments. The time to a mean MIC of 4 µg/mL was 3, 17, 25, and 156 min for plasma, subcutaneous adipose tissue, vertebral cancellous bone, and the intervertebral disc, respectively. A mean MIC of 8 µg/mL could not be reached in the intervertebral disc, whereas it was reached after 7, 37, and 81 min in plasma, subcutaneous adipose tissue, and vertebral cancellous bone, respectively.

The vancomycin tissue and plasma concentration-time profiles are shown in Figure 2. The pharmacokinetic parameters are presented in the Table. C_{max} (95% CI) was 6.6 µg/mL (3.6–9.6) for the intervertebral disc, 12 µg/mL (9.6–15) for vertebral cancellous bone, 18 µg/mL (14–22) for subcutaneous adipose tissue, and 40 µg/mL (36–44) for plasma. The T_{max} findings revealed delayed tissue penetration, particularly to the intervertebral disc and vertebral cancellous bone. Furthermore, $T_{1/2}$ was approximately 3 times longer in the intervertebral disc in comparison with the other compartments ($p < 0.001$). Finally, $AUC_{0–last}$ and C_{max} were lower in the intervertebral disc than in the vertebral cancellous bone ($p < 0.01$).

Discussion

To our knowledge, this is the first study to investigate single-dose vancomycin intervertebral disc and vertebral cancellous bone concentrations using microdialysis. Insufficient perioperative antimicrobial target site penetration might play an important role for the rather high incidence of postoperative spondylodiscitis. A key finding of this study was therefore incomplete and delayed intervertebral disc and vertebral cancellous bone penetration of vancomycin, with the lowest and most delayed penetration found in the intervertebral disc. However, using standard recommendations for prevention of surgical site infections and planktonic MICs of commonly encountered bacteria in spine surgery (0.5–4 µg/mL), adequate mean concentrations were achieved in all compartments, although a considerable delay was found in the intervertebral disc (Gouliouris et al. 2010, EUCAST 2017). Advantageously for a perioperative prophylactic setting, it should be noted that an approximately 3 times longer vancomycin elimination rate was found in the intervertebral disc in comparison with the other compartments. Thus, if vancomycin is administrated in due time, adequate intervertebral disc concentrations may be

sustained throughout even long surgical procedures and for some time after. This makes vancomycin attractive because it continues to kill bacteria even after surgery has ended. On the other hand, our data suggest a rather narrow or no margin at all in individuals with low intervertebral disc concentrations to bacteria exhibiting high MICs. Moreover, in the case of MRSA, increasing vancomycin MICs have been demonstrated over the last decades (Steinkraus et al. 2007). The traditional target recommendations for prevention of surgical site infections lack scientific evaluation and may in fact be insufficient for spine surgery, particularly when considering the possible devastating complications of infection in spine surgery. Higher and prolonged tissue concentrations cannot be achieved by means of increasing intravenous vancomycin doses as this is restricted by toxicity (Rybak et al. 2009). Consequently, our findings call for some considerations regarding vancomycin dosing, timing, and administration in the perioperative spine setting. Preoperative administration of 1,000 mg of vancomycin may provide adequate vancomycin tissue concentrations with a considerable delay. However, in order also to achieve adequate intervertebral disc concentrations in all individuals and accommodating a potentially higher MIC target, supplemental application (e.g. as perioperative powder) of vancomycin may be necessary (Murphy et al. 2017).

Incomplete and heterogeneous tissue distribution of antimicrobials has been demonstrated for a diverse combination of drugs and tissues and under different conditions, including infections (Joukhadar et al. 2001, Joukhadar and Muller 2005, Schintler et al. 2009, Hutschala et al. 2013, Tottrup et al. 2015, 2016). In our study, the tissue penetration ratios for vancomycin were lower for all tissues in comparison with an analogous cefuroxime study (Hanberg et al. 2016). These findings suggest that the choice of antimicrobial prophylaxis should not only be based on the characteristics of the infectious bacteria and plasma pharmacokinetics, but also on the tissue pharmacokinetics for the specific drug and the specific setting. In terms of infected tissue, it has previously been shown that vancomycin bone penetration may decrease with progression of an infection (Bue et al. 2018a). This emphasizes the need for spine tissue pharmacokinetic studies of relevant antimicrobials under different conditions.

The penetration of vancomycin into a human intervertebral disc may vary from our findings in the study, as juvenile pigs (aged 5 months) differ from adult humans in several ways (Alini et al. 2008). First, blood vessels in the human annulus fibrosus are only present in the first part of life; thereafter, the perfusion relies upon only diffusion from the endplates (Roberts et al. 2006, Gouliouris et al. 2010). Second, the intervertebral disc is thinner in pigs than in humans, indicating shorter diffusion distances (Alini et al. 2008). Third, the body mass and weight-bearing impact on the vertebral bodies and intervertebral disc differs between humans and pigs. Moreover, higher vancomycin concentrations have been demonstrated in pig bone and tissue concentrations in comparison with male patients undergoing total knee replacement surgery (Bue et al. 2015, Bue et al. 2018b).

Until now, vancomycin bone and intervertebral disc concentrations have only been assessed using bone and disc tissue samples and discectomy (Scuderi et al. 1993, Conaughty et al. 2006, Landersdorfer et al. 2009, Komatsu et al. 2010). In contrast, microdialysis allows for serial sampling of the unbound extracellular concentrations of drug in bone and in the intervertebral disc, and it provides dynamic concentration-time profiles (Joukhadar and Muller 2005, Hanberg et al. 2016). Therefore, the pharmacokinetic parameters obtained by microdialysis are useful for evaluating pharmacodynamic/pharmacokinetic targets. In addition to the inherent inter-species limitations of a porcine study, a certain methodological aspect of the microdialysis approach should be considered when evaluating microdialysis data. Thus, to obtain absolute tissue concentrations, the actual measured concentrations are corrected for relative recovery. This leads to a magnification of the variations associated with pre-analytical sample handling and chemical assay. These variations will increase exponentially as the relative recovery decreases. The variances in plasma and tissue pharmacokinetics found in the present study were comparable, indicating acceptable precision of the measurements within the biological variation.

In summary, vancomycin penetration into healthy pig intervertebral disc and vertebral cancellous bone was found to be incomplete and delayed, with the lowest and most delayed penetration to the intervertebral disc. However, applying standard recommendations for prevention of postoperative spondylodiscitis, preoperative administration of 1,000 mg of vancomycin may provide adequate vancomycin tissue concentrations with a considerable delay. Nonetheless, in order also to achieve adequate intervertebral disc concentrations in all individuals and accommodating a potentially higher MIC target, supplemental application of vancomycin may be necessary. Validation of these findings in a clinical setting is warranted.

MB, PH, MT, MBT, HBS, TMT, and KS initiated and designed the study. MB, MBT, and PH conducted the surgery and MB placed all the probes. MB, MBT, and PH collected the data. TLA performed the chemical analyses. Statistical analysis and interpretation of data was done by MB, PH, MT, HBS, TMT, TLA, and KS. All authors drafted and revised the manuscript.

Acta thanks Volker Alt and Ivar Rossvoll for help with peer review of this study.

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