Plasma, Subcutaneous Tissue and Bone Pharmacokinetics of Cefuroxime

PhD dissertation

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List of Papers

This PhD thesis is based on the following papers:

Paper I: Tøttrup M, Hardlei TF, Bendtsen M, Bue M, Brock B, Fuursted K, Soballe K, Birke-Sorensen H. Pharmacokinetics of cefuroxime in porcine cortical and cancellous bone determined by microdialysis. Antimicrobial Agents Chemotherapy. 2014 Jun; 58(6): 3200-5. doi: 10.1128/AAC.02438-14.

Paper II: Tøttrup M, Bibby BM, Hardlei TF, Bue M, Kerrn-Jespersen S, Fuursted K, Søballe K, Birke-Sørensen H. Continuous versus Short-term Infusion of Cefuroxime - Assessment of Concept Based on Plasma, Subcutaneous Tissue and Bone Pharmacokinetics in an Animal Model. Antimicrobial Agents Chemotherapy. 2015 Jan; 59(1): 67-75. doi: 10.1128/AAC.03857-14

Paper III: Tøttrup M, Bue M, Bibby BM, Hardlei TF, Hansen P, Fuursted K, Birke-Sørensen H Søballe K. Bone, Subcutaneous Tissue and Plasma Pharmacokinetics of Cefuroxime in Total Knee Replacement Patients – a Randomized Controlled Trial Comparing Continuous and Short-term Infusion. Submitted to: Antimicrobial Agents and Chemotherapy.

Preface

This PhD thesis consists of three papers and a comprehensive review dealing with plasma, subcutaneous tissue and bone pharmacokinetics of cefuroxime. The review is structured as a standard scientific paper, but in the materials and methods section, special attention has been put on the weaknesses and limitations of the applied methods.

The experimental studies were conducted at the Institute of Clinical Medicine, Aarhus University Hospital, Denmark, while the clinical study was conducted at the Department of Orthopaedic Surgery, Horsens Regional Hospital. Chemical analyses were performed at the Department of Clinical Biochemistry, Aarhus University Hospital.

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Abbreviations

AUC	Area under the concentration-time curve
C _{max}	The highest concentration reached (or estimated) in the compartment of reference
C _{min}	The minimum (or trough) concentration reached in the compartment of reference
CI	Continuous infusion
EI	Extended infusion
IAI	Implant-associated infection
LLOQ	Lower limit of quantification
MD	Microdialysis
MIC	Minimum inhibitory concentration
NCA	Non-compartmental analysis
РК	Pharmacokinetics
PD	Pharmacodynamics
PTA	Probability of target attainment
RCT	Randomised controlled trial
RR	Relative recovery
RR _{gain}	Relative recovery by gain
RR _{loss}	Relative Recovery by loss
SCT	Subcutaneous tissue
STI	Short-term infusion
T _{max}	Time to C _{max}
$T_{50\% \ of \ Cmax}$	Time to 50% of Cmax
T _{1/2}	Half-life
TDM	Therapeutic drug monitoring
TKR	Total knee replacement
UHPLC	Ultra High Performance Liquid Chromatography

Summary

Assessment of antimicrobial bone pharmacokinetics remains a challenging task. The predominant and traditional bone specimen approach suffers from methodological limitations, and may not be ideal for the task. The well-known pharmacokinetic tool, microdialysis, offers an attractive alternative to bone specimens. The key advantages of microdialysis are that it only samples the unbound and thus pharmaceutically active fraction of antimicrobial, and that serial measurements can be obtained even after surgery. Today, microdialysis has been validated for measurement of a number of antimicrobials in a variety of tissues, but only a few studies have assessed the applicability for bone.

The overall aim of this project was to validate and apply microdialysis for measurements of cefuroxime in bone, and to compare the findings with those in plasma and subcutaneous tissue. Cefuroxime was chosen because of its widespread use in orthopaedic surgery.

The project comprised three studies, of which two were experimental studies and one was a clinical study. All study animals/subjects received a total dose of 1,500 mg of cefuroxime irrespective of the mode of administration, and the maximum observation period was 8 hours. For all studies, a highly sensitive, specific and precise ultra high performance liquid chromatography assay was used to quantify cefuroxime. Data was analysed by either non-compartmental analysis or by population pharmacokinetic modelling.

The first study consisted of an *in vitro* part and an *in vivo* experimental (pigs) part. In the *in vitro* part, microdialysis was shown to be suited for sampling of cefuroxime. In the lack of a gold standard, the findings in the *in vivo* part suggested that measurements of cefuroxime in drill holes in bone reflect the actual bone concentration. Additionally, incomplete cancellous and cortical bone penetration of cefuroxime was found.

Prompted by the time-dependency and short half-life of cefuroxime, the second experimental study compared standard short-term infusion (STI) with continuous infusion (CI). The primary endpoint was the time that concentrations could be sustained above minimum inhibitory concentrations (MIC) for relevant pathogens. The findings in this study indicated that longer times with concentrations above a range of MICs could be achieved with CI. Nevertheless, the MICs for which CI was superior to STI would have been insufficient in a clinical setting. For both STI and CI, bone penetration was found to be incomplete.

In the clinical study, STI was again compared to CI. The population of choice was male patients scheduled for a total knee replacement. CI of cefuroxime resulted in improved tissue exposure in all tissues compared to STI. Nevertheless, both CI and STI of 1,500 mg of cefuroxime were inadequate for high MIC organisms. Statistically incomplete tissue penetration was found for cortical bone and subcutaneous tissue in the CI group. Low tissue penetration ratios were also found for the same tissues with CI, but in this group, the findings were not significant. Cefuroxime distributed well into cancellous bone in both groups.

In conclusion, the results suggest that microdialysis is a valuable tool for measurements of cefuroxime in bone. Cefuroxime tissue distribution was found to be uneven, and except for the CI group in the clinical study, cortical bone penetration was incomplete in all experiments. Finally, CI of cefuroxime results in improved tissue exposure in all tissues compared to STI in newly operated male total knee replacement patients.

Danish summary/Dansk resumé

Der er store metodologiske udfordringer forbundet med bestemmelse af antibiotikakoncentrationer i knoglevæv. Traditionelt har man målt koncentrationen af antibiotika i knogle ved hjælp af knoglebiopsier. Denne tilgang er dog forbundet med en række fundamentale metodologiske svagheder, og der er efterhånden enighed om, at metoden ikke er optimal. Det farmakologiske redskab mikrodialyse har potentialet til generere mere valide anvendelige og koncentrationsmålinger af antibiotika i knogle. Fordelen ved mikrodialyse er, at der kan foretages serielle koncentrationsmålinger af den ubundne og dermed farmakologiske aktive del af antibiotika. I modsætning til knoglebiopsi-metoden kan der desuden foretages målinger efter afslutning af kirurgiske indgreb.

Det overordnede formål med dette ph.d. projekt var at validere og anvende mikrodialyse til bestemmelse af cefuroximkoncentrationer i knogle, samt at sammenholde disse fund med koncentrationen i plasma og subkutant fedtvæv. Interessen for netop cefuroxim skyldes dets udbredte anvendelse indenfor ortopædkirurgien.

Afhandling omfatter tre studier; to eksperimentelle og et klinisk studie. Alle dyr/patienter modtog en samlet dosis cefuroxim på 1500 mg uafhængig af administrationsmåden. Den maksimale observationsperiode var 8 timer i alle studierne. Cefuroximkoncentrationen blev kvantificeret vha. ultra high performance liquid chromatography. Forsøgsresultaterne blev enten analyseret vha. non-kompartmental analyse eller vha. populationsmodellering.

Det første studie bestod af *in vitro forsøg* og eksperimentelle *in vivo* forsøg. *In vitro* forsøgene viste, at forudsætningerne for at foretage *in vivo* sampling af cefuroxim vha. mikrodialyse var til stede. Resultaterne fra *in vivo* forsøgene godtgjorde, at koncentrationen af cefuroxim i borehuller i knogle rent faktisk afspejler koncentrationen i knogle. Endelig blev der fundet nedsat penetration af cefuroxim til både spongiøs og kortikal knogle.

I det andet eksperimentelle studie blev traditionel bolus infusion sammenlignet med kontinuerlig infusion af cefuroxim med hensyn til tiden hvor koncentrationen oversteg en række relevante "minimum inhibitory concentrations" (MIC). Det viste sig, at det var muligt at opnå længere tid over visse MIC-værdier med kontinuerlig infusion. I klinisk sammenhæng ville disse MIC-værdier dog have været for lave. Igen blev der fundet nedsat knoglepenetration af cefuroxim for både bolus og kontinuerlig infusion.

I det tredje kliniske studie blev bolus infusion igen sammenlignet med kontinuerlig infusion. Forsøgspersonerne var nyopererede mandlige knæprotesepatienter, og knoglemålingerne blev fortaget i tibia. Det viste sig, at kontinuerlig infusion øgede sandsynligheden for at opnå effektive behandlingsmål i både plasma, subkutant fedtvæv og knogle i forhold til bolus infusion. Uafhængig af administrationsmåde viste det sig dog også, at en standarddosis på 1500 mg gav anledning til utilfredsstillende sandsynligheder for at opnå effektive behandlingsmål for bakterier med høje MIC-værdier. Der var nedsat penetration af cefuroxim til subkutant fedtvæv og kortikal knogle i bolus gruppen. I gruppen som fik kontinuerlig infusion, blev der også fundet lave vævspenetrations ratioer i de samme væv, men for denne gruppe var fundene ikke statistisk signifikante. Der var god cefuroximpenetration til spongiøs knogle i begge grupper.

Sammenfattende har dette ph.d. projekt vist, at mikrodialyse er en god metode til at foretage koncentrationsmålinger af cefuroxim i knoglevæv. Under de givne omstændigheder blev der påvist uens vævsfordeling af cefuroxim. Bortset fra forsøgspersonerne, som modtog kontinuerlig infusion, blev der påvist nedsat cefuroximpenetration til kortikal knogle i de øvrige forsøg. Endelig viste det kliniske forsøg, at der er større sandsynlighed for at opnå effektive behandlingsmål i både plasma, subkutant fedtvæv og knogle hos nyopererede mandlige knæprotesepatienter når cefuroxim administreres som kontinuerlig infusion i stedet for bolus infusion.

1 Introduction

1.1 Basic principles of antimicrobial pharmacokinetics/pharmacodynamics

Pharmacokinetics (PK) refers to the sub-branch of pharmacology that deals with the fate (i.e. absorption, distribution, metabolism and excretion) of a drug in the organism. Pharmacodynamics (PD), on the other hand, describes the relationship between exposure and the pharmacologic and toxicologic effects of drugs(1, 2). In 1982, Holford and Sheiner suggested the following simplified distinction between PK and PD: "Pharmacokinetics is what the body does to the drug; pharmacodynamics is what the drug does to the body"(3). In the particular case of antimicrobial PD, "measures of drug exposure is" linked "to the microbiological and clinical effects that are observed once an antiinfective drug has been administered"(1). The ultimate goal is to maximise the probability of attaining an effective response while keeping unwanted side effects at a minimum. Compared to other aspects of PD, the relationship between exposure and effect in antimicrobial PD is more straightforward. Other areas of PD are challenged by a between-patient difference in drugreceptor affinity, whereas in antimicrobial PD, the receptor (i.e. the pathogen) can be isolated, and the potency of a drug for the specific pathogen can be easily quantified (1, 2). This provides for a more direct association between drug exposure and effect(1, 2). Commonly used parameters of the potency include minimum inhibitory concentration (MIC), which is defined as the lowest drug concentration that results in stasis, and minimum bactericidal concentration (MBA), which signifies the lowest concentration required to kill a specific microorganism(1). While these measures do not allow for a description of the time-course and magnitude of bacterial killing, studies of time-kill curves have identified two primary patterns of bacterial killing(4, 5):

- Concentration-dependent drugs display a marked linear relationship between concentration and bacterial killing over a wide range of concentrations. Flouroquinolones and aminoglycosides are examples of drugs belonging to this group.
- *Time-dependent drugs* exhibit only very limited concentration-dependent killing. Instead, bacterial killing is primarily determined by the time of exposure. Beta-lactams display time-dependent killing, and maximal bacterial killing is generally achieved at concentrations of only two four times the MIC(6, 7).

After a meeting in 2002 in Nijmegen arranged by the International Society of Anti-infective Pharmacology (ISAP), it was agreed that the quantitative relationship between a pharmacokinetic parameter (such as area under the concentration-time curve (AUC)) and a microbiological parameter (such as MIC) should be referred to as a PK/PD index(8). It was also stated that all PK/PD indices should be expressed as the non-protein bound fraction of the drug, and unless stated otherwise, these PK/PD indices should refer to a 24-hour interval at steady state(8). Depending on the specific drug, the PK/PD indices that best predict efficacy for concentration–dependent drugs are C_{max} /MIC (C_{max} ; the highest concentration reached in the compartment of reference) or AUC/MIC(1, 2, 4, 9). For a number of specific combinations of concentration-dependent drugs, bug and disease, specific PK/PD targets have been determined. For time-dependent drugs, the time that the free concentration is sustained above the MIC ($T_{>MIC}$) is the best predictor of efficacy, and, depending on the drug, it is generally recommended that $T_{>MIC}$ is achieved for 30-70% of a dosing interval (10). It should be emphasized that PK/PD relationships are generally reported using plasma pharmacokinetics rather than tissue pharmacokinetics(11).

Different inhibitory effects on microorganisms that persist after antimicrobial drug exposure have been described(1, 2, 5, 12, 13); the post-antibiotic effect (PAE), the post-antibiotic sub-MIC effect (PAE-SME) and post-antibiotic leucocyte enhancement (PALE). Briefly, PAE is the phenomenon of persistent suppression of bacterial regrowth after intermittent drug exposure, PAE-SME describes the effect of an enhanced PAE at sub-MIC concentrations, while PALE describes the increased susceptibility of bacteria in PAE phase to human leucocytes(1, 2, 5, 12, 13). A detailed description of these parameters is beyond the scope of this work.

These persistent effects can be used to further subdivide the two primary patterns of bactericidal activity into three distinct groups(5, 13). The first group exhibits concentration-dependent killing with moderate to prolonged persistent effects. Group two and three both exhibit time-dependent killing, but differ with respect to the persistent effects. Group two displays limited or no persistent effects, whereas in the third group, moderate to prolonged persistent effects are present(5). Beta-lactams and thus cefuroxime belong to the second group.

On a more general level, antibacterials can be separated into two groups; bacteriostatic and bactericidal drugs. This distinction is based on the ability to kill bacteria, i.e. bactericidal drugs kill

bacteria, whereas bacteriostatic drugs only inhibit bacterial growth. This distinction suggests that bactericidal drugs are more efficacious than bacteriostatic drugs, but in a clinical setting, no such difference has been confirmed(14).

Obviously, the different patterns of antimicrobial activity have significant clinical impact with respect to selecting optimal dosing regimens(5).

1.2 Tissue distribution of antimicrobials

For the majority of bacterial infections, the pathogen resides in the interstitial space of a solid tissue. Nevertheless, inference on dosing regimens is commonly based on plasma PK/PD indices(11, 15, 16). Indeed, the unbound plasma concentration was previously, and in many situations, it still is considered to provide a solid surrogate marker for the unbound interstitial tissue concentration(5, 11, 15, 17, 18). During the last two decades, however, a growing number of studies have found incomplete tissue penetration for different combinations of drug and tissue under both physiological and pathological conditions(19-25). For other combinations, tissue concentrations have exceeded unbound plasma concentrations(26, 27). These findings indicate that a homogenous tissue-plasma distribution cannot be taken for granted, and it can be speculated if incomplete tissue distribution may account for some therapeutic failures. Accordingly, clinical studies on beta-lactam drugs have suggested that aggressive plasma targets of 100% $T_{>1-5\times MIC}$ are more predictive of a successful outcome than traditional targets(28-30). Application of aggressive targets like these obviously reduces the risk of insufficient exposure at the target site due to incomplete tissue penetration. In any case, it seems reasonable to characterize not only the pharmacokinetics of a drug in a specific tissue, but also for specific conditions that may affect tissue penetration. Accordingly, the US Food and Drug Administration (US FDA) recommends tissue distribution studies to be part of antimicrobial drug development(31, 32).

Recognizing the importance of antimicrobial tissue penetration, a number of different techniques have been applied over the years in order to estimate tissue concentrations of antimicrobials. Examples include the skin blister method(33, 34), concentration measurements in wound exudates(35, 36), tissue (37, 38) and fibrin clots(39). These methods are considered to share notable methodological limitations, and they are often lacking a pathophysiological counterpart(15, 40). A major disadvantage of the skin blister technique is that the drug concentration has been shown to

vary with size and surface area to volume ratio(41, 42). The tissue specimen method, which almost exclusively has been the method choice for bone(43), also suffers from a number of limitations. This approach includes a homogenization procedure of the entire tissue specimen. The antimicrobial concentration is then measured in the homogenate, not appreciating the fact that tissues comprise a number of different compartments (i.e. interstitial space, cells, organelles within the cells and possibly remaining blood). As explained above, the unbound fraction of antimicrobials is considered to be pharmaceutically active for non-intracellular infections(1). Hence, application of this approach for a drug that accumulates intracellularly will lead to an overestimation of the unbound extracellular fraction, and vice versa for a drug that mainly distributes to the interstitial space. Due to the inherent invasiveness, it is generally only possible to harvest one or a rather limited number of specimens resulting in poor temporal resolution. Additionally, concentrations are reported per weight and not per volume. Consequently, PK parameters obtained by means of tissue specimens cannot straightforwardly be related to relevant PK/PD indices. Based on similar considerations, it has been advocated that pharmacokinetic data obtained by means of tissue specimens may be misrepresentative and at worst harmful to the patients(44, 45).

1.2.1 The particular case of antimicrobial bone penetration

The vast majority of data on bone pharmacokinetics of antimicrobials originates from bone specimen studies. In 2009, Landersdorfer et al. reviewed antimicrobial bone penetration studies published between 1997 and 2007 focusing on bone specimen studies(43). The bone specimen technique suffers from the same methodological limitations as described above(43, 45). Moreover, Landersdorfer et al. noted a lack of standardised procedures in terms of sample preparation, drug analysis, data handling and reporting, further contributing to the difficult interpretation of the results(43). Indeed, a considerable variability between drugs and between studies of the same drug was found(43). Nevertheless, for most of the investigated drugs, incomplete bone penetration was found as expressed by a bone/serum concentration ratio of less than 1.

Compared to well-vascularised organs like the kidneys and the lungs, bone blood flow is poor(43, 46). Osteomyelitis and implant-associated infections (IAI) are difficult to treat. Despite extensive surgical debridement and removal of implants, long lasting antimicrobial therapy is needed for therapeutic success. Combined with the findings of bone specimen studies, these circumstances suggest that bone penetration of antimicrobials may be incomplete.

In order to increase the limited current knowledge on bone pharmacokinetics, it appears that identification, validation and implementation of new techniques are warranted. The well-known probe-based pharmacokinetic tool microdialysis (MD) represents a potential candidate. A significant advantage of MD is that only the extracellular unbound fraction of a drug is measured. When the present PhD project commenced, MD had successfully been applied for measurement of gentamycin and linezolid in bone in a series of experimental studies (47-50), and for measurement of daptomycin, fosfomycin and linezolid in a clinical setting(26, 51, 52). The experimental studies suggested that MD is a suitable method for measurements of gentamycin in bone. In another approach, radiolabeled antimicrobials in bone are quantified using positron emission tomography(53, 54). This method lacks the ability to distinguish intracellular from extracellular concentrations, but concentration-changes over time may be assessed. Irrespective of approach, the lack of a solid gold standard presents a challenge for development and validation of new methods for measurement of antimicrobials in bone.

1.3 Cefuroxime

Cefuroxime is a second-generation semisynthetic cephalosporin(55). More than 90% of a dose is excreted in the urine, and it is recommended that dose is reduced for creatinine clearances of less than 20 ml/min(56-58). Protein binding and half-life are generally reported to be in the range of 33-50% and 60-90 minutes, respectively(56-60). Cefuroxime exerts its bactericidal effect by inhibiting peptidoglycan synthesis, which leads to disruption of the bacterial cell wall and hence bacterial death(57). Like other beta-lactams, the bactericidal activity of cefuroxime is well established to be time-dependent. Cefuroxime is widely used as antimicrobial prophylaxis for orthopaedic procedures because it is effective against a broad spectrum of both gram-positive and gram-negative pathogens including those most frequently encountered for prosthetic infections (Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli)(61). Based primarily on findings from *in vitro* and animal infection models, $T_{>MIC}$ targets for cephalosporins are reported to be in the range of 40-70%(10, 56, 62-64). $T_{>MIC}$ of approximately 40% generally leads to bacteriostasis, while targets of 60-70% are needed for maximum bactericidal effect. In recent clinical studies, however, more aggressive targets of 100% $T_{>1.5\timesMIC}$ have been associated with improved outcomes for cefepime, ceftazidime and meropenem(28-30). In accordance with this, targets of 100% $T_{>MIC}$ are widely

adopted as thresholds for dose increase in intensive care units where therapeutic drug monitoring of beta-lactams is routinely used(65).

Cefuroxime is manufactured and delivered as cefuroxime sodium. It should be stored below 25°C and protected from sunlight(57, 58). Before administration, cefuroxime sodium is to be reconstituted in a relevant solution(57, 58). After reconstitution, various manufacturers report stability up to 12-24 hours at room temperature, but stability of more than 24 hours at room temperature has been demonstrated(55). Long stability in plasma has also been demonstrated(66). Various analytical methods have been used to quantify cefuroxime. These include microbiological assays, high performance liquid chromatography coupled with UV detection(67) and liquid chromatography coupled with mass- and tandem mass spectroscopy(66). The modern methods are superior in terms of analysis time, sensitivity, accuracy and precision, which are important advantages in MD pharmacokinetic studies and therapeutic drug monitoring.

In Denmark, cefuroxime is widely used in orthopaedic surgery, and is, for example, currently recommended as antimicrobial prophylaxis in total hip replacements(68). All available studies on cefuroxime bone penetration have used the bone specimen method(69-73). Bone/serum concentration ratios in the range of 0.09-0.55 have been found. However, these studies all suffer from the inherent methodological limitations of the bone specimen method, and therefore, the results are difficult to interpret and they may be unsuited for clinical application.

As for other beta-lactams, the combination of time-dependency and short half-life suggests that extended (EI) or continuous (CI) infusion of cefuroxime may be favourable compared to short-term infusion (STI) in terms of $T_{>MIC}$, and thus clinical efficacy. Despite the methodological limitations, the bone specimen pharmacokinetic studies on cefuroxime indicate incomplete bone penetration of the drug. CI of cefuroxime may improve bone penetration because the maintenance of a steady state concentration provides longer time for plasma/bone equilibration, which may be reached too late or not at all with STI. The excellent stability of cefuroxime also makes it well suited for a CI approach. Convincing evidence for the clinical superiority of EI or CI of beta-lactams with short half-lives is, however, still lacking(74-79). This may be related to the fact that in the majority of studies, the total daily dose was reduced for patients receiving EI or CI(75-77). In a subset of RCTs with equivalent doses in the two intervention arms, clinical failure was lower for patients treated

with CI(75). Another factor that may contribute to the limited evidence supporting a CI approach is the potentially minimal "gap" between steady state concentrations and MICs. Given the substantial inter-individual variation observed for beta-lactams, particularly in critically ill patients (80, 81), combined with potential incomplete tissue penetration(22, 23), it is not surprising that therapeutic concentrations may not be reached at the target site for all patients, especially when the total daily dose is reduced. Based on these speculations, it seems rational that selection of dosing regimens for RCTs comparing STI and CI should be guided by findings from tissue pharmacokinetic studies. Today, no studies have assessed bone pharmacokinetics of cefuroxime administered as CI.

1.4 Objectives and hypotheses

The overall objective of this PhD project was to validate and apply MD for *in vivo* measurements of cefuroxime in subcutaneous tissue (SCT), cancellous and cortical bone, and ultimately application in a clinical setting. This would allow for estimation of key PK parameters and $T_{>MIC}$ in the tissues of interest. In turn, STI could be compared to CI in terms of tissue $T_{>MIC}$, and the probability of attaining relevant pre-specified targets could be calculated. These objectives called for a 3-step approach comprising in vitro experiments, experimental studies and a clinical study, with each separate step depending on the preceding. The specific hypotheses of the three studies were

Study 1

- *In vitro* part of the study
 - Relative recovery (RR) of cefuroxime by gain (RR_{gain}) equals relative recovery by loss (RR_{loss}).
 - RR of cefuroxime is independent of the concentration over a relevant concentration range.
 - The effect of physiological temperature changes on RR of cefuroxime is negligible.
- *In vivo* part of the study
 - RR of cefuroxime remains constant over an adequate period of time.
 - Sealing of cortical drill holes is unnecessary, indirectly indicating that MD measurements of cefuroxime in these drill holes reflect the actual concentration in the cortical bone.
 - Bone penetration of cefuroxime is incomplete.

Study 2

- CI of cefuroxime increases T_{>MIC} for relevant MICs in plasma, SCT and bone in pigs compared to STI.
- CI of cefuroxime improves bone penetration of cefuroxime compared to STI

Study 3

- CI of cefuroxime increases the probability of attaining relevant T_{>MIC} in plasma, SCT and bone in patients undergoing total knee replacement (TKR) compared to STI.
- CI of cefuroxime improves bone penetration of cefuroxime compared to STI

2 Materials and methods

In this chapter, the materials and methods on which this thesis is based will be presented. Briefly, MD was used as a tool to sample cefuroxime in subcutaneous tissue and bone. Cefuroxime was quantified using an ultra high performance liquid chromatography (UHPLC) method with UV detection. This setup was applied for *in vitro* feasibility assessment, in an experimental setting and in a clinical study. In the *in vivo* studies, the drug concentration data were analysed using either a non-compartmental- or a population PK approach. This chapter will outline the basic principles of the methods with focus on the advantages, weaknesses and limitations of the various methods in relation to the specific application. A detailed description of the more practical application of the methods can be found in the separate papers in the appendix.

2.1 Microdialysis

Microdialysis is a minimal invasive probe-based technique that allows for continuous sampling of non-protein-bound water-soluble molecules in the interstitial space of a variety of tissues(22, 48, 51, 52, 82, 83). A number of probe-designs exist, but in clinical pharmacokinetic research, the concentric design is frequently preferred(23, 25, 51, 84-86). The sampling (or delivery) of molecules occurs as diffusion along the concentration gradient across a semipermeable membrane at the tip of the probe (see fig 1).



Figure 1. Schematic drawing of a microdialysis probe illustrating diffusion of molecules across the semipermeable membrane at the tip of the probe. Reprinted from Wikipedia (http://en.wikipedia.org/wiki/Microdialysis#mediaviewer/File:Schematic illustration of a microdialysis probe.png)

Under experimental conditions, the probe is continuously perfused with a physiologically compatible solution. The solution that exists the probe (referred to as the dialysate) can be collected for immediate analysis or stored for late use. Given that the probe is continuously perfused, concentration equilibrium will never occur. Consequently, the concentration of molecules or compounds in the dialysate will only represent a fraction of the concentration in the investigated tissue. This fraction is referred to as relative recovery (RR). In some studies, relative changes from baseline may provide adequate information, but in antimicrobial pharmacokinetic studies, estimation of absolute tissue concentrations is generally of essence. Absolute tissue concentrations can only be calculated by correcting the measured concentrations for RR. RR can be determined by various routine and well-described calibrations methods, see below. Consequently, implementation of a calibration procedure is imperative in MD pharmacokinetic studies.

A typical MD system in antimicrobial studies consists of a MD probe, a precision pump and perfusion fluid. Apart from probe design, MD catheters differ with respect to dimensions (shaft, inlet and outlet etc.), composition and size of the semipermeable membrane. The precision pump is to provide a constant and exact perfusate flow (typically in the range of $0.1 - 5 \mu$ L/min) through the probe. The composition of the perfusate can be varied according to objective, but it is generally recommended that it mimics the ionic composition of the interstitial space surrounding the probe(87, 88). In the present PhD project, CMA 107 precision pumps (μ -Dialysis AB, Stockholm, Sweden) and CMA 63 catheters (membrane length 10 mm, molecular cut-off 20 kilo Daltons) were used throughout all studies.

Insertion of a MD catheter into a tissue of interest will inevitably traumatize the tissue, and this may affect the subsequent measurements. For a variety of tissues, elevations of markers of tissue trauma and changes in local blood circulation have been described(15, 87, 88). These alterations usually return to baseline within 30-60 min. The alterations associated with insertion of a MD probe in bone tissue have not been described.

It appears that MD is merely a sampling technique that has to be linked to an appropriate analytical assay. The inherently low volumes and low concentrations calls for very sensitive, accurate, and precise assays with low volume demands. In the present PhD project, cefuroxime was quantified using an UHPLC method with UV-detection. A presentation of this method and considerations on analytical aspects in MD pharmacokinetic experiments can be found in section 2.2.

2.1.1. Probe recovery and calibration methods

RR has been shown to be dependent on experimental conditions, properties of the analyte of interest and tissue specific properties. Among others, experimental conditions that affect recovery include perfusion rate, composition of the perfusate, area of the semipermeable membrane and temperature(15, 88, 89). Physiochemical properties of the analyte and changes in the peri-probe environment may affect the diffusion coefficient in the interstitial space and hence also recovery(15, 88, 89). Importantly, it should be emphasized that RR is independent of the concentration gradient across the semipermeable membrane(15, 88, 89). It appears that some of the experimental conditions can be adjusted in order to achieve a RR that matches specific

experimental needs. On the other hand, changes in the peri-probe interstitial space may also cause changes in the diffusion coefficient leading to unwanted changes in RR(90, 91). A number of methods can be used to assess probe RR. They all rely on the assumption that RR_{gain} equals RR_{loss} . RR_{gain} and RR_{loss} can be calculated using the following equations

$$RR_{gain} = C_{out}/C_m \tag{1}$$

$$RR_{loss} = 1 - \frac{c_{out}}{c_{in}} \tag{2}$$

where C_{in} is the concentration in the perfusate, C_{out} the concentration the dialysate and C_m the concentration in the media surrounding the probe. Equation 1 relies on the assumption that $C_{in} = 0$, while equation 2 relies on the assumption that $C_m = 0$. In turn, absolute tissue concentrations can be calculated as

$$C_{tissue} = \left(\frac{C_{out}}{RR}\right) \tag{3}$$

Frequently used calibration methods include the no-net-flux method, the low-flow-rate method, and retrodialysis by calibrator or by drug (15, 88, 89). In pharmacokinetic studies, the most commonly used method is retrodialysis(22, 23, 25, 51, 86, 92). Retrodialysis by drug, which has been used in the present PhD project, can be performed either at the beginning or at the end of the experiment by adding a known concentration of drug to the perfusate. The concentration in the dialysate can be quantified, and thus RR_{loss} can be calculated according to equation 2. The method was originally proposed by Stahle et al.(93). Compared to other methods, it is advantageous that RR determination is 1) based on the drug of interest and 2) that the procedures are simple and not very time-consuming. On the other hand, possible changes in RR over time cannot be assessed. Moreover, if performed at the beginning of the experiment, a washout period is needed to prevent spill over of drug to the actual experiment, whereas if performed at the end of the experiment, remnants of drug from the experiment may still be present in the tissue, violating the assumption that $C_m = 0$. If the difference between C_{in} and C_m is large, C_m may be neglected resulting only in a minimal error(23, 94).

Advantages

Compared to other methods for determination of tissue pharmacokinetics, MD has the important advantage of measuring the extracellular unbound fraction of the drug, which is known to be pharmaceutically active(1). Consequently, drug pharmacokinetics can be compared directly to relevant PK/PD indices. Moreover, the ability to continuously measure the drug of interest provides relatively high-resolution concentration-time profiles compared to other approaches like tissue specimens. This provides more solid data and reduces the number of patients/experimental animals needed.

Weaknesses and limitations

It is generally recognized that a compromise between the ideal setup and experimental requirements is often unavoidable in MD studies. An important factor that may be compromised by experimental needs is RR. The correction of the measured concentrations for RR will lead to a magnification of the variations associated with the pre-analytical sample handling and the chemical assay. This magnification will increase exponentially with decreasing recovery. Consequently, measures should be taken to maintain RR as high as possible. Experimental factors that contribute to lower the RR are short membrane length and high perfusion flow. A short membrane length may be needed because of limitations in space, for example due to anatomical factors. When frequent sampling is needed to achieve high temporal resolution as in the case of short half-lived drugs, flow rate has to be relatively high to produce a sufficient volume of dialysate for the chemical analysis.

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. Additionally, low volumes and low concentrations provide a need for a sensitive assay with low volume demands. Newer analytical methods have improved this aspect of MD pharmacokinetic studies significantly, but it is still important to integrate information about the performance of the available analytical assay in the adjustment of experimental factors like flow rate, membrane length and sampling interval in order to achieve a feasible methodological setup(15).

As dialysates are continuously gathered, the concentration in the dialysates represents the average concentration in the tissue during the sample period. The actual measured concentration is commonly attributed to the midpoint of the sampling interval, but this remains a simplification.

Currently, MD sampling is almost restricted to water-soluble molecules with limited molecular sizes. This is explained by the fact that the aqueous perfusates are incompatible with lipophilic molecules that also tend to stick to the tubing and probe components, while large pore-size membranes suited for macromolecules and proteins cause excessive fluid shift(15, 87, 88)

Given the solid nature of bone, MD probes cannot be introduced using a standard introducer. This obstacle has been overcome by introducing the probes into drill holes in the bone(26, 47-52). This approach fosters the question whether MD measurements obtained in drill holes in bone really do reflect bone concentrations, or rather a combined concentration derived partly from the presence in the bone, and partly from the presence in the adjacent soft tissues. Furthermore, it is a challenge to generate and document that drill holes in cortical bone are strictly intra-cortical, and that the MD probes are not dislocated during the study period. Particularly in a clinical setting, where immobilization may be unethical, dislocation of the probes poses a considerable risk. Further considerations on MD measurements in drill holes in bone can be found in section 2.4.

2.2 Ultra High Performance Liquid Chromatography

As outlined above, an appropriate analytical assay is a prerequisite for a successful MD pharmacokinetic study. Consequently, assessment of the performance of the assay of choice should be conducted prior to the actual pharmacokinetic experiment. This is important not only in order to evaluate feasibility of the experiment, but also in order to judge the resulting findings.

In the present PhD thesis, an *Ultra High Performance Liquid Chromatography* (UHPLC) method with UV detection at 275 nm was applied for quantification of cefuroxime in plasma and dialysates. The method was validated according to selected relevant recommendations set forth in the US FDAs "Guidance for Industry: Bioanalytical Method"(95). More specifically, method validation was performed with respect to selectivity, linearity, precision, accuracy, lower limit of quantification (LLOQ), stability and recovery (M. Tøttrup and TF. Hardlei, unpublished data). The practical steps of the analysis are described in paper 1. Quality controls (QC) were included in all runs. Briefly, the standard volume demand is 15 μ L, and the overall chromatographic run time was 3.5 min, which resulted in 4.5 min between each injection. In the following, selected elements in the validation will be discussed focusing on aspects of particular importance for the present application.

Selectivity

Selectivity was evaluated by analyzing plasma samples spiked with components, suspected to interfere with the assay. The investigated compounds were: Amoxicillin, acetylsalicylic acid, salicylic acid, paracetamol, piperacillin, benzylpencillin, phenoxymethylpencillin, dicloxacillin, oxacillin, moxifloxacin, ciprofloxacin and ampicillin. Additionally, 5 samples from healthy donors were investigated in order to elucidate whether endogenous compounds in human plasma would interfere. There was no chromatographic interference between any of the tested compounds suspected to interfere with the assay, and no endogenous compounds in blank human plasma, were found to elute as did cefuroxime or the internal standard (IS) ceftriaxone. Figure 2 and 3 represents typical chromatograms for the measurement of the concentration of cefuroxime in dialysate and the free plasma concentration. Cefuroxime and the internal standard ceftriaxone were separated from unspecific matrix compounds, and showed to elute as two distinct, narrow and symmetric peaks



Figure 2. Dialysate. 1, ceftriaxone (IS); 2, cefuroxime



Precision, accuracy and lower limit of quantification

Intra- and interrun precision for the quantification of cefuroxime in dialysates was assessed at three different concentrations that were expected to cover a relevant range of concentrations. Each concentration was measured four times in one batch on 5 different days. Precisions are given as per cent coefficients of variations (CV), and can be found in table 1. A LLOQ of 0.06 µg/mL was considered to be sufficient. At this level a CV of 7.9% confirmed a LLOQ of at least 0.06 µg/mL for this assay. Analytical accuracy estimated by measurements of cefuroxime formulations with a nominal concentration of 5 μ g/mL, was found to be in the range; -3,3 - 5,8 %.

Nominal conc.	Intrarun precision	Nominal conc.	Interrun precision
$(\mu g/mL)$	(CV%)	(µg/mL)	(CV%)
0.25	5.6	0.25	6.8
2.5	4.3	2.5	4.7
10	2.6	10	2.8

 Table 1. Precision for dialysate measurements

Stability of cefuroxime

The stability of cefuroxime in plasma during freeze/thaw cycles and during different storage conditions is adequate for the present assay, and has previously been well documented(66). Stability of cefuroxime in plasma for up to 48 hours at 5°C would, however, allow for convenient handling-protocols. Consequently this storage condition was evaluated for a cefuroxime plasma concentration of 52 μ g/mL. Additionally, the stability of cefuroxime in 0.9% NaCl spiked to a concentration of 0.38 μ g/mL was assessed at 22°C, 5°C and – 20°C, as these conditions were considered to reflect likely storage and working conditions. Except for cefuroxime stability in 0.9% NaCl after 48 hours at 22° C, no significant degradation of cefuroxime was found under the specified conditions. Acceptable stability was defined as a difference between the baseline measurements and measurement of the stored samples of less than the intrarun precisions of the baseline concentrations.

In summary, the presented UHPLC-UV method is specific, sensitive and accurate, and thus a reliable choice for quantification of cefuroxime in dialysates. Previously described chromatographic UV-detection methods, showed LLOQs between 0.1 μ g/mL for dialysate(83) and 2 μ g/mL for plasma(96). This new UHPLC-method showed chromatographic profiles with narrow symmetrical peaks, with a baseline width of about 0.1 min for both plasma and dialysate. Other published chromatographic methods showed considerably wider peaks, demonstrating that UHPLC-analysis reduces the risk of interference from other compounds in the sample matrix(96, 97). The stability of cefuroxime was found to be fully compatible with the present method.

Limitations and weaknesses

Depending on sampling interval and perfusion rate, dialysate volumes in MD pharmacokinetic studies are generally less than 80 μ L, and often in the range of 20 – 40 μ L. In the present study, dialysate volumes ranged from 40 - 60 μ L. Given the standard volume demand of 15 μ L of

dialysate, and the fact that not all dialysate can be de-pipetted from the microvials, only a limited number of analyses can be conducted. Pipetting of volumes in the present ranges is demanding, and even small deviations will affect the end result.

2.3 In vitro feasibility assessment

In order to assess the feasibility of using MD as a sampling tool for cefuroxime, a number of *in vitro* experiments were conducted. A selection of the results of these experiments can be found in paper I. The purpose of the experiments was to confirm that RR_{gain} equals RR_{loss}, that cefuroxime did not adhere to the tubings of the probe, that movement of cefuroxime across the semipermeable membrane of the probe was fast, and that RR remains constant over a relevant range of concentrations. These matters are prerequisites for performing measurements of transient compound concentrations and calibrating probes by means of retrodialysis, as it was planned in the present series of studies(87). Moreover, the effect of temperature on RR was assessed, as physiological temperature changes are likely to occur during an experiment, particularly when measurements are performed on extremities. These experiments were also used to identify a suitable flow rate.

The in vitro experiments were conducted in 0.9% NaCl spiked to concentrations of 1, 10 and 50 μ g/mL. The investigated temperatures were 22, 30 and 40 °C.

Limitations and weaknesses

As discussed previously, the coefficient of diffusion in the medium or tissue surrounding the probe is partly decisive of RR. Additionally, inter-probe RR variations are well known(98). Therefore, in vitro assessment of recovery cannot replace *in vivo* calibration. Due to the inter-probe variation it is also imperative that *in vivo* RR is determined separately for each probe even if they are placed in identical tissues.

2.4 The porcine model

It was decided to make the initial *in vivo* applications of our methodological setup in porcine experiments. This decision was governed by the following considerations. Firstly, it had to be assessed whether RR remained constant for a sufficient period of time, i.e. for the entire observation time of eight hours corresponding to a standard dosing interval of cefuroxime. If this was not the

case, retrodialysis would be unsuited for calibration. Secondly, it had to be evaluated whether concentrations measured in drill holes in bone reflected the actual concentration in the bone satisfactorily (see below). A safe methodological setup for investigating this issue in a clinical setting could not be identified. Thirdly, based on the lack of experience with MD pharmacokinetic studies, it appeared most ethically sound to explore the basic concepts of bone pharmacokinetics and different dosing regimens in an animal model. The experiences and results of these experiments were expected to lead to an optimized design of the planned clinical trial.

A porcine model was chosen because pigs have been shown to resemble man in terms of physiology(99). Pig bone composition, density and quality have also been found to compare reasonably with that of man(100). Additionally, a long tradition of conducting pig experiments has resulted in excellent local facilities for this purpose. The experiments were approved by The Danish Animal Experiments Inspectorate and carried out in accordance with existing laws.

MD measurements of cefuroxime in drill holes in bone

Introduction of MD probes into bone tissue requires creation of drill holes, in which the probes can be placed. The diameter of the drill holes must exceed that of the probe (0.6 mm) in order to position the probe without damaging the membrane. Additionally, there is a considerable risk of breaking the drill when drilling in cortical bone. Particularly in a clinical setting this should be avoided. After thorough considerations, trade-off was a 2 mm (i.e. diameter of 2 mm) drill. The difference in diameter between the drill holes and the probes results in a "dead space" around the catheter. The question was if the concentrations measured in such a drill hole dead space would be reflective of the actual concentration in the bone. In case of higher concentrations in the adjacent soft tissue, this may lead to overestimation of the actual bone concentration, whereas lower concentrations in the surroundings would lead to the opposite. The former scenario was considered most likely. However, the basic law of diffusion states that diffusion time increases proportionally with the square of the distance, making a significant contribution from the surroundings unlikely considering the much larger diffusion distance from the surroundings to the membrane compared to the distance from the bone the membrane. In 2011, Bøgehøj et al. assessed this issue by comparing measurements of metabolites in a blood clot with measurements obtained in a drill hole in the femoral head of a minipig(101). It was concluded that the measurement in bone reflected bone metabolism whereas in the blood clot, a clear washout pattern was found. It was decided to further

address the issue by comparing cefuroxime concentrations between sealed and unsealed drill holes in cortical bone. Bone wax was considered an appropriate material for such an experimental sealing procedure. As a paired design is statistically stronger than a non-paired, and thus more likely to demonstrate a significant difference, this was chosen. The paired drill holes were created bilaterally in the tibiae, and it was decided to create two symmetric pairs in each pig in order to reduce the number of animals. The creation of two drill holes in close vicinity could potentially reduce bone penetration of cefuroxime, which could theoretically increase a potential concentration gradient between the drill holes and the surroundings. However, a finding of no difference between sealed and unsealed drill holes would only be strengthened by the latter. Each symmetric pair of drill holes was randomly allocated to sealing of either the left or the right drill hole.

Verification of drill hole location and location of probes

The thickness of cortical bone is limited. An anatomical location where bone was easily accessible and had a thick cortex was needed. The best-suited bone was the tibia, which has a rather thick cortex at its anterior margin. Nevertheless, even at this location, a limited and rather challenging amount of bone is present. The depth of cortical drill holes is limited to approximately 16-18 mm, while only 1-2 mm of cortical bone will be present adjacent to a drill hole with a diameter of 2 mm. Obviously, only a few degrees deviation could lead to penetration to either the surroundings or to the bone marrow. Consequently, post-mortem CT scans of all bones with intra-cortical drill holes were conducted. At the end of all experiments, it was assessed by autopsy that the probe had not been displaced from the drill hole.

Limitations and weaknesses

Despite the fact that pigs resemble man with respect to physiology and anatomy, the foremost limitation of the present porcine model is that pigs remain a different species than humans. Whereas the findings in different infectious models (i.e. drug – bug interaction for a specific infection) to some extent may be generalized to a clinical setting(102), this is not the case for PK studies. Even small interspecies PK differences will have an impact on the probability of attaining desired PK/PD targets.

Preventing displacement of probes in awake and freely moving animals would have been an extremely challenging, and most likely an impossible task. In agreement with other authors(47-50,

82, 88), the animals were therefore kept under general anaesthesia during the entire measurement period. General anaesthesia is known to cause physiological alterations, which may in turn affect pharmacokinetics. General anaesthesia also limits the length of the experiments. However, for a short half-lived drug like cefuroxime no different steady state is likely to be achieved after multiple doses.

An animal weight resembling that of the average human being was chosen. However this animal weight corresponds to juvenile pigs. Again, this may limit generalizability.

2.5 The clinical total knee replacement model

Several different patient categories were considered for a clinical application of the methodological setup. Prompted by differences in cortical and cancellous bone penetration of cefuroxime in the experimental studies, it was also decided to conduct separate measurements in these two compartments in the clinical setting. As a result of this decision, a patient category had to be identified, in which bone with a thick and easily accessible cortex was available. Another very important prerequisite for the feasibility of such a study was to ascertain that enough patients could be included within a foreseeable period of time. After thorough considerations, male patients scheduled for a total knee replacement (TKR) were chosen, as this patient category fulfilled both criteria. Well aware that it would limit the subsequent generalizability, it was decided only to include males in order to safely create intra-cortical drill holes. Inclusion and exclusion criteria are listed in paper III. Using an open-labelled randomised controlled design, the concept of CI vs. STI was pursued again. Block randomisation was applied with blocks of 6 patients, and an allocation ratio of 1:1. The investigators were blinded during the surgical procedures and placement of the MD probes, while the actual administration of cefuroxime was unblinded. This approach was chosen so that placement of the MD probes during surgery would not be affected by the mode of cefuroxime administration. Based on a sample size calculation (see section: 2.6), a total of 18 patients were included. The total cefuroxime dose was equivalent in both intervention arms. At the department of Orthopaedic Surgery, Horsens Regional Hospital, approximately 70-80 male patients undergo TKR each year. As such, it was anticipated that all patients could be included within 1 year. The primary outcome was the key PK/PD index for cefuroxime T_{>MIC}. Based on this measure, the probability of attaining specified targets could be determined for various MICs.

In order not to overheat the bone, which potentially could cause necrosis, drilling was ceased every few seconds, and water was continuously applied. Additionally, a brand new drill was used for each patient.

At the end of the TKR, a combination 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 around the knee, intraarticularly and in the posterior joint capsule of the knee. This is a standard procedure following TKR, which provides excellent pain relief, and for this reason, the procedure was maintained in the study. However, it cannot be ruled out that the vasoconstrictive effect of adrenaline may affect at least the SCT pharmacokinetics. Accordingly, the findings in SCT are at least reflecting circumstances where SCT infiltration with adrenaline is being used, whereas a conclusion concerning SCT in general must await further studies.

Before removal of the probes, a CT scan of the cortical drill hole was conducted in order to verify that the drill hole had not penetrated to the bone marrow, and that the catheter had not been displaced. This procedure was considered mandatory as no restriction to mobilization was dictated by the study protocol.

For practical reasons, the cancellous bone probes had to enter the bone via the knee joint. This raised a concern for an increased risk of prosthetic infection, one of the most serious complications of TKR. However, intraarticular drains are routinely used in TKR. It has been shown that bacteria cannot be cultured from the tip of drains removed after 24 hours(103), and generally, MD related infections are not reported in clinical MD studies(25, 26, 51, 52, 84, 85, 104, 105). In order to minimize the risk of infection, all probes were tunnelated 2-3 cm. Altogether, the risk of infection was considered negligible. Nevertheless, the average risk of prosthetic infection following TKR is reported to be approximately 1-2%(106-108). Accordingly, there was approximately an 18-36% chance that one of the study participants would acquire a prosthetic infection.

The study was approved by the Ethics Committee of the Central Denmark Region (registration number 1-10-72-161-13) and the Danish Health and Medicines Authority (EudraCT number 2013-001138-17). The study was conducted in accordance with the Declaration of Helsinki and the ICH Harmonised Tripartite Guideline for Good Clinical Practice. The GCP unit at Aalborg and Aarhus

University Hospitals conducted the mandatory monitoring procedures. The results of the study are reported according to the CONSORT recommendations for reporting randomized trials (http://www.consort-statement.org)

Limitations and weaknesses

The major limitation of this model is that male patients having a TKR are a rather selected population. For obvious practical reasons, measurements could only be conducted postoperatively, and not prior to and during surgery. Consequently, generalization to the pre/perioperative administration of antimicrobials, and to the average population, is debateable, while generalization to osteomyelitis and IAI should be avoided. Moreover, the anatomical area in which the measurements were obtained had been subjected to a substantial surgical trauma, and adrenaline had been injected locally as described above. While these factors obviously are reflective of the study population, they may not be for other clinical situations.

2.6 Statistical analysis

Different approaches can be used to analyse PK data. In the present PhD project, noncompartmental analysis (NCA) and population PK modelling have been applied. In this section, the basic principles of these approaches will be outlined. Additionally, considerations on sample size calculation will be presented.

2.6.1 Non-compartmental analysis

In NCA, key PK parameters (such as AUC, elimination rate constant etc.) are calculated from the individual concentration – time profiles for the different compartments. Subsequently, descriptive and comparative statistics can be performed, and different measures such as tissue-plasma penetration ratios, clearance and other PK parameters can be calculated. The advantages of NCA are that it does not require the assumptions of compartmental models, and that it is relatively simple to apply(109). On the other hand, the information that can be derived is limited to the actual data, i.e. concentration-time profiles and PK parameters for other dosing regimens cannot be predicted(109). Based on the parameters of a NCA, the major determinant of efficacy for cefuroxime and other beta-lactams, $T_{>MIC}$ (plasma), can be calculated based on the following equation suggested by Turnidge(110):

$$\%T_{>MIC} = \left(\frac{Dose}{V_d \times MIC}\right) \times \frac{T_{1/2}}{\ln(2)} \times \frac{100}{DI}$$

Where ln is the natural logarithm, V_d volume of distribution, $T_{1/2}$ elimination half-life and DI the dosing interval.

However, the equation only applies for bolus or very short infusions with a short distribution phase as it only considers the elimination phase. As such, the equation is not suited for calculation of tissue $T_{>MIC}$ and $T_{>MIC}$ for CI.

Alternatively, $T_{>MIC}$ can be estimated by means of linear interpolation. As indicated by the name, this approach relies on the assumption that the increase or decrease between the coordinates surrounding the point of interest, is linear. This assumption is obviously violated for cefuroxime leading to under- or overestimation of selected points of time. The magnitude of this violation is related to the temporal resolution of sampling. Our dialysates were collected over 30 minutes. The concentration in the dialysates is subsequently attributed to the midpoint of the sampling interval, but this remains a simplification. Particularly if the concentration-time profile crosses the MIC value several times, as it may be the case for CI, the resulting error may be significant. These weaknesses and limitations were considered unacceptable.

 $AUC_{0 - last}$ was computed in Stata (version 12.0; Statacorp, USA) using the linear trapezoidal rule. The AUC is calculated as the sum of each trapezoid. This approach is obviously not exact. The associated error depends on the widths of the trapezoids (i.e. the sampling interval), and the shape of the true concentration-time profile. In the case of first order kinetics, the linear trapezoidal method will overestimate the area during the elimination phase, while the area during the ascending/infusion phase will be underestimated, see figure 4(109). It appears that the relationship between sampling interval and half-life is decisive for size of this estimation error.



Figure 4. Concentration (*C*) versus time during and after infusion. The shaded area represents underestimation of the area during ascending concentrations and overestimation of the area during descending concentrations. By decreasing the sampling interval (Δt), this under- or overestimation of the area is reduced. *Obtained from*(109).

If the drug is not fully eliminated at the end of sampling, different methods exist to extend the curve to infinity and calculate the resulting additional area under the curve. In Stata, three different methods are available; linear fit extension, exponential fit extension and linear extension of the natural log(Stata version 12.0; Statacorp, "*Methods and formulas*"). When NCA was applied in this study, practically no drug was present at the end of sampling, or application of curve extension would not provide extra information. Consequently, curve extension was not applied in the present studies.

The terminal half life ($t_{1/2}$) 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 (Stata version 12.0; Statacorp, *Methods and formulas''*), USA. The appropriate number of points used for the calculation was determined by inspection of the individual concentration-time profiles.

2.6.2 Population pharmacokinetic modelling

As explained above, $T_{>MIC}$ in tissues cannot be accurately determined by simple methods in case of delayed plasma-tissue equilibrium. One way to solve this problem is to apply a population pharmacokinetic approach. In a US FDA Guidance for Industry, population modelling is defined as

"the study of the sources and correlates of variability in drug concentrations among individuals, who are the target patient population receiving clinical relevant doses of a drug of interest(111)". Due to the low number and the homogeneity of study subjects/animals, no attempts were, however, made to identify any covariates potentially influencing cefuroxime pharmacokinetics. The primary objective was to obtain a good description of the drug concentration - data in order to determine good estimates of $T_{>MIC}$ and the PK parameters. Various approaches can be used to fit specific models to drug-concentration data. In the present PhD project, the non-linear mixed effects (NLME) approach was used to fit two-compartment models to the drug concentration data. The equations describing the drug-concentration over time can be found in paper II and III. Both one-and two-compartment models with different kinetics were explored to obtain the best description of the drug-concentration in each tissue. Observed vs. fitted cefuroxime concentrations – plots and individually and population fitted concentration-time profiles were used to assess if the models provided a satisfactory description of the cefuroxime concentration data.

 $T_{>MIC}$, target attainment for specific targets and standard PK parameters can be determined from the equations describing the model. In turn, 95%-confidence intervals for $T_{>MIC}$, probability of target attainment (PTA) and 95%-confidence intervals for the PK parameters can be determined using Monte Carlo Simulations(112, 113).

Unless stated otherwise, $T_{>MIC}$ and thus PTA are commonly considered to reflect a 24-hour steady state situation(114). Our models were based on measurements in newly operated animals/patients. In the immediate postoperative period, PK parameters may be rather versatile. Consequently, prediction of alternative scenarios like a 24-hour steady state situation was considered inappropriate, and it was decided only to simulate the doses that were actually administered. Another uncertainty was related to the selection of tissue $T_{>MIC}$ targets for estimation of PTAs. Specifically, PK/PD relationships are commonly described using plasma and not tissue pharmacokinetics. Moreover, particularly in relation to the prevention of surgical site infections in an orthopaedic setting, tissue targets for cefuroxime and other antimicrobials are unknown(115). Nevertheless, for time-dependent drugs, which are most commonly used for antimicrobial surgical prophylaxis, it is recommended that tissue and plasma concentrations exceed *in vitro* MIC values of relevant pathogens throughout the procedure(115). In the lack of established tissue targets for the present setting, traditional plasma targets were applied.
Population modelling is a rather complex process that requires trained modelling scientists and high computational capacity. Moreover, the available software packages (i.e. NONMEM and R) are not user-friendly. The population pharmacokinetic modelling and Monte Carlo simulations applied in the present PhD project was conducted by associate professor Bo Martin Bibby from department of biostatistics at Århus University sparring with the undersigned. More specifically, the analyses were conducted using the statistical software R (R v 3.0.2, R core team, Vienna, Austria) with the package nlme. In depth description of population modelling is beyond the scope of this thesis, and can be found elsewhere(111, 116, 117).

Population PK modelling generally offers a number of advantages compared to traditional NCA(111, 116, 117). For the present application, the primary advantage was the ability to provide good estimates of $T_{>MIC}$ and PTA. As described above, no other dosing regimens were simulated, as this would have called for additional assumptions, which were not considered justified. The primary weaknesses of the approach is the assumptions (i.e., regarding the distribution of parameter estimates) made during the modelling process(117) and its complexity which calls for trained modellers.

2.6.3 Sample size

In this project, sample size calculations were conducted for studies II and III with respect to the primary endpoint, which was $T_{>MIC}$ in bone. The single-point measurements inherent to the bone specimen method do not, however, allow for estimation $T_{>MIC}$. Estimates of difference and standard deviation were therefore based on transposition/translation (based on an average cefuroxime bone-plasma ratio of 0.2(43)) and visual inspection of cefuroxime plasma concentration-time profiles from a previous pharmacokinetic study of cefuroxime(25). It has to be acknowledged that the bone/plasma ratio is probably not constant throughout the duration of measurements. The above-mentioned manoeuvre suggested a 50% difference in bone $T_{>MIC}$ between CI and STI with a standard deviation of 25% for relevant bacterial MICs. Additionally, it was decided to use a standard alpha of 0.05 and a power of 0.9. Based on these estimates, a sample size of 6 patients in each intervention arm was calculated (Stata, version 12.0; Statacorp, USA). In order to accommodate drop-out of patients and/or catheters and potential non-normality, the sample size in the clinical study was increased to 9 patients in each intervention arm.

3 Summary of papers

Paper 1

"Pharmacokinetics of Cefuroxime in Porcine Cortical and Cancellous Bone Determined by Microdialysis"

This study was separated in an *in vitro* and an *in vivo* part. The *in vitro* part of the study was designed to investigate the basic prerequisites for determining transient cefuroxime concentrations across a relevant range of concentrations using MD, and for calibration by means of retrodialysis. At 30°C, RR_{gain} was compared to RR_{loss} at concentrations of 1, 10 and 50 µg/mL. Moreover, the impact of temperature on RR was investigated by assessing RR_{gain} at three different concentrations (1, 10 and 50 µg/mL) and three different temperatures (22, 30 and 40°C).

In the *in vivo* experiments, the applicability of the retrodialysis method for calibration was further evaluated by assessing the stability of RR_{loss} . This was done by calculating the relation of RR_{loss} to mean RR_{loss} for the entire observation period (RR_{loss}/mRR_{loss}) for every separate probe at each time point. In the subsequent evaluation, data were pooled for all probes and for every distinct anatomical location, respectively. Furthermore, the cefuroxime concentrations obtained in unsealed drill holes in cortical bone were compared to those of bone wax-sealed drill holes in order to assess whether the concentration of cefuroxime in drill holes reflects the actual concentration in bone. Under analogous experimental conditions, but in a separate series of experiments, plasma, SCT and cancellous bone pharmacokinetics of cefuroxime were then assessed. In both series of experiments, a clinical relevant dose of 1,500 mg of cefuroxime was administered, but the observation periods were 5 and 8 hours, respectively.

Results

In vitro part

At 30°C, mean RR_{gain} and RR_{loss} for cefuroxime were 42.1% and 42.9% at 1 µg/mL, 46.0% and 48.8% at 10 µg/mL and 51.3% and 48.0% at 50 µg/mL, respectively. An average increase in

recovery of 7.5% (95%-CI: -2.4 to 17.4, P = 0.12) was found when the temperature was increased from 20°C and 40°C.

In vivo part

Over a 7 hours observation period, the pooled relation of RR_{loss} to mean RR_{loss} fluctuated in the range of 0.96 – 1.04. For the separate tissues, the corresponding ranges were 0.93 – 1.10, 0.93 – 1.12 and 0.92 – 1.12 for subcutaneous tissue cortical and cancellous bone, respectively (see figure 5). The graphical presentation revealed no distinct patterns for the pooled relation or any of the separate anatomical locations.



Figure 5. The relation of RR_{loss}/mRR_{loss} for each anatomical location (dashed lines), and the mean for all locations (solid line). The horizontal solid line represents a relation of 1. Bars represent SEM

The mean concentration-time profiles for sealed (n = 10) and unsealed drill holes (n= 10) are shown in figure 6. The corresponding pharmacokinetic parameters can be found in table 1. Regardless of pharmacokinetic parameter, no differences between sealed and unsealed drill holes could be demonstrated. Additionally, RR was identical for sealed (18.7 \pm 2.5%) and unsealed drill holes (17.7 \pm 1.7%)



Figure 6. Mean concentration-time profiles for sealed an unsealed drill holes. Bars represent SEM

Table 1

Key pharmacokinetic parameters for sealed and unsealed drill holes in cortical bone

Pharmacokinetic parameter	Sealed drill holes	Unsealed drill holes	P-value
AUC_{0-last} (min µg/mL)	868±233	1037±318	0.38
C_{max} (µg/mL)	4.8±1.0	5.7±1.4	0.42
T _{max} (min)	84±12.7	105±11.8	0.33
T _{50% of Cmax} (min)	31.8 ±5.8	28.7±4.7	0.61
t _{1/2} (min)	136±42.0	142.2±59.3	0.93

 C_{max} , peak drug concentration; T_{max} , time to C_{max} ; $T_{50\% \text{ of } Cmax}$, time to 50% of Cmax; $t_{1/2}$, half-life at β - phase; AUC_{0-last}, area under the concentration–time curve from 0 to the last measured value. Values are given as mean±SEM.

Mean concentration-time profiles for plasma, subcutaneous tissue and cancellous bone are depicted in figure 7. As the observation period in cortical bone was only 5 hours, $AUC_{0-5 hour}$ was used to compare the different anatomical locations. The $AUC_{0-5 hours}$ (± SEM) were 6013±1339, 3222±1086, 2232±635 and 952±290 min µg/mL for free plasma, subcutaneous tissue, cancellous and cortical bone, respectively (ANOVA P < 0.01). In a subsequent pairwise comparison, cortical bone AUC was found to be significantly lower than that of cancellous bone (P = 0.04).



Figure 7. Mean concentration-time profiles for plasma, subcutaneous tissue and cancellous bone. Bars represent SEM

Paper 2

"Continuous versus Short-term Infusion of Cefuroxime - Assessment of Concept Based on Plasma, Subcutaneous Tissue and Bone Pharmacokinetics in an Animal Model"

The objective of this study was to compare $T_{>MIC}$ (primary endpoint) and key pharmacokinetic parameters of cefuroxime in plasma, subcutaneous tissue, cancellous and cortical bone after administration of 1,500 mg cefuroxime as either STI or CI. The resulting data were analysed using a population pharmacokinetic approach.

Results

Mean observed concentrations and population fitted concentration-time profiles are depicted in figure 8. Observed vs. fitted cefuroxime concentrations are shown in figure 9. These plots demonstrate that the model provided a satisfactory description of the cefuroxime concentration data.



Figure 8. Mean observed concentrations (dots) and population fitted concentration-time profiles (lines) for short-term infusion (upper plots) and continuous infusion (lower plots).



Figure 9. Observed versus simulated individual- and population cefuroxime concentrations for free plasma, SCT, cortical and cancellous bone.

Mean concentration-time profiles are shown in figure 10. Tissue penetration, defined as the ratio of free AUC_{0-last} tissue to free AUC_{0-last} plasma, was found to be significantly impaired for all tissues in the CI group. The tissue penetration ratios (95%-confidence intervals) were 0.53 (95%-CI: 0.33; 0.84), 0.38 (95%-CI: 0.23; 0.57) and 0.27 (95%-CI: 0.13; 0.48) for subcutaneous tissue, cancellous and cortical bone, respectively. In the STI group, tissue penetration was significantly impaired for cancellous bone (0.61 (95%-CI: 0.51; 0.73)) and cortical (0.45 (95%-CI: 0.36; 0.56)), but not for subcutaneous tissue (0.97 (95%-CI: 0.67; 1.39)). For cancellous bone, the tissue penetration ratio for CI was significantly lower than that of STI (P = 0.02), while for subcutaneous tissue and cortical bone, this relation only just failed to be significantly lower in the CI group (P = 0.05 and 0.07, respectively).



Figure 10. Mean concentration-time profiles for short-term and continuous infusion of cefuroxime for free plasma, SCT, cancellous and cortical bone. Bars represent standard deviations.

The relationships between $T_{>MIC}$ and MIC for free plasma, SCT, cancellous and cortical bone are depicted in figure 11. Significantly longer $T_{>MIC}$ was found for CI up to MICs of 4 µg/mL for plasma, 2 µg/mL for SCT and cancellous bone and 0.5 µg/mL for cortical bone. It should be noted, however, that for MICs of 1 and 1.5 µg/mL, cortical bone $T_{>MIC}$ only just failed to be significantly higher for CI. For higher MICs, the advantage of CI in the tissues is leveled out or reversed. For MICs of 4 µg/mL, significantly higher cancellous and cortical bone $T_{>MIC}$ is achieved with STI.



Figure 11. Time with concentrations above MIC – MIC profiles for free plasma, SCT, cancellous and cortical bone after administration of 1,500 mg cefuroxime as either STI or CI. Measurements were conducted for 8 hours. The dotted lines represent 95%-confidence intervals.

Paper 3

"Bone, Subcutaneous Tissue and Plasma Pharmacokinetics of Cefuroxime in Total Knee Replacement Patients – a Randomized Controlled Trial Comparing Continuous and Short-term Infusion"

The objective of this randomized controlled clinical trial was to compare $T_{>MIC}$ (primary endpoint) and key pharmacokinetic parameters of cefuroxime in plasma, subcutaneous tissue, cortical and cancellous bone after administration of 1,500 mg of cefuroxime as either STI or CI. The resulting data were analysed using a population pharmacokinetic approach. Instead of reporting $T_{>MIC}$, the

probability of attaining specified targets of 65% (low target) and 90% $T_{>MIC}$ (high target) were reported. This seems to be an intuitive and clinically applicable measure.

Results

Eighteen patients (the pre-specified recruitment target) were included in the study, nine in each group. In the STI group, all dialysates from 1 cancellous bone and 1 cortical bone probe had to be excluded. In the CI group, all dialysates from 3 cortical bone probes had to be excluded. Except for 2 individual blood samples and 1 individual dialysate, all other dialysates and blood samples were eligible for analysis. A CONSORT flow diagram can be found in paper III.

Observed concentrations and modelled concentration-time profiles are depicted in figure 12. Observed vs. fitted cefuroxime concentrations are shown in figure 13. These plots demonstrate that the model provides a satisfactory description of the cefuroxime concentration data.



Figure 12. Observed concentrations (dots) and modelled concentration-time profiles (solid lines).



Figure 13. Observed versus fitted individual- and population cefuroxime concentrations for free plasma, SCT, cortical and cancellous bone.

Tissue penetration was incomplete for SCT and cortical bone in the STI group. In the CI group, low SCT and cortical bone tissue penetration was also found, but in this group, the findings were not statistically significant. Additionally, there were no significant differences in AUCs and tissue penetration ratios between the two groups, see table 2.

Table 2

Comparison of AUC and tissue penetration

Parameter	STI	CI	P-value
Free plasma $AUC_{0-\infty}(\min mg/L)$	5801 (4902; 7277)	5415 (4625; 6670)	P = 0.63
SCT AUC _{0-∞} (min mg/L)	3016 (1929; 4675)	3764 (2164; 6426)	P = 0.56
Cancellous bone $AUC_{0-\infty}$ (min mg/L)	6035 (3718; 9831)	6256 (4276; 8954)	P = 0.91
Cortical bone AUC _{0-∞} (min mg/L)	2630 (1746; 3945)	3557 (1375; 7262)	P = 0.56
SCT fAUC _{tissue} /fAUC _{plasma}	0.52 (0.32; 0.83)	0.69 (0.38; 1.21)	P = 0.48
Cancellous bone fAUC _{tissue} /fAUC _{plasma}	1.03 (0.61; 1.74)	1.15 (0.74; 1.71)	P = 0.76
Cortical bone fAUCtissue/fAUCplasma	0.35 (0.28; 0.70)	0.65 (0.25; 1.36)	P = 0.50

 $AUC_{0-\infty}$, area under the concentration-time curve from 0 to infinity; $fAUC_{tissue}/fAUC_{plasma}$, tissue penetration expressed

as the ratio of free AUC tissue to free AUC plasma. Values are given as mean (95%-confidence intervals).

In figure 14, the PTA in the different tissues for the low (65% $T_{>MIC}$) and the high (90% $T_{>MIC}$) targets are depicted as a function of relevant MICs. Irrespective of tissue and target, CI leads to improved PTA compared to STI. Nonetheless, even for the low target, high organism MICs of 8 mg/L leads to inadequate (<90%) PTA in all tissues for both STI and CI. Apart from for cortical bone, CI leads to adequate target attainment in the remaining tissues for a MIC of 4 mg/L. In cortical bone, the PTA for this MIC is 80%. Figure 14 illustrates that, except for cancellous bone, application of the high target instead of the low generally only reduces the PTAs for CI slightly, whereas this reduction is considerable for STI.



Figure 14. Probability of target attainment (proportion of patients with concentrations above MIC) in the different tissues.

4 Discussion

4.1 In vitro and in vivo methodological feasibility assessment

The preliminary *in vitro* studies in this PhD project were designed to assess whether cefuroxime was suited for MD sampling in order to provide a sound foundation for the subsequent *in vivo* experiments. In the *in vitro* studies, RR ranged from 42.1% to 51.3%. A variation of this magnitude is most likely attributable to the variations associated with the pre-analytical sample handling and the chemical assay, and indicates a satisfactory methodological setup. As such, it was concluded that RR was independent of the concentration, and that RR_{gain} equalled RR_{loss} within an appropriate range of concentrations. No time-delay in solute movement was observed. Increasing temperature from 20°C to 40°C resulted in a statistically insignificant increase in RR of 7.6% suggesting that physiological temperature changes are unlikely to result in significant changes in RR. Based on these findings, the fundamental prerequisites for conducting *in vivo* MD sampling of cefuroxime were fulfilled.

The preliminary *in vivo* experiments were designed to assess two important aspects. Firstly, retrodialysis is only a valid method of calibration if RR remains constant for the entire duration of the experiment in question. Application of the retrodialysis method for calibration is attractive because of its simplicity and the short duration of the involved procedures. Maintenance of steady tissue concentrations *in vivo* is challenging and difficult to document. Consequently, prolonged *in vivo* RR_{gain} experiments are unlikely to be successful. Having demonstrated equality of RR_{gain} and RR_{loss} in vitro, *in vivo* RR_{loss} was considered a valid surrogate marker for *in vivo* RR_{gain}. As RR-changes may be tissue dependent, RR_{loss} was investigated in all tissues of interest. The pooled relation of RR_{loss} to mean RR_{loss} fluctuated in the range of 0.96 - 1.04 indicating that RR remains constant for a sufficient period of time. Combined with the findings of the in vitro experiments, this suggests that MD is a valid tool for evaluating tissue pharmacokinetics of cefuroxime in studies lasting several hours, and with potential physiological changes in temperature. This is in accordance with previous findings(25, 83, 118).

Secondly, the compact nature of bone calls for creation of drill holes, into which the probes can be introduced. In order to avoid damaging the membrane during insertion, the diameter of the drill hole must exceed that of the MD probe. The result is a dead space surrounding the membrane. This

raised the fundamental question if MD measurements of cefuroxime obtained in drill holes reflect the actual bone concentration or rather a mixed concentration measure derived from the actual presence of cefuroxime in bone and a contribution of cefuroxime diffusing into the drill hole from the adjacent soft tissues. Based on the dimensions of the drill holes and the basic law of diffusion, our hypothesis was that the potential contribution from the surroundings would be negligible and not clinically relevant. Indeed, previous studies have suggested that bone concentrations of gentamycin and metabolites can be assessed by means of MD measurements in a drill hole dead space in the bone. In study I, similar pharmacokinetic parameters for sealed and unsealed drill holes were found. Additionally, the corresponding concentration-time profiles were parallel and almost overlapping. If a significant diffusion of cefuroxime should have occurred from the surroundings, the unsealed drill hole pharmacokinetics should have resembled those of subcutaneous tissue and/or plasma, which they did not.

The rather flat course of the concentration-time profiles around C_{max} combined with a temporal resolution of 30 minutes, leaves T_{max} as an insensitive measure to detect differences in the kinetics. Consequently, it was decided to include the non-standard pharmacokinetic parameter $T_{50\% \text{ of } Cmax}$ (time to 50% of C_{max}), which is situated on the steepest part of the concentration-time profile, and therefore a more sensitive and accurate measure to detect differences in the kinetics. $T_{50\% \text{ of } Cmax}$ was found to be similar for sealed and unsealed drill holes. In conclusion, these findings suggest that MD measurements of cefuroxime in drill holes do reflect the actual bone concentration, and that sealing of these drill holes is unnecessary.

It is appreciated that both the present attempt to validate MD for measurement of cefuroxime in bone and alternative attempts to validate MD for measurement of other compounds in bone suffer from the lack of a validated reference method. Consequently, the different validation attempts remain indirect, and this should be remembered when interpreting the results. Nevertheless, MD offers significant advantages compared to bone specimens, particularly because it samples the unbound pharmaceutically active fraction of the drug, and that serial measurements can be obtained even after surgery. Accordingly, MD therefore appears to be superior compared to bone specimens. Having assessed linezolid bone penetration in an experimental setting using both bone specimens and microdialysis, Stolle et al. also suggested MD to be the favourable approach(50).

4.2 Tissue penetration

Regardless of the fact that most bacterial pathogens reside in the interstitial space of solid tissues, dosing regimens are commonly based on plasma PK/PD indices. Accordingly, the free plasma concentration of antimicrobials was previously, and in many situations it still is, considered to reflect the free concentration in the interstitial space of solid tissues (17, 18). Over the last decades, however, this assumption has been challenged by repeated findings of incomplete and heterogeneous tissue distribution of a number of different antimicrobials(19-25). Consequently, it is increasingly being appreciated that sufficient antimicrobial exposure not only in plasma, but also at the site of infection, is a prerequisite for a successful therapeutic outcome. Indeed, an increasing number of studies investigating tissue distribution of various antimicrobials under different conditions are emerging. For the particular case of bone, however, antimicrobial penetration remains poorly investigated. So far, the majority of studies addressing this issue have done so by measuring the concentration in homogenized bone specimens(43). This approach does, however, suffer from a number of substantial methodological limitations, which reduce the value of the findings(43, 45). These limitations have been outlined previously in section 1.2 of this thesis. Despite an aggressive approach including removal of implants and surgical debridement, longlasting antimicrobial therapy is needed for successful management of IAI and osteomyelitis. This need for prolonged antimicrobial therapy may, among other factors, be related to incomplete antimicrobial tissue penetration.

In this PhD project, cefuroxime bone penetration was investigated in two experimental studies, and in one clinical study. The preliminary experiments indicated differences in cancellous and cortical bone pharmacokinetics. Consequently, measurements of cefuroxime were obtained from cancellous and cortical bone separately in all studies. The cortical bone concentration data in study I was obtained in a separate experimental series, and the reported values represent the mean of all sealed and unsealed drill holes. Accordingly, unpaired statistical analysis was applied when comparing cortical bone with the other compartments. In the second experimental study, a fully paired design was applied, and the animals were further randomized to receive cefuroxime as either traditional STI or CI. The total dose was the same in the two intervention arms. In the first study, cortical and cancellous bone AUCs were significantly lower than that of plasma, and in a subsequent pairwise comparison, cortical bone AUC was significantly lower than cancellous bone AUC. The corresponding tissue penetration ratios were approximately 1/6 and 1/3 for cortical and cancellous

bone, respectively. In the second experimental study, tissue penetration ratios after STI were 0.45 and 0.61 for cortical and cancellous bone, respectively. After CI the corresponding ratios were 0.27 and 0.38. Direct comparison between the results of the two experimental studies is restricted for a number of reasons. In the first study, 1,500 mg of cefuroxime was administered over 30 minutes, while in the STI group in the second study, the same dose was administered over 15 minutes. The observation period in cortical bone in the first study was only 5 hours compared to 8 hours in study 2. Regarding tissue penetration ratio, this difference in observation time is unlikely to have a major impact, but for absolute AUCs it will be of some importance. Moreover, the average weight of the pigs in the second study was approximately 25% higher than that of the first study (60 kg vs. 75 kg). This corresponds to an age difference of approximately 20 days (130 vs. 150 days, information from Påskehøjgård Centret, Aarhus University). A difference in age and weight of this magnitude may be associated with physiological and pharmacokinetic differences. Finally, the two drill holes in cortical bone in the first study were located in close vicinity, which may have an influence on cefuroxime bone penetration. In spite of this, the results of the experimental studies strongly indicate that bone penetration of cefuroxime in pigs is incomplete.

In the clinical study, cortical bone and SCT tissue penetration was incomplete in the STI group (0.35 and 0.52, respectively). In the CI group, low cortical bone and SCT penetration was also found (0.65 and 0.69, respectively), but this was not statistically significant. Cefuroxime was found to distribute well into cancellous bone in both groups. No significant differences in AUCs and tissue penetration ratios were found between the two groups. It can be speculated that incomplete SCT penetration may be related to the vasoconstrictive effect of adrenaline. Such an effect would obviously be more pronounced with an STI approach, where the largest amount of cefuroxime is available for tissue penetration in the beginning of the dosing interval. This hypothesis remains speculative, however, and it will need confirmation in an appropriately designed experiment. Only one study with a comparable methodological and analytical approach has previously assessed cefuroxime tissue distribution using MD(25). In this study, SCT and muscle concentrations were investigated in morbidly obese (body mass index > 44) patients undergoing abdominal surgery. The free plasma cefuroxime concentration was not measured. Instead, the total plasma concentration was corrected for a protein binding of 33%. Mean (\pm SD) tissue penetration ratios of 0.94 \pm 0.78 and 1.53 ± 0.36 for SCT and muscle tissue, respectively, were found. As for our data, the variation for SCT was substantial, and as such, no conclusions on differences can be made. Comparison with

results from bone specimen studies of cefuroxime seems irrational given the different and questionable methodology of this approach(43, 45). Nevertheless, bone/serum concentration ratios in the range of 0.09-0.55 support that cortical bone penetration of cefuroxime may be incomplete(69-73).

Though obtained under different experimental conditions, the differences in AUC (see paper I-III) and tissue penetration ratios between pigs and humans indicate that experimental porcine pharmacokinetic data on cefuroxime cannot readily be extended to the clinical setting.

In summary, the present findings suggest that a homogeneous tissue distribution of cefuroxime cannot be taken for granted. This emphasizes the risk of insufficient antimicrobial exposure at the site of infection if dosing is merely based on plasma pharmacokinetics. Consequently, assessment of target site tissue pharmacokinetics for specific combinations of drug and tissue is attractive and may prove to be important for optimizing dosing strategies for prevention of orthopaedic surgical site infections and for treatment of serious infections in general. At present, it seems reasonable that dosing schemes of antimicrobials for serious infections should be able to accommodate for potential incomplete tissue penetration.

4.3 Time with concentrations above the MIC and probability of target attainment

The bactericidal activity of cefuroxime is well defined to be time-dependent. *In vitro* and animal infection model studies of cephalosporins have shown that bacteriostasis is achieved at approximately 40% T_{>MIC}, while maximum bactericidal effect is reached around 60%-70% T_{>MIC}(10, 56, 62-64). Nevertheless, recent clinical studies of other cephalosporins and meropenem have suggested that aggressive plasma targets of 100% T_{>1-5×MIC} are more likely to result in a successful outcome(28-30). More specifically, Mckinnon et al. found significantly improved microbiological and clinical cure with 100% T_{>MIC} compared to T_{>MIC} < 100% in patients with bacteraemia and sepsis treated with cefepime and ceftazidime(30). For cefepime, Tam et al. demonstrated that microbiological success correlated significantly with the proportion of time that concentrations exceeded 4.3 x MIC in patients treated for gram-negative infections(29). In patients with lower respiratory tract infections treated with meropenem, a C_{min}/MIC ratio > 5 was found to be most predictive of clinical and microbiological cure(28). In situations with incomplete tissue penetration, aggressive targets like these are obviously less liable to result in insufficient

antimicrobial exposure at the target site. Interestingly, the results from the clinical studies are in accordance with data from in vitro time-kill studies of beta-lactams showing that killing rate can be improved by increasing drug concentrations up to approximately 4 - 6 times the MIC(6).

Having established MD for measurements of cefuroxime in bone, it was decided to investigate $T_{>MIC}$ for cefuroxime in bone. Though not convincingly demonstrated in a clinical setting, the time-dependency and short half-life of cefuroxime and the majority of other beta-lactams suggest that a CI- or EI-approach may be favourable compared to STI in terms of optimizing $T_{>MIC}$. Consequently, it was decided to compare STI and CI.

Analogous methodological approaches were applied in the experimental and the clinical study. The primary endpoint was $T_{>MIC}$ and key pharmacokinetic parameters were secondary endpoints. Population pharmacokinetic modelling and Monte Carlo simulations were used to analyse the concentration-time data of cefuroxime. The main reason for this was the ability to obtain good estimates of $T_{>MIC}$ and to determine PTA for specified targets. As described by Turnidge(110), $T_{>MIC}$ may be calculated by simpler means, but this approach assumes a rapid tissue distribution. This assumption is obviously violated in tissue pharmacokinetic studies with delayed tissue distribution. Another simple alternative to determine $T_{>MIC}$ is linear interpolation, but for reasons stated in section 2.6.1, this was not an attractive approach. In the clinical study, we reported PTA, but refrained from this in the experimental study, as pig PTA was unlikely to be of clinical relevance. Rather, the experimental study was designed to provide basic pharmacokinetic information on CI.

Tissue targets for cefuroxime and other antimicrobials for prevention of surgical site infections in an orthopaedic setting are unknown, but for time-dependent drugs like cefuroxime, it is recommended that plasma as well as tissue concentrations exceed *in vitro* MIC values of relevant pathogens throughout the procedure(115). In the lack of well-established PK/PD tissue targets for the clinical study, traditional plasma targets were applied. Moreover, in the pharmacokinetic analysis, only the actual administered doses were simulated. This decision was governed by considerations outlined in section 2.6.2. While simulation of multiple dose steady-state situations with different doses were considered inappropriate, it may be somewhat reasonable to extend the findings for the observation period to the first 24 postoperative hours. The latter seems reasonable because of the short half-life of cefuroxime and the initiation of CI with a bolus injection, but it relies on the assumption that the model parameter estimates are reasonably stable within this period of time.

In the experimental study (paper II), interesting concentration-time profiles for cefuroxime were found (see figure 10). An important finding was that stable tissue concentrations could be achieved using CI, but due to incomplete penetration, these were lower than those found in plasma. For low MICs, CI was superior to STI in terms of tissue $T_{>MIC}$, whereas the opposite was found in bone for higher MICs. This pattern is to be expected when comparing STI and CI, as high MICs may not be exceeded at any time during CI. Depending on tissue and MIC of the pathogen, the present CI data suggests a risk of attaining a limited or even inversed gap between steady-state tissue concentrations and the MIC. For STI, mean $T_{>MIC}$ was less than 50% in all tissues for a MIC of 2 µg/mL. Accordingly, the MICs for which acceptable tissue $T_{>MIC}$ was achieved in the present setting would have been somewhat insufficient in a clinical setting considering the breakpoints of relevant bacteria (www.eucast.org). The significance and possible implications of the gap between plasma and tissue $T_{>MIC}$ for CI will be discussed later in this chapter.

Population pharmacokinetic modelling and Monte Carlo simulations were performed in the clinical study (paper III) in order to estimate PTA for targets of 65% (low target) and 90% $T_{>MIC}$ (high target) up to MICs of 8 µg/mL. It was found that CI led to higher PTAs compared to STI for all tissues. Nonetheless, even for the low target, a dose of 1,500 mg of cefuroxime was insufficient for MICs of 8 µg/mL in all tissue for both CI and STI. For MICs of 4 µg/mL (EUCAST breakpoint for staphylococcus aureus) and aplication of the low target, CI leads to adequate PTA in plasma, subcutaneous tissue and cancellous bone, whereas in cortical bone, only an 80% PTA was achieved for this MIC. Importantly, application of the high target only reduced plasma, SCT and cortical bone PTAs slightly for CI. For STI, the reduction was more marked. The observation of a reduction in cancellous bone PTA for CI similar to that of STI when applying the high instead of the low target may be explained by some rather low concentrations for some patients within the first 105 minutes of the observation period for CI (see figure 13). The low target may seem somewhat high for a cephalosporin. Nevertheless, maximum bactericidal effect is often achieved with $T_{>MIC}$ in the range of 60 – 70%(10, 56, 63, 64). Additionally, the persistent effects are limited for gram-negative pathogens, which may also be encountered in an orthopaedic setting(10, 110). The low target is also

consistent with a recent population PK study on cefuroxime, thus allowing for easy comparison of results(119). The high target, on the other hand, may seem rather low given that CI aims at attaining 100% $T_{>MIC}$. As such, it would have seemed rational to use this as the high target instead of 90% $T_{>MIC}$. We did, however, assess the first dosing interval of CI, which was initiated with a 5-min bolus infusion of 500 mg. Moreover, tissue distribution was also associated with a time delay. Consequently, attaining 100% $T_{>MIC}$ was impossible, and accordingly not a meaningful target. Yet, the 90% $T_{>MIC}$ target may reflect a 100% $T_{>MIC}$ for later observation periods, again under the assumption of stable pharmacokinetics. The acceptable level of PTA is under debate, and varies between 90-99%(120). Well aware that 10% of the population are unlike to be optimally covered, a 90% PTA was considered adequate, which is in agreement with a previous study on cefuroxime(119).

From a PK/PD point of view, the findings in this clinical study support that CI of a short half-lived and time-dependent drug like cefuroxime may be favourable compared to STI. Two previous studies on meropenem and piperacillin also found advantageous subcutaneous tissue exposure after CI(86, 92). On this background, it is somewhat surprising that meta-analyses assessing clinical outcomes after STI and CI have been unsuccessful in demonstrating superiority of CI(74-79). Traditionally, however, CI has been considered as a way to lower the total drug dose. This is reflected in the majority of studies included in these meta-analyses, where the total daily dose was lower for CI than for STI. In fact, a subset of RCTs using the same dose in the two intervention arms did find lower clinical failure rates for patients treated with CI(75). Another possible explanation for the lack of evidence supporting CI is that targets of approximately 40-50% T_{>MIC} may actually have been sufficient for the specific combinations of drug and bug in immunecompetent individuals, and that complete antimicrobial target site penetration was present. Targets of this magnitude are likely to be achieved with intermittent infusion, and in that case, CI is obviously of limited additional value. Additionally, concentrations of higher multiples of the MIC are achieved with STI compared to CI. Despite the fact that this is only the case for a limited part of the dosing interval, it may be of significance.

Recently, a population pharmacokinetic study in critically ill patients found that intermittent infusion of a standard dose of 1,500 mg cefuroxime is inadequate(119). Even for low MICs, creatinine clearances above 50 ml/min were associated with high probabilities of underdosing.

Though obtained in a different setting in healthy but newly operated patients, our findings for STI support that current routine dosing schemes of cefuroxime may be inadequate. Particularly if more aggressive targets are pursued, alternative dosing strategies like CI or EI and higher doses should be considered.

To some extent, CI follows an "all or nothing" principle in the individual patient, i.e. $T_{>MIC}$ is either 100% or 0%. Our data confirms that for high MICs, $T_{>MIC}$ may very well be 0% using CI. This aspect should be remembered when applying CI. STI, on the other hand, will lead to $T_{>MIC}$ for at least a small fraction of the dosing interval in most situations.

For total hip replacement surgery, lower infections rates have been found when antimicrobials were administered 4 times on the day of surgery(121). Accordingly, it is recommended that antimicrobial prophylaxis be continued for 24 hours following total joint replacement surgery in general(61). Under the assumption that the chosen targets are representative for cefuroxime prophylaxis in relation to total joint replacement, application of an appropriately adjusted CI dosing regimen in this setting may lower the infection rates further.

Application of a CI approach may also prove to be an important measure in the management of serious deep-seated orthopaedic infections if aggressive targets of the magnitude outlined previously in this section are to be achieved. Though obtained in healthy tissue and therefore not readily extendable to an infection situation, our findings suggest that a high dose CI approach would be needed to achieve acceptable PTA for these aggressive targets. A drug sparring CI approach on the other hand, seems to be associated with a considerable risk of permanent under dosing, particularly if susceptibility of the invading pathogen cannot be determined.

4.4 TDM and target achievement

Therapeutic drug monitoring (TDM) of beta-lactams is increasingly becoming available as routine analyses in the daily clinical setting, and particularly in intensive care units(65). Traditionally, TDM has primarily been used for antimicrobials with at narrow therapeutic index and to avoid toxicity (i.e. for aminoglycosides). Over the last decade, however, the pharmacokinetics of beta-lactams has been shown to be unpredictable and display considerable inter-individual variation, particularly in critically ill patients(80, 81). TDM is therefore expected to improve the likelihood of

beta-lactam PK/PD target attainment, and thus ultimately to improve treatment of serious infections(122-124). Our findings of incomplete tissue penetration combined with lower $T_{>MIC}$ and PTAs in tissues compared to free plasma after CI, suggest that caution should be observed when adjusting antimicrobial dosing based upon free plasma concentrations and MIC values alone. In situations with incomplete tissue penetration, this approach may lead to sub-therapeutic concentrations at the site of infection if a safety margin is not included. For CI in particular, there is a risk of permanent sub-therapeutic target site concentrations, while STI in most situations will lead to $T_{>MIC}$ for at least a discrete part of the dosing interval. As mentioned above, more aggressive targets of 100% $fT_{>1-5\times MIC}$ have been shown to be more predictive of a successful clinical outcome (28-30). These observations may partly be related to incomplete tissue penetration. Our findings support similar aggressive targets for bone infections, which seems reasonable given the difficulty in management of these infections. Individual measurement of target site tissue concentrations is obviously not feasible, but for drug-infection combinations, for which incomplete tissue penetration has been documented, this knowledge should be included in the associated dosing and TDM regimens.

4.5 Osteomyelitis and IAI

The present thesis deals with cefuroxime pharmacokinetics in healthy bone. Accordingly, the data are confined to reflect similar clinical situations, which essentially only include the use of cefuroxime as antimicrobial prophylaxis in relation to orthopaedic procedures. Currently, only limited antimicrobial pharmacokinetic data on infected bone is available, and the effect of osteomyelitis and IAI on antimicrobial bone penetration remains unclear(43, 125). The fact that the method of choice used in previous studies almost exclusively has been bone specimens, contributes further to the uncertainty(43). At least in chronic bone infections and IAI, biofilm, sequestrated and ischemic bone may be present. In such lesions and matrices, antimicrobial penetration is presumably poor compared to healthy and acutely infected bone. In addition to antimicrobial penetration, the ability of Staphylococcus aureus to be internalized in osteoblast may play a role in the challenging management and high recurrence rate of bone infections(126). Altogether, it seems prudent to investigate infected bone antimicrobial penetration with alternative methodological approaches like microdialysis. For time-dependent and short half-lived drugs, comparison of STI and EI or CI seems relevant.

4.6 Limitations

Limitations and weaknesses of the methods applied in the present studies have been discussed on a general level the methodology section. In this section, these limitations will be discussed in relation to the actual findings in the separate studies.

To some extent, pig physiology and anatomy resemble that of man(99). Specifically, bone composition, density and quality have also been shown to compare reasonably with that of man(100). Nonetheless, the present findings suggest that cefuroxime pharmacokinetics in the porcine model are not analogous to that in man. The pigs had to be kept under general anaesthesia during the entire experiments, and as such, the differences may not exclusively be attributed to interspecies variation. The use of porcine cefuroxime pharmacokinetic data to adjust dosing in a clinical setting, however, seems unadvisable. Nevertheless, the porcine model provides a valuable tool for feasibility assessment and identification of possible pitfalls before progressing with clinical experiments.

Healthy senior males undergoing TKR represent a very selected group, and for practical reasons, measurements of cefuroxime were conducted after the surgical procedures. Consequently, generalizability to the actual peri/preoperative cefuroxime prophylaxis is questionable, whereas the results are more likely to be reflective of the 24-hour postoperative continuation of antimicrobial prophylaxis. Accordingly, external validity beyond the actual population and situation is uncertain. Particularly the absolute estimates of PTA, $T_{>MIC}$ and key pharmacokinetic parameters seem to be restricted to the present setting. The findings for tissue penetration ratios, on the other hand, may be more generalizable to other populations.

All MD experiments are dependent on a sensitive, accurate and precise chemical assay. Our UHPLC-UV method fulfilled these demands. Given the rather low RRs (mean RRs were in the range of 10.8 - 29.4%) encountered with our methodological setup, this was of outmost importance. Inherent in the mandatory correction for RR lies a magnification of the variations related to the pre-analytical sample handling and the chemical assay. This magnification increases exponentially with decreasing RR, which explains the need for a solid chemical assay and careful sample handling. In the experimental studies, comparable variations were found in plasma and the remaining compartments, indicating that the applied setup was adequately reliable in terms of precision. As

such, a substantial part of the variations may be considered as biological. In the clinical study, tissue variations exceeded those of plasma, which is in agreement with findings in other clinical antimicrobial MD studies(27, 127). In addition to the well-known biological variations in antimicrobial pharmacokinetics, the surgical trauma and local injection of adrenaline are likely to have contributed to the variations. As such, combined with the limited variations in the experimental studies, the variations in the clinical study are unlikely to represent inadequate precision of the methodological setup. Another possible explanation of the variation is a poor model description of the data. However, the model-diagnostic plots demonstrate a satisfactory description of the data.

Despite the considerations above, higher RRs are attractive and will contribute to more precise pharmacokinetic parameter estimates. In the present studies, RR could have been increased by increasing membrane length or by choosing a lower perfusion rate. Due to decreasing volume, temporal resolution change proportionally with perfusion rate, i.e. lower perfusion rate results in poorer temporal resolution. The short half-life of cefuroxime called for high temporal resolution, and accordingly, the selected perfusion rate of 2 μ L/min was considered a reasonable trade-off. A perfusion rate of 1 μ L/min would have reduced temporal resolution from 30 minutes to 60 minutes, which potentially would have had a significant impact on estimates of T_{>MIC}. Increasing membrane length of the probes was not possible due to the anatomical limitations of drill hole depth.

In the experimental study, RR was found to be constant over 7 hours in SCT, cancellous and cortical bone. Assuming that this would also be the case in a clinical setting, RR was estimated by means of retrodialysis by drug. The participants in the clinical study were subject to major surgery, administration of local adrenaline, and for ethical reasons, no restrictions regarding mobilization were applied. Consequently, transient changes in local perfusion, and thus in RR, cannot be excluded. Transient and/or permanent decreases in RR would have resulted in underestimation of the associated absolute concentrations and vice versa. Application of an internal calibrator would have elucidated this issue, and for future studies such an approach seems recommendable.

In the experimental studies, the animals were kept under general anaesthesia, and therefore the observation period was confined to one dosing interval. In the clinical study, the observation period was also confined to one dosing interval, but in this setting, safety (risk of infection) and ethical

concerns were limiting factors. Drug steady state is generally achieved after 4-6 half-lives. Consequently, if the intra-individual pharmacokinetics remains constant over time, it is unlikely that the findings would have differed significantly after a few repeated doses due to the short halflife of cefuroxime, but this remains speculative.

5 Conclusions and perspectives

MD was successfully applied for evaluation of cefuroxime bone concentrations. The present results suggest that measurements obtained in small drill holes in bone reflect the actual bone concentration, and that sealing of these drill holes is unnecessary. Calibration is imperative, and the simple and easy-to-use retrodialysis approach was found to be adequate for this task in an experimental setting, even when measurements were conducted for several hours.

In the experimental studies, cortical as well as cancellous bone cefuroxime penetration was found to be incomplete. CI resulted in attractive concentration-time profiles though the steady state concentrations would have been insufficient in a clinical setting.

In the clinical study, cefuroxime was found to distribute well into cancellous bone. Both SCT and cortical bone penetration was significantly incomplete in the STI group. The same tendency was observed in the CI group, but the findings were not as marked, and they were not significant. CI of cefuroxime led to improved tissue exposure in all tissues compared to STI in terms of PTA. As such our findings suggest that CI of cefuroxime is favourable compared to STI. Nevertheless, a standard dose of 1,500 mg results in insufficient tissue exposure for high MICs regardless of mode of administration.

Investigating antimicrobial bone penetration by means of bone specimens is associated with a number of substantial limitations. Consequently it has been advocated that results from these studies may be misleading and ultimately harmful if applied uncritically in a clinical setting. The findings in the present thesis suggest that MD may become a very useful alternative tool for assessment of antimicrobial bone pharmacokinetics. After preliminary feasibility assessment, MD can be used to sample a variety of antimicrobials. As for other tissues, increased pharmacokinetic knowledge can be used to optimize dosing regimens, which may in turn optimize clinical outcomes.

In the present thesis, only healthy bone was investigated. Future studies should include large animal infectious models, in which the effect of infection on antimicrobial penetration can be assessed. Ultimately, similar studies should be conducted in a clinical setting, though the heterogeneity of osteomyelitis and IAI will present a methodological challenge.

The favourable tissue exposure obtained with CI in the clinical study calls for application in a large randomized clinical trial with clinical and not pharmacokinetic endpoints. An obvious study population would be patients with early infection of hip and knee prostheses, as these conditions provide significant therapeutic challenges and high recurrence rates.

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Appendix



Pharmacokinetics of Cefuroxime in Porcine Cortical and Cancellous Bone Determined by Microdialysis

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Traditionally, the pharmacokinetics of antimicrobials in bone have been investigated using bone biopsy specimens, but this approach suffers from considerable methodological limitations. Consequently, new methods are needed. The objectives of this study were to assess the feasibility of microdialysis (MD) for measuring cefuroxime in bone and to obtain pharmacokinetic profiles for the same drug in porcine cortical and cancellous bone. The measurements were conducted in bone wax sealed and unsealed drill holes in cortical bone and in drill holes in cancellous bone and in subcutaneous tissue. As a reference, the free and total plasma concentrations were also measured. The animals received a bolus of 1,500 mg cefuroxime over 30 min. No significant differences were found between the key pharmacokinetic parameters for sealed and unsealed drill holes in cortical bone. The mean \pm standard error of the mean area under the concentration-time curve (AUC) values from 0 to 5 h were 6,013 \pm 1,339, 3,222 \pm 1086, 2,232 \pm 635, and 952 \pm 290 min $\cdot \mu$ g/ml for free plasma, subcutaneous tissue, cancellous bone, and cortical bone, respectively (P < 0.01, analysis of variance). The AUC for cortical bone was also significantly different from that for cancellous bone (P = 0.04). This heterogeneous tissue distribution was also reflected in other key pharmacokinetic parameters. This study validates MD as a suitable method for measuring cefuroxime in bone. Cefuroxime penetration was impaired for all tissues, and bone may not be considered one distinct compartment.

Osteomyelitis and periprosthetic bone and joint infections (PJIs) are among the most severe orthopedic conditions. Treatment includes surgical debridement, removal of implants, and long-lasting antimicrobial therapy, and it calls for a multidisciplinary approach (1). Nevertheless, treatment failure is common. One of the reasons for this may be incomplete or heterogeneous tissue distribution of antimicrobials, which has been demonstrated in a number of studies for different combinations of drug and tissue (2-8).

Determining the penetration of antimicrobials into bone remains a difficult task. Traditionally, bone biopsy has been the predominant method used. Obtaining pharmacokinetic data by means of tissue biopsy specimens may, however, be misleading and ultimately harmful to patients (9, 10). When analyzing tissue biopsy specimens, no selective measurement of the free extracellular concentration or distinction between the intra- and extracellular compartments can be made. Furthermore, temporal resolution is poor or nonexistent, and the concentrations are given by weight and not by volume. Consequently, pharmacokinetic parameters obtained by tissue biopsy specimens are difficult to relate to relevant pharmacodynamic endpoints.

In several studies, microdialysis (MD) has been used to determine the concentrations of antimicrobials in the tissue of interest (11–16). By being minimally invasive, the MD technique permits clinical investigations, and at the same time, it provides the possibility of continuous sampling of the unbound fraction of a drug in the interstitial space. From this perspective, MD offers an attractive alternative to using bone biopsy specimens in order to assess antimicrobial penetration into the bone. However, due to the compact nature of bone, MD probes cannot readily be implanted. This issue has been overcome by inserting the probes into drill holes in the bone (17–23). This approach obviously raises the question of whether MD measurement of antimicrobials in drill holes truly reflects bone drug concentration or rather a mixed concentration stemming partly from the presence in the bone, and partly from the presence of the substance in the surrounding soft tissue. Additionally, it is a challenge to create drill holes in cortical bone and verify that they are strictly intracortical and that the MD catheters remain in the drill holes during the entire study period.

In the present study, we investigated the suitability of MD for cefuroxime measurements in a laboratory setting. Second, the feasibility of applying MD to measure cefuroxime in cortical bone was investigated in anesthetized pigs. With an identical methodological setup, parallel *in vivo* measurements of cefuroxime were also performed in cancellous bone and subcutaneous tissue. Studies evaluating the distribution of cefuroxime in cortical and cancellous bone by use of MD have not been published.

MATERIALS AND METHODS

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

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Address correspondence to Mikkel Tøttrup, mikkel.tottrup@ki.au.dk. Copyright © 2014, American Society for Microbiology. All Rights Reserved. doi:10.1128/AAC.02438-14 **Microdialysis.** An in-depth description of MD can be found elsewhere response (24, 25). Briefly, the basic principles of MD rely on the facts that diffusion across the semipermeable membrane of the microdialysis probe is quantitatively equal in both directions, and that relative recovery of solutes is independent of the concentration gradient between the tissue and perfusate. In this study, the MD equipment from M Dialysis AB (Stockholm, 3) Sweden) was used in all the experiments. Specifically, the catheters used μ

were CMA 63 (membrane length, 10 mm; molecular cutoff, 20 kDa), and CMA 107 precision pumps produced a flow rate of 2 μ l/min.

When calculating the relative recovery by loss (RR_{loss}) and relative recovery by gain (RR_{gain}) , the following equations were applied:

$$RR_{gain}(\%) = 100 \times (C_{out}/C_m)$$
(1)

$$RR_{\rm loss}\left(\%\right) = 100 \times \left(1 - \frac{C_{\rm out}}{C_{\rm in}}\right) \tag{2}$$

where C_{in} is the concentration in the perfusate, C_{out} is the concentration of the dialysate, and C_m the concentration in the medium surrounding the catheter.

As microdialysates are gathered continuously, the measured concentrations were attributed to the midpoint of the sampling intervals for the subsequent data analysis. In the *in vivo* studies, absolute tissue drug concentrations were obtained by correcting for relative recovery using the following equation:

$$C_{\text{tissue}} = \left(\frac{C_{\text{out}}}{RR}\right) \tag{3}$$

Individual *in vivo* probe calibration was performed for all animal experiments.

Handling of samples. The dialysates were immediately frozen and stored at -80° C until analysis. Venous blood samples were stored at 5°C for a maximum of 20 h before being centrifuged at 3,000 × *g* for 10 min. The plasma aliquots were then frozen and stored at -80° C until analysis.

UHPLC analysis of cefuroxime. The assessment of cefuroxime was performed with ultrahigh-performance liquid chromatography (UHPLC). For measuring the total plasma drug concentrations, 100 μ l plasma was added to 300 μ l acetonitrile (Sigma-Aldrich, Denmark) containing 30 μ g/ml ceftriaxone (Sigma-Aldrich) as an internal standard and filtered through a protein precipitation plate (Captiva ND plate; Agilent Technologies, USA). Subsequently, 400 μ l of 10 mM phosphate buffer (PB) (pH 3) (NaH₂PO₄, H₂O adjusted with HCl; Merck, Germany) was added to the filtrate. Standards (6.25, 25, and 100 μ g/ml) were prepared by adding cefuroxime sodium (Fresenius Kabi AB, Sweden) to human donor plasma.

For measuring the free fraction of cefuroxime, 300 µl plasma was placed into an ultrafilter 96-well plate with a 30-kDa molecular mass cutoff (AcroPrep 30K Omega; Pall Corporation, USA) and centrifuged for 30 min at 1,000 \times g. Fifteen microliters of plasma ultrafiltrate, dialysate, or in vitro sample was added to 20 µl PB containing 10 µg/ml ceftriaxone. For these measurements, standards of cefuroxime (0.6, 1.3, 2.5, 5.0, and 10 µg/ml) in 0.9% NaCl-water were used. The UHPLC system (Agilent 1290 Infinity; Agilent Technologies, USA) was equipped with a 1.7 µm 100 by 2.1 mm C₁₈ column (Kinetex; Phenomenex, USA), and chromatography was performed with a gradient of acetonitrile (5 to 10% over 4 min) in PB as the eluent. For analysis, 5 μ l prepared sample was injected, and the analytes were detected at 275 nm. Quantification was based on the areas of the cefuroxime and ceftriaxone peaks and was performed with the ChemStation software (Agilent Technologies). The limit of quantification was 0.25 μ g/ml for the measurement of the total cefuroxime concentration in plasma and 0.06 µg/ml for the measurement of the free concentrations in plasma, dialysate, and samples from the in vitro study. Intrarun (interrun) imprecisions (percent coefficients of variation [%CV]) were 5.3% (8.2%) at 12.5 µg/ml and 4.1% (4.3%) at 50 μ g/ml for quantification of the total plasma drug concentrations, and 4.3% (4.7%) at 2.5 µg/ml and 1.6% (6.2%) at 38 μ g/ml for the quantification of the free concentrations. The accuracy of the assay was judged by repeated measurements of 5 different cefuroxime formulations obtained from the pharmacy at Aarhus University Hospital and was found to be between -3.3% and 5.8%.

In vitro experiments. *In vitro* relative recovery by gain (RR_{gain}) and by loss (RR_{loss}) were determined using isotonic saline solutions containing cefuroxime concentrations of 1, 10, and 50 µg/ml. Using 20-min intervals, 3 samples of 40 µl were harvested for each concentration, starting at 1 µg/ml. An equilibration period of 15 min was allowed whenever the concentrations were changed. The same catheter was used for both RR_{gain} and RR_{loss} at all concentrations, and the entire experiment was conducted on the same day. The temperature was maintained at 30 ± 1°C.

The effect of temperature was also assessed. This was done in a series of RR_{gain} experiments, where the temperature was increased in a stepwise manner at fixed concentrations of 1, 10, and 50 µg/ml, respectively. Three samples of 40 µl were harvested at each temperature step: 22°C ± 1°C, 30°C ± 1°C, and 40°C ± 1°C.

In vivo studies. (i) Animal, anesthetic, and surgical procedures. Fifteen pigs were included in the study (60-kg Danish Landrace breed). The animals were kept under general anesthesia during the entire study using a combination of fentanyl (0.25 to 0.5 mg/h, continuous infusion), propofol (150 mg/h, continuous infusion), and sevoflurane (minimal alveolar concentration, $1.1\% \pm 0.1\%$). pH, which is known to affect RR (24), was evaluated with arterial gas analysis and kept within the reference range (7.36 to 7.42) throughout the study by controlling ventilation. Normal kidney function, assessed by plasma creatinine, was confirmed for all pigs before inclusion in the study. Body temperature was kept within the range of 36.5°C to 39.5°C. Immediately after the induction of anesthesia, the surgical procedures were performed. MD catheters were placed either in drill holes in cortical bone of the anterior margin of the tibia or in cancellous bone within the femoral condyles. The tibia was accessed by an anteromedial approach, while the femur was accessed by a lateral approach. The depths of the drill holes were 15 mm and 20 mm for the cortical and cancellous drill holes, respectively. Regardless of the anatomical location, the holes were made using a 2-mm drill. Cessation of drilling occurred every few seconds to prevent overheating the tissue. The catheters were fixed to the skin with sutures. The correct locations of the catheters were verified by autopsy. For all pigs, in places where a catheter was implanted into cortical bone, postmortem computed tomography (CT) scans of the tibia were performed in order to document the correct intracortical location of the drill hole.

(ii) Assessment of stability of recovery over time. In order to assess whether RR_{loss} remains constant over a relevant period of time, retrodialysis was performed for 7 h in three pigs. The first pig had two catheters implanted in cortical and cancellous bone, the second had three catheters implanted in cortical bone, cancellous bone, and subcutaneous tissue, and the third pig had catheters implanted in cancellous bone and subcutaneous tissue. The cefuroxime concentrations in the perfusates were 5 µg/ml for cortical bone catheters and 10 µg/ml for cancellous bone and subcutaneous tissue catheters. The samples were collected at 60-min intervals.

(iii) Assessment of the effect of bone wax sealing of drill holes. This part of the study was designed to assess whether MD measurements of cefuroxime in drill holes in cortical bone solely reflect bone drug concentrations. In six pigs, four holes were drilled in the cortical part of the tibia, two at each side. Each hole was symmetrical with a hole on the contralateral side, leaving a total of two symmetrical pairs. When all holes were fitted with a catheter, one hole in each pair was randomly allocated for sealing with bone wax, while the corresponding contralateral hole was left open. Prior to implantation, the catheters were perfused with Ringer's acetate containing cefuroxime at a concentration of 10 µg/ml. When surgery was completed, a 30-min tissue equilibration period followed. The probes were then calibrated using the retrodialysis method (26) by collecting a sample over a 30-min interval. Following calibration, the perfusate was changed to blank Ringer's acetate, and a 75-min washout period was conducted. A dialysate was collected during the last 20 min of this period in order to assess the efficacy of the washout. Fifteen hundred milligrams of cefuroxime was then administered intravenously over a



FIG 1 (a) Mean RR_{gain} and RR_{loss} values at different concentrations of cefuroxime. (b) Effects of temperature on recovery at different concentrations of cefuroxime. Bars represent the SEM.

30-min period. The dialysates were collected at 30-min intervals for the first 2 h and at 60-min intervals at 3 to 5 h.

(iv) Measurement of cefuroxime in cancellous bone and subcutaneous tissue. The methodological setup of this part of the study is analogous to the one outlined above. However, the MD catheters were placed in cancellous bone of the femur and in subcutaneous tissue of the abdomen. The dialysates were collected every 20 min for the first 3 h and every 30 min for the next 5 h, giving a total sampling time of 8 h. Additionally, venous blood samples were collected in the middle of every dialysate sampling interval. The blood samples were drawn from a central venous catheter. Six pigs were included in this part of the study.

Pharmacokinetic analysis and statistics. The pharmacokinetic parameters were determined separately for each subject by noncompartmental analysis (NCA) using Stata (version 12.0; StataCorp, USA). The exception is the time to 50% of C_{\max} ($T_{50\% \text{ of } C_{\max}}$), which was determined using WinNonlin software (version 5.3; Pharsight Corporation, Mountain View, CA). The washout concentrations were low and as such were neglected in the analysis. The area under the concentration-time curve (AUC) values for the sampling periods were calculated using the trapezoidal rule. As cefuroxime measurements were conducted for only 5 h in cortical bone, the AUC from 0 to 5 h (AUC₀₋₅) was also calculated for the other compartments to allow for a relevant statistical comparison. The terminal half-life ($t_{1/2}$) was calculated as \ln_2/λ_{eq} , where λ_{eq} is the terminal elimination rate constant estimated by linear regression of the log concentration on time. The appropriate number of points used for the calculation was determined separately by inspection of the concentration-time profiles.

In order to assess whether RR_{loss} remains constant, the relation of RR_{loss} to mean RR_{loss} for the entire 7 h (RR_{loss}/mRR_{loss}) was calculated for every catheter at each time point. In the following analysis, the data were pooled for all catheters and for each distinct location, respectively.

All values are given as the mean \pm standard error of the mean (SEM) unless stated otherwise. An unpaired *t* test was used for comparing relative recovery at room temperature and 40°C. A paired *t* test was used for comparing the pharmacokinetic parameters between sealed and unsealed drill holes within the same animal. A general comparison of the pharmacokinetic parameters was conducted using one-way analysis of variance (ANOVA) with a random animal effect. Finally, *post hoc* pairwise comparisons were made for selected pairs of pharmacokinetic parameters. When comparisons were made across the two animal experiments, the variability of C_{max} and AUC increased slightly. Normality improved and was confirmed when transforming to the log scale, and as such, comparisons for these parameters were made on a log scale. A *P* value of <0.05 was considered significant. As a measure of tissue penetration, the ratio of the free tissue AUC (*f*AUC_{tissue}) to free plasma AUC (*f*AUC_{plasma}) was also

calculated for subcutaneous tissue and cancellous bone. Statistical analyses were also performed using Stata (version 12.0; StataCorp, USA).

RESULTS

Effects of concentration, temperature, and time. The mean RR_{gain} and RR_{loss} for cefuroxime were 42.1% and 42.9% at 1 µg/ml, 46.0% and 48.8% at 10 µg/ml, and 51.3% and 48.0% at 50 µg/ml, respectively (Fig. 1a). Figure 1b shows RR_{gain} using different concentrations of cefuroxime at different temperatures. When pooling data for the three different concentrations, the average difference in recovery between 20°C and 40°C was 7.5% (95% confidence interval [CI], -2.4 to 17.4) (P = 0.12).

Over 7 h, the pooled relationship of RR_{loss}/mRR_{loss} ranged from 0.96 to 1.04, while the ranges for subcutaneous tissue, cortical, and cancellous bone were 0.93 to 1.10, 0.93 to 1.12, and 0.92 to 1.12, respectively (see Fig. 2). No distinct patterns were recognized for the pooled relationship or for any of the separate anatomical locations.

Assessment of the effect of bone wax sealing of drill holes. Of the 6 pigs included in this part of the study, only 5 were eligible for analysis. In the excluded pig, one pair of drill holes was excluded because the postmortem CT scan revealed penetration to the surroundings in the distal part of the hole. For one of the catheters in the other pair of drill holes, two concentration analyses failed, and



FIG 2 The relationship of RR_{loss}/mRR_{loss} for each anatomical location (dashed lines) and the means for all locations (solid line). The horizontal solid line represents a relationship of 1. Bars represent the SEM.



FIG 3 Mean concentration-time profiles for sealed and unsealed drill holes. Bars represent the SEM.

not enough material was left for a third reanalysis. In another pig, the calibration resulted in an RR of 4%. This was considered unreliably low, and therefore, all the samples from this catheter were reanalyzed. The mean RR values were $18.7\% \pm 2.5\%$ and $17.7\% \pm 1.7\%$ for the sealed and unsealed holes, respectively. The mean concentrations in the washout samples were $0.26 \pm 0.08 \mu$ g/ml and $0.31 \pm 0.05 \mu$ g/ml for the sealed and unsealed drill holes are depicted in Fig. 3. No significant differences were detected between the key pharmacokinetic parameters (Table 1).

Pharmacokinetics of cefuroxime in subcutaneous tissue and cancellous bone. Of the 6 pigs included in this part of the study, only 5 were included in the analysis. For the excluded pig, the perfusate accidentally was not changed to pure Ringer's acetate following calibration. Thus, it was not possible to calculate absolute tissue drug concentrations. For another pig, the RR of the subcutaneous catheter was not reliably determined. Consequently, the subcutaneous measurements in this pig were also left out of the analysis. The mean RR values were 29.1% \pm 11.0% and $29.4\% \pm 14.1\%$ for the cancellous and subcutaneous catheters, respectively. The mean concentrations in the washout samples were $0.11 \pm 0.05 \,\mu$ g/ml and $0.10 \pm 0.05 \,\mu$ g/ml for the same holes, respectively. The concentration-time profiles of the two locations and for plasma are depicted in Fig. 4. The pharmacokinetic parameters are shown in Table 2. Significant differences among the means were found for AUC, Cmax, and T50% of Cmax values. Significant differences were also found for pairwise comparisons of free plasma versus subcutaneous tissue and free plasma versus cancellous bone for the same pharmacokinetic parameters.

 TABLE 1 Key pharmacokinetic parameters for sealed and unsealed drill holes in cortical bone

Pharmacokinetic parameter ^a	Sealed drill holes (mean \pm SEM)	Unsealed drill holes (mean \pm SEM)	Р
\overline{AUC}_{0-last} (min $\cdot \mu g/ml$)	868 ± 233	$1,037 \pm 318$	0.38
$C_{\rm max}$ (µg/ml)	4.8 ± 1.0	5.7 ± 1.4	0.42
$T_{\rm max}$ (min)	84 ± 12.7	105 ± 11.8	0.33
$T_{50\% \text{ of } Cmax}(\min)$	31.8 ± 5.8	28.7 ± 4.7	0.61
$t_{1/2}$ (min)	136 ± 42.0	142.2 ± 59.3	0.93

 a $C_{\rm max}$ peak drug concentration in serum; $T_{\rm max}$ time to $C_{\rm max}$, $T_{50\%~{\rm of}~Cmax}$, time to 50% of $C_{\rm max}$; $t_{1/2}$, half-life at β phase; AUC_0-last, area under the concentration-time curve from 0 to the last measured value.



FIG 4 Mean concentration-time profiles for plasma, subcutaneous tissue, and cancellous bone. Bars represent the SEM.

Comparison of AUC₀₋₅, C_{max} , and time to 50% of C_{max} for free plasma, subcutaneous tissue, and cancellous and cortical bone. The AUC₀₋₅ values were 6,013 ± 1,339, 3,222 ± 1,086, 2,232 ± 635, and 952 ± 290 min µg/ml for free plasma, subcutaneous tissue, cancellous bone, and cortical bone, respectively (P <0.01, ANOVA). The value for cortical bone is the average of the sealed and unsealed drill holes. A subsequent comparison of cancellous versus cortical bone showed a P value of 0.04. Statistically significant differences among the means were also found for the C_{max} (P < 0.01) and $T_{50\% \text{ of } Cmax}$ (P < 0.01) values for plasma and the three different tissues (data not shown). For these parameters, a significant difference was found for the C_{max} (P < 0.01) for the subsequent pairwise comparison of cancellous and cortical bone but not for the $T_{50\% \text{ of } Cmax}$.

DISCUSSION

In this study, we have demonstrated that *in vitro* RR_{gain} equaled RR_{loss} over a relevant range of concentrations and that RR was independent of the concentration. When temperature was increased from 20°C to 40°C, an insignificant increase in the recovery of 7.6% was found. It was also shown that *in vivo* RR_{loss} remained constant over a relevant period of time. Accordingly, MD seems to be a valuable tool for assessing tissue distribution of cefuroxime in studies lasting several hours and with possible physiological changes in temperature. This is in agreement with the findings in previous studies (7, 27, 28).

Bone is a compact tissue. Thus, it is necessary to create drill holes in order to implant MD catheters. This inevitably raises the question as to whether MD measurements of cefuroxime in drill holes solely reflect bone drug concentrations or rather a mixed tissue drug concentration stemming from the actual presence of cefuroxime in bone and a contribution of cefuroxime diffusing into the drill hole from the surrounding soft tissues. Our results show identical pharmacokinetic parameters for sealed and unsealed drill holes, with parallel and almost overlapping time-concentration profiles. If a substantial diffusion of cefuroxime should have occurred from the surroundings to the unsealed drill holes, the pharmacokinetic parameters for these holes should have resembled findings in subcutaneous tissue and/or plasma, which was not the case. In addition to the common pharmacokinetic parameters, we included $T_{50\% \text{ of } Cmax}$ in the analysis for the follow-

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TABLE 2 Nev pharmacokinetic parameters for plasma, subcutaneous tissue, and cancellous bon	TABLE 2 Key pharmacokinetic	parameters for plasma	, subcutaneous tissue,	and cancellous bone
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Pharmacokinetic					
parameter ^a	Total plasma	Free plasma	Subcutaneous tissue	Cancellous bone	P^{c}
AUC_{0-last} (min · µg/ml)	8,450 ± 1,911	$6,227 \pm 1,449$	$3,386 \pm 1,120^d$	$2,292 \pm 660^{e}$	0.01
$C_{\rm max}$ (µg/ml)	154.4 ± 45.7	109.9 ± 31.4	24.0 ± 6.5^{e}	22.9 ± 6.3^{e}	< 0.01
$T_{\rm max}$ (min)	30.0 ± 0	30.0 ± 0	50.0 ± 8.2	50.0 ± 0	
$t_{1/2}$ (min)	60.8 ± 9.6	66.6 ± 11.5	85.4 ± 34.9	56.4 ± 6.6	0.40
$T_{50\% \text{ of } \text{Cmax}}(\text{min})$	11.0 ± 1.7	10.7 ± 2.2	16.8 ± 1.4^{e}	22.5 ± 1.5^{e}	< 0.01
fAUC _{ISF tissue} /fAUC _{plasma}			0.58 ± 0.19	0.45 ± 0.13	0.57^{f}

 a C_{max} peak drug concentration in serum; T_{max} , time to C_{max} ; $t_{1/2}$, half-life at β phase; AUC_{0-last}, area under the concentration-time curve from 0 to the last measured value; fAUC_{1SF tissue}/fAUC_{plasma}, free area under the concentration-time curve ratio of interstitial fluid (ISF) tissue to free plasma.

^b All values (other than P values) are expressed as the mean \pm SEM.

^c One-way ANOVA for free plasma, subcutaneous tissue, and cancellous bone.

 $^{d}P < 0.05$ for comparison with the corresponding free plasma value.

 e P < 0.01 for comparison with the corresponding free plasma value.

f By t test.

ing reasons. Considering the fact that the concentration-time profiles are rather flat around C_{\max} combined with a temporal resolution of 30 min, T_{\max} is an insensitive measure for detecting differences in the kinetics. $T_{50\% \text{ of } C\max}$, on the other hand, is situated on the steepest part of the curve, making evaluation and comparison of tissue penetration more accurate.

Over the last 2 decades, an increasing number of studies have demonstrated incomplete tissue penetration for different combinations of drug and tissue under both physiological and pathological conditions (2-8). This emphasizes the need not only to characterize the pharmacokinetics of an antimicrobial drug in a specific tissue but also under specific conditions. In this study, tissue distribution was analyzed using a number of pharmacokinetic parameters. For all extravascular tissues, a heterogeneous tissue distribution was demonstrated. Significant differences in AUC values were found for all tissues compared to free plasma. The lowest AUC was found in cortical bone, reaching only about 1/6 of the corresponding free plasma value. The same ratio for cancellous bone was just >1/3. Both the AUC and C_{max} values were significantly higher in cancellous than in cortical bone, suggesting that bone may not be considered one distinct compartment. It is noteworthy that cefuroxime penetration was impaired for all investigated tissues, as expressed in several pharmacokinetic parameters. Altogether, the findings in this study support the fact that complete tissue penetration cannot be taken for granted. In turn, it can be speculated that poor bone penetration may partly account for the prolonged treatment needed for osteomyelitis and PJIs and for the high failure rate when treating these infections.

Determining the concentration of antimicrobials in bone remains a difficult task. The vast majority of studies assessing this challenge have done so using bone biopsy specimens. This method, however, has considerable limitations, not only regarding the method but also because of the lack of standardized procedures in terms of sample preparation, drug analysis, data handling, and reporting (10, 29). Regarding the method as such, it allows only for measurement of the total tissue concentration and not the free and unbound extracellular fraction, which is known to be pharmaceutically active (30, 31). Due to the inherent invasiveness of bone biopsy specimens, samples can be harvested only during surgery, providing poor temporal resolution. Moreover, concentrations are given by weight and not by volume, which makes it difficult to relate the findings to established pharmacodynamic endpoints (10, 29). For β -lactams, it is generally recommended that the concentration of the drug exceeds the MIC for suspected microorganisms for \geq 50% of a dosing interval, leaving the time exceeding the MIC ($T_{\rm MIC}$) as the most important pharmacokinetic parameter for this group of antimicrobials (32). Due to the restricted temporal resolution provided with bone biopsy specimens, $T_{\rm MIC}$ cannot be assessed with this method. The present study suggests that microdialysis can solve these major limitations that are encountered with bone biopsy specimens.

When performing MD experiments, it is important to realize that there will often be a trade-off between experimental needs and the ideal setup. Examples of factors contributing to the limitations are the analytical lower limit of quantification, injection volume, membrane length, and flow rate. Also, these are the adjustable experimental factors that will ultimately decide the RR and the temporal resolution. Our setup resulted in an in vivo RR of approximately 18% for cortical bone MD measurements. It is generally recommended that recovery should be >20%, as lower levels of recovery are relatively more exposed to the standard deviations associated with the preanalytical handling as well as chemical analysis (33). The resulting variations will increase exponentially with decreasing recovery. This disadvantage should be remembered when interpreting results obtained with MD. Nevertheless, in our case, where the depth of the drill holes limits membrane length and the relatively short half-life of cefuroxime calls for high temporal resolution, an in vivo RR of 18% seems acceptable.

From a clinical perspective, the findings of the present study are of considerable importance. A drug like cefuroxime reaches a high peak concentration in plasma after a bolus injection, but it is rapidly cleared from plasma because of excretion and redistribution. For the drug to exceed the MIC in its target site for a sufficient period of time, quick tissue equilibration seems mandatory for obtaining relevant antimicrobial action. For cortical bone in particular, penetration seems to be incomplete and delayed, as shown in this study, and it can be questioned if bolus injections of drugs with short half-lives are suitable when treating or preventing infections in bone.

In conclusion, the findings in the present study demonstrate that MD is a valuable and reliable method for evaluating the tissue distribution of cefuroxime. Calibration can be performed by means of retrodialysis, and studies can be prolonged for several hours. The problem of assessing cefuroxime concentrations in bone can be overcome by placing the MD catheters in drill holes, and sealing of these seems unnecessary. As such, we find that MD might be a valuable tool for clinical studies on bone pharmacokinetics. The uneven tissue distribution that was demonstrated in this study is important and may account for treatment failures in the clinical setting.

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Continuous versus Short-Term Infusion of Cefuroxime: Assessment of Concept Based on Plasma, Subcutaneous Tissue, and Bone Pharmacokinetics in an Animal Model

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The relatively short half-lives of most β -lactams suggest that continuous infusion of these time-dependent antimicrobials may be favorable compared to short-term infusion. Nevertheless, only limited solid-tissue pharmacokinetic data are available to support this theory. In this study, we randomly assigned 12 pigs to receive cefuroxime as either a short-term or continuous infusion. Measurements of cefuroxime were obtained every 30 min in plasma, subcutaneous tissue, and bone. For the measurements in solid tissues, microdialysis was applied. A two-compartment population model was fitted separately to the drug concentration data for the different tissues using a nonlinear mixed-effects regression model. Estimates of the pharmacokinetic parameters and time with concentrations above the MIC were derived using Monte Carlo simulations. Except for subcutaneous tissue in the short-term infusion group, the tissue penetration was incomplete for all tissues. For short-term infusion, the tissue penetration ratios were 0.97 (95% confidence interval [CI], 0.67 to 1.39), 0.61 (95% CI, 0.51 to 0.73), and 0.45 (95% CI, 0.36 to 0.56) for subcutaneous tissue, cancellous bone, and cortical bone, respectively. For continuous infusion, they were 0.53 (95% CI, 0.33 to 0.84), 0.38 (95% CI, 0.23 to 0.57), and 0.27 (95% CI, 0.13 to 0.48) for the same tissues, respectively. The absolute areas under the concentration-time curve were also lower in the continuous infusion group. Nevertheless, a significantly longer time with concentrations above the MIC was found for continuous infusion up until MICs of 4, 2, 2, and 0.5 µg/ml for plasma and the same three tissues mentioned above, respectively. For drugs with a short half-life, like cefuroxime, continuous infusion seems to be favorable compared to short-term infusion; however, incomplete tissue penetration and high MIC strains may jeopardize the continuous infusion approach.

The relatively short half-lives of most β -lactams suggest that extended infusion (EI) or continuous infusion (CI) of these time-dependent antimicrobials may be favorable compared to short-term infusion (STI). Nevertheless, different meta-analyses evaluating CI versus STI of various time-dependent antimicrobials have failed to convincingly demonstrate improved clinical outcomes on mortality and clinical cure (1–6). It is noteworthy, however, that in the majority of studies included in these metaanalyses, the total daily dose of antimicrobials was lower for patients treated with EI or CI (1, 2, 5). In a subset of randomized controlled trials (RCTs) in which the total daily dose was equivalent in the two intervention arms, the clinical failure rate was lower for patients treated with CI (1).

Inferences about the dosing regimens of antimicrobials are commonly based on plasma pharmacokinetics-pharmacodynamics (PK-PD) indices, despite the fact that the majority of bacterial pathogens reside in the interstitial space of solid tissues. However, incomplete and uneven tissue distribution was eventually demonstrated in a number of studies for different combinations of drug and tissue (7–13). As the gap between steady-state plasma concentrations and MICs may be rather limited using CI, incomplete tissue penetration may partly explain why improved clinical outcomes for CI have been difficult to demonstrate, particularly when the total daily dose is reduced.

Deep-seated orthopedic infections, like osteomyelitis and implant-associated infections (IAI), are difficult to treat, often requiring extensive surgical debridement and long-lasting antimicrobial therapy (14). In a recent porcine study, we demonstrated substantially impaired bone and subcutaneous tissue (SCT) penetration of cefuroxime (15). The pharmacokinetic profiles suggested that EI or CI of the drug might attain increased time with tissue concentrations above the MIC (T>MIC) for relevant microorganisms.

Ultimately, the dosing regimens of antimicrobials should be based on results of RCTs for a specific combination of drug, bug, and disease. However, in order to increase the probability of obtaining useful information from such trials, the selection of dosing regimens should be guided by the results from tissue pharmacokinetic studies.

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FIG 1 Mean observed concentrations (dots) and population-fitted concentration-time profiles (lines) for short-term infusion (top) and continuous infusion (bottom).

In the present study, we used the microdialysis (MD) technique to obtain the pharmacokinetic parameters of cefuroxime in the SCT and bone of pigs receiving 1,500 mg of cefuroxime as either traditional STI or CI. The primary endpoint of this randomized trial was the T>MIC, which is the key PK-PD index for cephalosporins (16).

MATERIALS AND METHODS

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

Animals, anesthesia, and surgical procedures. Twelve female pigs were included in the study (Danish Landrace breed, weighing 73 to 79 kg). Anesthesia was maintained during the entire study period using a combination of propofol (200 to 550 mg/h, continuous infusion) and fentanyl (0.4 to 0.85 mg/h, continuous infusion). Body temperature was kept within the range of 37.5 to 39.0°C. Normal kidney function, assessed by plasma creatinine level, was confirmed for all pigs before inclusion in the study. pH was monitored during the entire study using arterial gas analysis and was kept within a range of 7.36 to 7.54 by regulating tidal volume

and respiratory frequency. The surgical procedures were initiated immediately after the induction of anesthesia. Using two distinct anteromedial approaches, MD catheters were placed in drill holes in the cortical bone of the anterior margin of the tibia and in cancellous bone within the tibial condyles. The depths of the drill holes were 14.5 ± 0.5 mm and 20 ± 1 mm for the cortical and cancellous drill holes, respectively. A 2-mm drill was used for both sites. Drilling was stopped every few seconds in order not to overheat the bone. Before wound closure, the catheters were fixed to the skin with a single suture. At the end of each experiment, it was verified by autopsy that the catheters had not been displaced from the drill holes. The intracortical location of the cortical drill holes was assessed by postmortem computed tomography (CT) scans of the tibia.

In addition to the two bone catheters, a reference catheter was placed in the SCT of the abdomen, according to the guidelines of the manufacturer.

Microdialysis and sampling procedures. The principles of MD have been described in detail elsewhere (17–19). Briefly, MD is a minimally invasive probe-based technique that allows for continuous sampling of small unbound water-soluble molecules in the interstitial spaces of virtually all tissues (10, 20–24). The diffusion of solutes takes place across a semipermeable membrane at the tip of the probe along the concentration gradient. As the probe is continuously perfused, equilibrium will never Downloaded from http://aac.asm.org/ on February 23, 2015 by STATE LIBRARY IN AARHUS



FIG 2 Observed versus simulated individual and population cefuroxime concentrations for free plasma, SCT, cortical bone, and cancellous bone.

Antimicrobial Agents and Chemotherapy

Pharmacokinetic	Mean (95% confidence interval) values in:				
parameter ^a	Free plasma	SCT^b	Cancellous bone	Cortical bone	
$AUC_{0-last} (min \cdot \mu g/ml)$	2,919 (2,615–3,263)	2,820 (1,986–3,986)	1,786 (1,557–2,049)	1,319 (1,096–1,586)	
$C_{\rm max}$ (µg/ml)	70.2 (64.5-76.4)	45.4 (31.7-64.7)	24.4 (20.7–28.8)	11.5 (8.1-16.4)	
$T_{\rm max}$ (min)	3.7 (3.7–3.8)	21.0 (16.6-26.5)	37.7 (34.0-41.8)	38.3 (26.8-54.7)	
$t_{1/2}$ (min)	26.1 (24.3–28.0)	35.1 (25.0–49.2)	36.0 (31.0-41.8)	65.9 (50.6-85.9)	
fAUC _{tissue} /fAUC _{plasma}		0.97 (0.67–1.39)	0.61 (0.51-0.73)	0.45 (0.36-0.56)	

 TABLE 1 Key STI pharmacokinetic parameters for free plasma, subcutaneous tissue, cancellous bone, and cortical bone

^{*a*} 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; fAUC_{tissue}/fAUC_{plasma}, tissue penetration expressed as the ratio of free AUC tissue to free AUC plasma.

^b SCT, subcutaneous tissue.

occur, and the concentration in the dialysate will represent only a fraction of the actual concentration in the tissue. This fraction is referred to as relative recovery (RR). Consequently, a calibration procedure, in which the RR is determined, is imperative if absolute tissue concentrations are to be determined.

The MD system in the present study consisted of CMA 63 catheters (membrane length, 10 mm; molecular cutoff, 20 kDa) and CMA 107 precision pumps (M Dialysis AB, Stockholm, Sweden). Following implantation, the catheters were perfused with 0.9% NaCl containing 5 μ g/ml cefuroxime. The perfusion rate was 2 μ l/min. When surgery was completed, a 30-min tissue equilibration period was allowed. The probes were then calibrated using the retrodialysis method (25) by collecting a sample over a 30-min interval. The RR was calculated using the following equation:

$$RR = 1 - \frac{C_{\text{out}}}{C_{\text{in}}} \tag{1}$$

where C_{in} is the cefuroxime concentration in the perfusate and C_{out} is the concentration in the dialysate. Individual *in vivo* calibration was performed for all catheters.

Following calibration, the perfusate was changed to blank 0.9% NaCl, and a 105-min washout period was allowed. A dialysate was collected during the last 20 min of this period in order to assess the efficacy of washout. The animals were then randomly assigned to receive 1,500 mg of cefuroxime (Fresenius Kabi AB, Sweden) as either STI (over 15 min) or CI (500 mg as a loading dose over 5 min, followed by CI of the remaining 1,000 mg over 7 h 55 min). Fifteen hundred milligrams was chosen because it is the standard dose for orthopedic procedures in Denmark, and because the weight of the animals resembled that of an average human being. In both groups, the dialysates were collected every 30 min for 8 h, starting at the beginning of the infusions. For the subsequent data analysis, the cefuroxime concentration in the dialysates was attributed to the midpoint of the sampling interval. The absolute tissue concentrations (C_{tissue}) were obtained by correcting for RR using the following equation:

$$C_{\rm tissue} = \frac{C_{\rm out}}{RR} \tag{2}$$

Blood samples were drawn from a central venous catheter halfway through every dialysate sampling interval.

Handling of samples. The dialysates were immediately frozen and stored at -80° C until analysis. The venous blood samples were stored at 5°C for a maximum of 20 h before being centrifuged at 3,000 × *g* for 10 min. The plasma aliquots were then frozen and stored at -80° C until analysis.

Quantification of cefuroxime concentrations. The dialysate and free plasma concentrations of cefuroxime were quantified using a validated ultrahigh-performance liquid chromatography assay (reference 15 and M. Tøttrup and T. F. Hardlei, unpublished data). The intrarun (interrun) imprecisions (in percent coefficients of variation [%CVs]) were 5.6% (6.8%) at 0.25 μ g/ml, 4.3% (4.7%) at 2.5 μ g/ml, and 2.6% (2.8%) at 10 μ g/ml for the dialysates. For the free plasma concentration, the intrarun

(interrun) imprecision rates were 1.8% (6.5%) at 9.2 µg/ml and 1.6% (6.2%) at 38 µg/ml. The lowest limit of quantification was defined as the lowest concentration to be measured with an intrarun %CV of <20% and was found to be 0.06 µg/ml for both dialysates and the free cefuroxime concentration in plasma.

Pharmacokinetic analysis and statistics. (i) Population PK modeling. We explored one- and two-compartment models with zero- and first-order kinetics in order to find the best description of the drug concentration in each tissue. A two-compartment model with zero-order appearance and first-order clearance was found to provide the best description of the cefuroxime concentrations in SCT, cancellous bone, and cortical bone. For the free plasma concentrations, an ordinary two-compartment model with first-order kinetics provided the best description of the drug concentration. For CI, the drug concentrations in SCT, cancellous bone, and cortical bone are given using the following equation:

$$C_{\text{tissue}}(t) = \begin{cases} \frac{k_1}{k_3}(1 - e^{-k_3 t}), & t \le \frac{x_0}{k_1 - I} \\ \frac{I}{k_3} + \left(\frac{k_1 - I}{k_3}e^{k_3\frac{x_0}{k_1 - I}} - \frac{k_1}{k_3}\right)e^{-k_3 t}, & t > \frac{x_0}{k_1 - I} \end{cases}$$
(3)

where k_1 is the appearance rate, k_3 is the clearance rate, t is time, I is the continuous infusion rate, and x_0 is the plasma concentration at time zero. For plasma, the drug concentration (C_{plasma}) is given by:

$$C_{\text{plasma}}(t) = \frac{k_1 x_0}{k_3 - k_1} (e^{-k_1 t} - e^{-k_3 t}) + \frac{I}{k_3 - k_1} \left(1 - e^{-k_1 t} - \frac{k_1}{k_3} (1 - e^{-k_3 t}) \right), \quad t \ge 0 \quad (4)$$

The drug concentration in the case of STI is obtained from the expressions above by putting I equal to zero.

From these expressions, it is possible to determine the *T*>MIC, the area under the concentration-time curve (AUC), peak drug concentration (C_{max}), time to C_{max}), and in the case of STI half-life ($t_{1/2}$) (calculated by \log_2/k_3), though in the case of the *T*>MIC for plasma, this has to be done numerically.

(ii) Statistical analysis. The two-compartment model was fitted to the drug concentration data separately for the different tissues using a nonlinear mixed-effects regression model with a random animal effect for each of the parameters k_1 , k_3 , x_0 , and *I*. The washout concentrations were low and as such were neglected in the analysis. Monte Carlo simulation was used to determine 95% confidence intervals for the *T*>MIC, AUC, C_{\max} , T_{\max} , $t_{1/2}$, and the ratio between the AUCs, as well as the test for no difference between bolus and continuous infusions with regard to these quantities. More specifically, this was done by simulating 50,000 curves from the joint asymptotic normal distribution of the parameter estimates, calculating the derived quantities for each set of parameters, and then determining the 95% confidence intervals from the empirical distribution of these. The confidence intervals for the ratio between the AUCs were derived under the additional assumption that parameter estimates corresponding to the different tissues were independent. The data were ana-

TABLE 2 Key CI pharmacokinetic parameter	rs for free plasma,	subcutaneous tissue,	cancellous bone, and cortical bone
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Pharmacokinetic	Mean (95% confidence int	Mean (95% confidence interval) values in:				
parameter ^a	Free plasma	SCT^b	Cancellous bone	Cortical bone		
AUC_{0-last} (min · µg/ml)	3,437 (2,586–4,578)	1,809 (1,240–2,636)	1,296 (859–1,759)	919 (471–1,545)		
$C_{\rm max}$ (µg/ml)	51.4 (28.0–94.2)	12.7 (9.0–17.8)	6.1 (3.8-8.3)	2.5 (0.9-5.8)		
$T_{\rm max}$ (min)	2.7 (2.4–3.1)	16.6 (14.3–19.4)	39.9 (33.2-48.9)	52.1 (31.4-95.8)		
fAUC _{tissue} /fAUC _{plasma}		0.53 (0.33-0.84)	0.38 (0.23-0.57)	0.27 (0.13-0.48)		

 a 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} ; fAUC_{tissue}/fAUC_{plasma}, tissue penetration expressed as the ratio of free AUC tissue to free AUC plasma.

^b SCT, subcutaneous tissue.

lyzed using R version 3.0.2 (R Core Team, Vienna, Austria) with the package nlme.

found in Tables 3 and 4, respectively. In Fig. 4, the relationship between the *T*>MIC and MIC is depicted for free plasma, SCT, cancellous bone, and cortical bone.

RESULTS

All 12 experiments were completed, and no MD-related problems were encountered. In one of the pigs receiving CI, the postmortem CT scan revealed that the cortical drill hole penetrated to the bone marrow. Thus, the measurements obtained from this hole were excluded from the analysis. The mean \pm standard deviation [SD] *in vivo* RRs were 15.4% \pm 6.5%, 20.9% \pm 10.4%, and 13.6% \pm 5.8% for cortical bone, cancellous bone, and SCT, respectively. The mean \pm SD concentrations in the washout samples were 0.09 \pm 0.07 µg/ml, 0.02 \pm 0.02 µg/ml, and 0.03 \pm 0.03 µg/ml for the same anatomical sites, respectively.

The mean observed concentrations and population fitted concentration-time profiles are depicted in Fig. 1. The observed versus fitted cefuroxime concentrations are shown in Fig. 2.

Estimates of the key standard pharmacokinetic parameters for free plasma, SCT, cancellous bone, and cortical bone are shown in Tables 1 and 2. The corresponding mean concentration-time profiles are displayed in Fig. 3. Comparisons of the AUC, tissue penetration ratios, and T>MIC between the STI and CI group can be

Except for SCT in the STI group, tissue penetration was incomplete for all tissues. Both the tissue AUCs and tissue penetration ratios were generally found to be lowest in the CI group. For cancellous bone, the tissue penetration ratio for CI was significantly lower than that of STI, whereas for SCT and cortical bone, this ratio only just failed to be significantly lower for CI. Nevertheless, a significantly longer T>MIC was found for CI up to MICs of 4 μ g/ml, 2 μ g/ml, 2 μ g/ml, and 0.5 μ g/ml for plasma, SCT, cancellous bone, and cortical bone, respectively. The same is true for lower MICs for all tissues, but with increasing MIC, the differences in T>MIC between STI and CI leveled out, with T>MIC eventually becoming higher for STI than that for CI for high MICs in the solid tissues (Fig. 4 and Table 4).

DISCUSSION

This is the first article to report concurrent pharmacokinetics of a β -lactam antibiotic in plasma, SCT, and bone administered as STI and CI. The main finding is that a longer *T*>MIC can be achieved using CI rather than STI of a drug with a short



FIG 3 Mean concentration-time profiles for short-term and continuous infusion of cefuroxime for free plasma, SCT, cancellous bone, and cortical bone. The error bars represent standard deviations.

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TABLE 3	Comparison	of AUC and	tissue	penetration
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	Mean (95% confidence interval)	for:	
Parameter ^a	STI	CI	P value
$\overline{AUC_{0-last} (\min \cdot \mu g/ml)}$ for:			
Free plasma	2,919 (2,615–3,263)	3,437 (2,586–4,578)	0.33
SCT	2,820 (1,986-3,986)	1,809 (1,240–2,636)	0.1
Cancellous bone	1,786 (1,557–2,049)	1,296 (859–1,759)	0.06
Cortical bone	1,319 (1,096–1,586)	919 (471–1545)	0.18
fAUC _{tissue} /fAUC _{plasma} for:			
SCT	0.97 (0.67–1.39)	0.53 (0.33-0.84)	0.05
Cancellous bone	0.61 (0.51-0.73)	0.38 (0.23-0.57)	0.02
Cortical bone	0.45 (0.36–0.56)	0.27 (0.13–0.48)	0.07

^{*a*} AUC_{0-last}, area under the concentration-time curve from 0 to the last measured value; *f*AUC_{tissue}/*f*AUC_{plasma}, tissue penetration expressed as the ratio of free AUC tissue to free AUC plasma.

half-life, like cefuroxime. Nevertheless, the data also clearly indicate that with a CI approach, the gap between tissue concentrations and MIC may be limited or even inversed, depending on the tissue type and MIC. A similar relationship has been found for piperacillin (26). From this point of view, it is not surprising that convincing evidence for the superiority of CI over STI has been difficult to establish, despite the apparent theoretical advantages. Accordingly, the practice of lowering the total daily dose for CI seems unsafe.

In recent years, therapeutic drug monitoring (TDM) has be-

TABLE 4 Comparison of time above the MIC

	Mean (95% confi	dence interval) for	
<i>T</i> >MIC for tissues	(min):		
by concn (µg/ml)	STI	CI	P value
0.5			
Plasma	190 (178-205)	465 (465-465)	< 0.001
SCT	249 (187-332)	464 (464-465)	< 0.001
Cancellous bone	239 (215-266)	463 (461-464)	< 0.001
Cortical bone	335 (279–401)	457 (450-460)	< 0.001
1			
Plasma	164 (153–177)	465 (465-465)	< 0.001
SCT	214 (162-283)	464 (463-464)	< 0.001
Cancellous bone	202 (183-224)	460 (457-462)	< 0.001
Cortical bone	268 (227–315)	448 (0-455)	0.12
1.5			
Plasma	149 (139–160)	465 (465-465)	< 0.001
SCT	193 (147-255)	463 (463-464)	< 0.001
Cancellous bone	180 (165–199)	457 (179-460)	< 0.001
Cortical bone	227 (195–263)	435 (0-448)	0.07
2			
Plasma	139 (129–149)	465 (465-465)	< 0.001
SCT	178 (136-234)	463 (189-463)	< 0.001
Cancellous bone	165 (151-182)	453 (113-458)	0.001
Cortical bone	199 (172–228)	185 (0-439)	0.9
4			
Plasma	113 (104-121)	465 (465-465)	< 0.001
SCT	142 (109–185)	118 (68–243)	0.6
Cancellous bone	127 (117–139)	61 (0-121)	0.03
Cortical bone	126 (106–145)	0 (0-122)	< 0.001

come increasingly available as a routine analysis method in the daily clinical setting. As the pharmacokinetics of β -lactams has been shown to be unpredictable and display considerable interindividual variation, particularly in critically ill patients (27, 28), TDM is expected to optimize PK-PD target attainment and thus ultimately improve the treatment of serious infections (29–31). Our data suggest that care should be taken when adjusting antimicrobial dosing that is based merely upon the plasma concentrations and MICs of isolated pathogens, as incomplete tissue penetration may result in subtherapeutic tissue concentrations at the site of the infection. CI obviously has the potential to improve target attainment. However, if tissue penetration is substantially incomplete, CI may result in subtherapeutic concentrations at the site of infection for the entire dosing interval. On the other hand, STI may provide therapeutic concentrations but only for a limited part of the dosing interval. Clearly, tissue concentrations cannot be measured in the individual patient, but the available data on tissue pharmacokinetics for the specific combination of drug and infection should be integrated in the clinical decision making in order to prevent treatment failure. It was recently argued that an aggressive target of obtaining free plasma concentrations of 4 to 5 times the MIC for the entire dosing interval may be more predictive of a successful clinical outcome (31). At least for critically ill patients, in whom heterogeneous tissue distribution has been well documented (10, 11), our findings for CI support this aggressive approach.

A key finding of this study is the heterogeneous tissue distribution of cefuroxime, which was present regardless of the type of administration of the drug. In agreement with our previous study, the poorest tissue penetration was found for bone (15). Somewhat surprisingly, bone penetration in the CI group was poorer than in the STI group, but this seems partly compensated for by higher free plasma AUCs. Based on the present data, it can be speculated that plasma-tissue equilibrium may be concentration dependent in a dynamic manner. However, this hypothesis obviously needs further investigation.

Our finding of bone penetration ratios of approximately 1:3 to 2:3 suggests that incomplete tissue penetration may partly explain the prolonged antimicrobial treatment needed for osteomyelitis and IAIs. Accordingly, in terms of T>MIC, a standard target of remaining at >2 µg/ml for 50% of the dosing interval was achieved in neither cancellous nor cortical bone using traditional STI. The majority of isolated *Staphylococcus aureus* exhibits MICs



FIG 4 Time with concentrations above MIC-MIC profiles for free plasma, SCT, cancellous bone, and cortical bone. The dotted lines represent 95% confidence intervals.

of 1 μ g/ml (http://mic.eucast.org/Eucast2/SearchController /search.jsp?action=performSearch&BeginIndex=0&Micdif =mic&NumberIndex=50&Antib=46&Specium=-1). Based on the *T*>MIC values for CI, it appears that increased *T*>MIC can be achieved using CI but only for low MICs. Nevertheless, in order to remain above the MIC for the entire dosing interval for pathogens with higher MICs, the total daily dose needs to be increased, which also seems reasonable for serious infections.

Although pigs resemble humans in terms of physiology and anatomy (32), the major limitation of this study is obviously that it is not a clinical study. Consequently, the findings cannot readily be extrapolated to humans or to pathological conditions. Moreover, the pigs had to be kept under general anesthesia during the entire study period, which is known to cause physiological alterations that may affect pharmacokinetics. This also precludes the opportunity to conduct measurements after the administration of multiple doses of cefuroxime. Nonetheless, it seems rational to use this large-animal model to explore the basic concepts of CI versus STI, focusing on the role of antimicrobial tissue penetration. This approach provides a sound foundation for future clinical studies, while attention is drawn to the possible pitfalls of CI and incomplete tissue penetration.

Over the last decade, MD has become the method of choice for obtaining antimicrobial tissue pharmacokinetics, including the particular case of bone (7, 10, 18, 20–24, 26, 33–37). Due to mandatory correction for RR in pharmacokinetic studies, a magnification of the variations associated with the preanalytical sample handling and chemical assay is inherent to the MD approach. These variations will increase exponentially with decreasing recovery (17). Consequently, MD studies should always be interpreted with this possible limitation in mind. Our finding of the comparable variations of the pharmacokinetic parameters in plasma and solid tissues suggests that our setup was adequately reliable in terms of precision and that a significant part of the variation can be regarded as biological.

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In conclusion, the findings in the present study indicate that CI of β -lactams with short half-lives may be favorable compared to STI, if dosed appropriately. These animal data cannot be applied uncritically in a clinical setting, but incomplete tissue penetration of antimicrobials should be considered when planning CI and using TDM. For bone, the tissue penetration was substantially incomplete. The high rates of treatment failure for osteomyelitis and IAIs may therefore partly be attributable to incomplete target site penetration of the antimicrobials. MD seems to be a valuable and reliable tool for investigating these matters, and as such, clinical studies with similar methodological setups are warranted.

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1	Bone, Subcutaneous Tissue and Plasma Pharmacokinetics of Cefuroxime in Total
2	Knee Replacement Patients – a Randomized Controlled Trial Comparing
3	Continuous and Short-term Infusion
4	
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16	Continuous versus Short-term Infusion of Cefuroxime
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27 Abstract

28 The objective of this study was to describe and compare plasma, subcutaneous tissue 29 and bone pharmacokinetics of cefuroxime after traditional short-term infusion (STI) and 30 continuous infusion (CI). Eighteen male patients undergoing total knee replacement 31 were randomly assigned to STI or CI of 1,500 mg of cefuroxime. Measurements of 32 cefuroxime were obtained in plasma, subcutaneous tissue (SCT), cancellous and cortical 33 bone every 30 minutes for 8 hours following surgery. For measurements in solid tissues, 34 microdialysis was applied. Population pharmacokinetic modelling and Monte Carlo 35 Simulations were performed in order to estimate AUCs, and to assess the probability of 36 attaining cefuroxime concentrations above the MIC for 65% (low target) and 90% (high target) of the dosing interval with the actual dose. Tissue penetration was incomplete for 37 SCT and cortical bone in the STI group. In the CI group, low SCT and cortical bone 38 39 penetration were also found, but in this group, the findings were not significant. No differences in AUCs and tissue penetration ratios were found between the two groups. 40 41 Irrespective of tissue and target, CI leads to improved probability of target attainment 42 (PTA) compared to STI. Nevertheless, even for the low target, inadequate PTA (<90%) 43 is achieved in all tissues for high organism MICs of 8 mg/L for both CI and STI. In 44 conclusion, CI of cefuroxime results in favourable tissue exposure in total knee 45 replacement patients compared to STI. Nonetheless, even with this approach, a standard dose of 1,500 mg leads to inadequate PTA in all tissues for high organism MICs. 46

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53 Introduction

54 Osteomyelitis and implant-associated infections (IAI) remain a significant therapeutic 55 challenge. Despite extensive surgical debridement and removal of implants, long-lasting 56 antibiotic therapy is required for successful management of these infections. 57 Nevertheless, treatment failure is not uncommon.

58

A number of attempts have been made towards determination of bone pharmacokinetics of antibiotics. The predominant and traditional bone biopsy method suffers from methodological limitations, and may not be ideal for the task(1-3). Recently, however, the well-known pharmacokinetic tool microdialysis (MD) has been successfully applied for measurement of various antibiotics in drill holes in healthy bone(4-7).

64

65 Cefuroxime is a second-generation cephalosporin. Like other beta lactams, the 66 bactericidal activity is time-dependent. This means that its efficacy is best related to the 67 time that the free concentration is sustained above the MIC $(fT_{>MIC})(8, 9)$. For cephalosporins, it is generally recommended that $fT_{>MIC}$ is sustained for approximately 68 69 40-70% of a dosing interval(8-11). Despite the fact that the majority of bacterial 70 pathogens reside in the interstitial space of solid tissues, inference on dosing regimens 71 of antibiotics is frequently based on plasma pharmacokinetics/pharmacodynamics 72 (PK/PD) indices. In situations with incomplete tissue penetration, this may account for 73 some treatment failures. Accordingly, clinical studies have suggested that a plasma targets of 100% $fT_{>1-5\times MIC}$ are more predictive of a successful outcome(12-14). 74 75 Aggressive targets like these are obviously more suited to accommodate potential 76 impaired tissue penetration.

78 In a recent population pharmacokinetic study of cefuroxime in critically ill patients, it 79 was found that non-standard dosing is necessary in order to achieve effective plasma 80 targets(15). As declared by the authors, a notable limitation of the study is that 81 concentrations at the actual site of infection were not measured. By employing the 82 microdialysis technique in a porcine model, we have recently demonstrated impaired 83 bone penetration of cefuroxime(6, 16). If this is also the case in a clinical setting, bone 84 concentrations of cefuroxime may be inadequate. In order to address this uncertainty, 85 we set out to investigate bone and subcutaneous tissue (SCT) concentrations of 86 cefuroxime using MD. As continuous infusion (CI) may provide an attractive 87 concentration profile for a short half-lived and time-dependent drug like cefuroxime, 88 continuous infusion (CI) was compared with traditional short-term infusion (STI).

89

90 Materials and methods

91 This study was conducted at the Department of Orthopaedic Surgery, Horsens Regional 92 Hospital between September 2013 and July 2014. Quantification of cefuroxime was 93 performed at the Department of Clinical Biochemistry, Aarhus University Hospital. The 94 study was approved by the Ethics Committee of the Central Denmark Region 95 (registration number 1-10-72-161-13) and the Danish Health and Medicines Authority 96 (EudraCT number 2013-001138-17). The study was conducted in accordance with the 97 Declaration of Helsinki and the ICH Harmonised Tripartite Guideline for Good Clinical 98 Practice. The GCP unit at Aalborg and Aarhus University Hospitals conducted the 99 mandatory monitoring procedures.

100

101 Study design and patients

102 The study was an open-labelled randomised controlled trial. Block randomisation was103 applied with blocks of 6 patients, and an allocation ratio of 1:1. The random allocation

104 sequence was implemented using numbered envelopes (provided by the Pharmacy at 105 Aarhus University Hospital). Competent male patients were offered enrolment in the 106 study if they were scheduled for a total knee replacement (TKR). The patients were 107 identified in the outpatient clinic by the two operating surgeons. Written informed 108 consent was obtained from all patients. Exclusion criteria included the following: 109 allergy to cefuroxime or vancomycin, on-going treatment with cefuroxime, and 110 clinically reduced renal function. Based on T_{>MIC}, the primary outcome is the 111 probability of attaining specified T_{>MIC} in the different tissues over an 8 hour period. 112 Area under the concentration-time curves (AUC) and tissue penetration ratios are 113 secondary outcomes.

114

115 Study drug

According to the randomisation, the patients were given 1,500 mg of cefuroxime (Fresenius Kabi AB, Sweden) intravenously in a peripheral catheter as STI (over 15 min) or CI (500 mg as loading dose over 5 min followed by CI of the remaining 1,000 mg over 7 hours and 55 min). Cefuroxime was administered after the surgical procedures and calibration of the MD catheters. As preoperative infection prophylaxis, all patients were given 1,000 mg of vancomycin prior to surgery.

122

123 Study procedures

124

125 Surgery

126 At the end of TKR surgery, MD catheters were placed in drill holes in cancellous bone 127 in the medial tibial condyle and in cortical bone in the anterior margin of the tibial 128 diaphysis. The medial tibial condyle was accessed via the TKR incision, while the 129 anterior margin of the tibia was accessed via a small anterior incision. A new 2 mm drill

130 was used for each patient. The depths of the drill holes were aimed to be 20 and 15 mm, 131 respectively. When drilling in cortical bone, saline was continuously applied, and 132 drilling was ceased every few seconds in order not to overheat the bone. At both 133 locations, the catheters were tunnelated approximately 2-3 cm before entering the drill 134 holes. In addition to the bone catheters, a SCT catheter was placed in the medial part of 135 the thigh according to the guidelines of the manufacturer. In order to prevent 136 displacement, all catheters were fixed to the skin with a single suture. At the end of 137 surgery, a mixture of 150 mL ropivacaine (2mg/mL), 1.5 mL toradol (30 mg/mL) and 138 0.75 mL adrenaline (1 mg/mL) was injected locally in the soft tissues surrounding the 139 knee, intraarticularly and in the posterior joint capsule of the knee.

140

141 Microdialysis and sampling procedures

142 MD is a probe-based method, which allows for continuous sampling of water-soluble 143 molecules, like the majority of antibiotics, in the interstitial space of most tissues(4, 5, 144 17-20). The diffusion of molecules follows the concentration gradient across a 145 semipermeable membrane at the tip of the probe. As the probe is continuously perfused, 146 equilibrium will never occur. Consequently, the concentration of solutes in the dialysate (i.e. the perfusate leaving the probe) only represents a fraction of the actual 147 148 concentration in the tissue. This fraction is referred to as relative recovery (RR). 149 Accordingly, determination of RR for each separate catheter is imperative if total tissue 150 concentrations are to be determined. Various routine calibration methods exist to solve 151 this task. A more detailed description of MD can be found elsewhere(21-23).

152

In the present study, the MD system consisted of CMA 107 precision pumps (μ-Dialysis
AB, Stockholm, Sweden) and CMA 63 catheters (membrane length 10 mm, molecular
cut-off 20 kilo Daltons). When surgery was completed, the MD catheters were perfused

with 0.9% NaCl containing cefuroxime at a concentration of 5 mg/mL (provided by the Pharmacy at Aarhus University Hospital) at a perfusion rate of 2 μ L/min. After a 30min tissue equilibration period, all catheters were individually calibrated using the retrodialysis method(24) by collecting a sample over a 30-min interval. RR was calculated using the following equation:

161

$$RR = 1 - \frac{C_{out}}{C_{in}}$$

162

163 where C_{in} is the cefuroxime concentration in the perfusate and C_{out} is the concentration 164 in the dialysate. Following calibration, the perfusate was changed to blank 0.9% NaCl, 165 and a 110-min washout period was allowed for. During the last 40 min of this period, 166 two 20-min dialysates were collected in order to evaluate and quantify the effectiveness 167 of washout. Cefuroxime was then administered according to the randomization, which took place during the washout period. This order of events was chosen so that 168 169 placement of the MD catheters during surgery would not be affected by the mode of 170 cefuroxime administration. Regardless of group, dialysates were collected with 30-min 171 intervals for 8 hours starting at the initiation of cefuroxime infusion. Dialysate 172 concentrations of cefuroxime were considered to represent the concentration at the 173 midpoint of the sampling interval. For the subsequent data analysis, the dialysates were 174 corrected for RR using the following equation:

175

176
$$C_{tissue} = \frac{C_{out}}{RR}$$

177

178 In the middle of every dialysate sampling interval, a blood sample was drawn from a179 peripheral venous catheter (cubital vein).

- 181 Before removal of the catheters, a CT scan of the cortical drill hole in the anterior aspect 182 of the tibia was conducted in order to verify that the drill hole had not penetrated to the 183 bone marrow, and that the catheter had not been displaced.
- 184

185 Handling of samples

186 Dialysates were immediately frozen and stored on dry ice for a maximum of 10 hours,

187 after which they were stored at -80°C until analysis. Venous blood samples were stored

188 at 2-8°C for a maximum of 20 hours before being centrifuged at 3,000 g for 10 minutes.

- 189 Plasma aliquots were then frozen and stored at -80°C until analysis.
- 190

191 Quantification of cefuroxime concentrations

Dialysate and plasma concentrations of cefuroxime were quantified using a validated ultra high performance liquid chromatography assay. Briefly, intra- and interrun imprecisions were all below 6.8%, 6.5% and 8.2% for dialysate (assessed at concentrations of 0.2, 2.5 and 10 mg/L), free (assessed at concentrations of 9.2 and 37.7 mg/L) and total plasma concentrations (assessed at concentrations of 12.5 and 50 μ g/mL), respectively. The lower limit of quantification was 0.06 mg/L in dialysates. A detailed description of the assay can be found elsewhere.(6, 16)

199

200 Pharmacokinetic analysis and statistics

201 *Population PK modelling*

202 One- and two-compartment models with zero'th and first order kinetics were explored 203 in order to obtain the best description of the drug concentration in each tissue. An 204 ordinary two-compartment model with first order kinetics, elimination from the second 205 compartment only, and measurement in the first compartment was found to provide the 206 best description of the free plasma concentrations. For the solid tissues, a twocompartment model with zeroth order appearance, first order clearance, no flow back
into the first compartment, elimination from the second compartment only, and
measurement in the second compartment provided the best description. For CI, the drug
concentration in the solid tissues is given by the following equation:

211

$$C_{tissue}(t) = \begin{cases} \frac{k_1}{k_3} (1 - e^{-k_3 t}) &, \quad t \le \frac{x_0}{k_1 - I} \\ \frac{I}{k_3} + \left(\frac{k_1 - I}{k_3} e^{k_3 \frac{x_0}{k_1 - I}} - \frac{k_1}{k_3}\right) e^{-k_3 t}, & t > \frac{x_0}{k_1 - I} \end{cases}$$

212

where k_1 is the appearance rate, k_3 is the clearance rate, t is the time, I is the continuous infusion rate, and x_0 is the plasma concentration at time 0. The restriction $k_I > I$ was made in order to exclude the scenario of a steady drug concentration increase in the first compartment. For free plasma, the drug concentration is given by:

217

$$C_{plasma}(t) = \frac{(k_1 + \beta)(\alpha x_0 + I)}{\alpha(\beta - \alpha)} e^{\alpha t} - \frac{(k_1 + \alpha)(\beta x_0 + I)}{\beta(\beta - \alpha)} e^{\beta t} + \frac{(k_2 + k_3)I}{k_1 k_3}$$

218 where

219
$$\alpha = \frac{-(k_1+k_2+k_3)+\sqrt{(k_1+k_2+k_3)^2-4k_1k_3}}{2}, \qquad \beta = \frac{-(k_1+k_2+k_3)-\sqrt{(k_1+k_2+k_3)^2-4k_1k_3}}{2}$$

and k_2 is the rate constant associated with the flow from the second to the first compartment. In the case of STI, the drug concentration is obtained from the expressions above by putting *I* equal to zero.

223

Based on these expressions, $AUC_{0-\infty}$ and target attainment for any given target could be determined.

226

227 Statistical analysis

228 Using a non-linear mixed effects regression model with a random patient effect for each 229 of the model parameters $(x_0, k_1, k_3 - k_2)$ in case of plasma - and I in connection with 230 continuous infusion) the two-compartment models were fitted to the drug concentration 231 data separately for the different tissues. The concentrations in the washout samples were 232 low, and therefore neglected in the analysis. Monte Carlo simulation was used to 233 determine the probability of target attainment (PTA) for targets of 65% (low target) and 234 90% fT > MIC (high target) for the observation period of 8 hours and 95%-confidence 235 intervals for AUC_{0- ∞} and the ratio between AUCs_{0- ∞}. More specifically, 50000 curves 236 were simulated from the asymptotic multivariate normal distribution of the parameter 237 estimates, and the relevant quantities were calculated for each simulation. Tests for no 238 difference between STI and CI with respect to AUC_{0- ∞} and the ratio between AUCs_{0- ∞} 239 were based on the simulated 95%-confidence intervals and the normal distribution. The 240 data were analysed using R (R v 3.0.2, R core team, Vienna, Austria) with the package 241 nlme.

242

243 Sample size

Selected estimates of difference and standard deviation were based on visual inspection of concentration-time profiles from a previous pharmacokinetic study of cefuroxime.(25) A 50% difference in $T_{>MIC}$ between CI and STI with a standard deviation of 25% for relevant Staphylococcus aureus MIC values was estimated. Based on this, a sample size of 6 patients in each intervention arm was calculated (Stata, version 12.0; Statacorp, USA). In order to accommodate drop-out of patients and/or catheters, the sample size was increased to 9 patients in each intervention arm.

251

252 **Results**

Eighteen patients were included in the study, and the study ended once this prespecified recruitment target was achieved. No MD or cefuroxime adverse events were encountered. One patient acquired prosthetic infection within a month after surgery, but this could not be related to the experiment. After replacement of mobile prosthetic components and antibiotic therapy, the patient was cured.

258

The flow of patients through the trial is shown in figure 1. Reasons for exclusion from analysis of all dialysates from a microdialysis catheter were: blood in all dialysates, displacement of the catheter from the drill hole, bone marrow penetration of the cortical drill hole, and no flow through the catheter after connection to the pump. Patient characteristics can be found in table 1.

264

Mean (\pm SD) *in vivo* RRs were 10.8 \pm 5.0%, 21.8 \pm 10.0% and 13.7 \pm 5.9%, for SCT, cancellous and cortical bone respectively. The mean (\pm SD) concentrations in the first and second washout samples were 0.15 \pm 0.28 mg/L and 0.11 \pm 0.16 mg/L, 0.09 \pm 0.19 mg/L and 0.06 \pm 0.12 mg/L and 0.24 \pm 0.26 mg/L and 0.17 \pm 0.17 mg/L for the corresponding anatomical sites, respectively.

270

Observed concentrations and modelled concentration-time profiles are depicted in
figure 2. Observed vs. fitted cefuroxime concentrations are shown in figure 3. These
plots demonstrate that the model provides a satisfactory description of the cefuroxime
concentration data.

275

276 Classic concentration-time profiles are displayed in figure 4. Comparisons of the 277 corresponding $AUCs_{0-\infty}$ and tissue penetration ratios can be found in table 2. Tissue 278 penetration was incomplete for SCT and cortical bone in the STI group. In the CI group, low SCT and cortical bone penetration were also found, but in this group, the findings
were not statistically significant. No significant differences in AUCs and tissue
penetration ratios were found between the two groups.

282

283 The PTA in the different tissues for the low and the high targets is shown in figure 4. 284 Irrespective of tissue and target, CI leads to improved PTA compared to STI. 285 Nevertheless, even for the low target, inadequate PTA (<90%) is achieved in all tissues 286 for high organism MICs of 8 mg/L (Eischeria coli breakpoint, see eucast.org). For MICs 287 of 4 mg/L (Staphylococcus aureus breakpoint, see eucast.org) and application of the low 288 target, CI leads to adequate PTA in all tissues but cortical bone, for which this 289 combination of target and MIC results in an 80% PTA. Using the low target, STI results 290 in adquate PTA for MICs of 4 mg/L in cancellous bone and MICs of 2 mg/L in cortical 291 bone, but in plasma and SCT, the PTA was just inadequate for MICs of 2 mg/L. Except 292 for cancellous bone, the differences in PTA when applying the high target instead of the 293 low are negligeable for CI, whereas for STI, af substantial reduction in PTA is seen for 294 all tissues.

295

296 **Discussion**

297 This is the first article to report concurrent clinical pharmacokinetics of an antibiotic in 298 plasma, SCT and bone administered as STI and CI. The main finding is that CI of 299 cefuroxime results in improved tissue exposure in all tissues compared to STI. 300 Nevertheless, both CI and STI of 1,500 mg of cefuroxime were inadequate for high 301 organism MICs of 8 mg/L, which may be encountered in an ortopaedic setting. Using 302 the 4 mg/L EUCAST breakpoint for Staphylococcus aureus and the low target, CI 303 resulted in adequate PTAs of over 90% for free plasma, SCT and cancellous bone. For 304 cortical bone, the PTA for this breakpoint and target was 80%. The correponding PTAs

were considerably lower for STI. Two previous studies on meropenem and piperacillin also found advantageous subcutaneous tissue exposure after CI(26, 27). From a PK/PD point of view, these findings support the use of CI for short half-lived and timedependent drugs. The lack of clinical evidence to support the use of CI may be related to the fact that CI traditionally has been considered a mean to lower the total daily drug dose(28-33). In fact, in a subset of RCTs where the total daily dose was equivalent in both intervention arms, clinical failure rates were lower for patients treated with CI(28).

312

313 Tissue targets for cefuroxime and other antimicrobials for prevention of orthopaedic 314 surgical site infections are somewhat unknown(34). Indeed, PK/PD relationships are 315 commonly described using plasma pharmacokinetics(35, 36). For time-dependent 316 drugs, which are commonly used for antimicrobial surgical prophylaxis, it is 317 recommended that tissue and plasma concentrations exceed in vitro MIC values of 318 relevant pathogens throughout the procedure (34). In relation to total hip replacement, a 319 practice of administering antimicrobials four times on the day of surgery has been 320 shown to result in lower infection rates compared to one perioperative dose(37). 321 Accordingly, antimicrobial prophylaxis is commonly continued for 24 hours following 322 joint replacement surgery (38). In our analyses, we only simulated the doses that were 323 actually administered. This decision was governed by the fact that the model parameter 324 estimates may be rather versatile in the immediate postoperative period. Consequently, 325 prediction of other scenarios was considered somewhat inappropriate. In the lack of 326 established tissue targets for the present situation, we used traditional plasma PK/PD 327 indices. Fundamental for a CI approach is a target of at least 100% fT > MIC. We did, 328 however, assess the first dosing interval of CI, which was initiated with a 5-min bolus 329 infusion of 500 mg. Moreover, tissue distribution was also associated with a time delay. Consequently, attaining 100% $fT_{>MIC}$ was impossible, and accordingly not a meaningful 330

target. Nevertheless, in case of unchanging pharmacokinetics, a 100% $fT_{>MIC}$ target would have resulted in similar PTA for subsequent dosing intervals. Assuming that the pharmacokinetics remain stable, the short half-life of cefuroxime suggests that our findings may in fact reflect the commonly used 24-hour postoperative continuation of antimicrobial prophylaxis following joint replacement surgery.

336

In a recent population pharmacokinetic study, it was found that standard intermittend infusion of 1,500 mg cefuroxime is inadequate for critically ill patients(15). Even for low MICs, creatinine clearance above 50 ml/min resulted in high probabilities of underdosing. Though obtained in a different setting, our findings for STI support that current routine dosing schemes of cefuroxime may be inadequate. Especially if higher than 65% fT > MIC targets are pursued, extended or CI seem mandatory, regardless of dose.

344

345 We recently investigated the applicability of MD for measurement of cefuroxime in 346 drill holes in bone in a porcine study(39). Our findings suggested that meaurements 347 obtained in drill holes in the bone do reflect the the actual bone concentration. At 348 present, however, no gold standard exist to validate the findings with certainty, and the 349 potential bias due to bone damage associated with the drilling remains unknow. 350 Nevertheless, MD offers advantages compared to bone biopsies, particularly because it 351 samples the unbound pharmaceutically active fraction of the drug, and that serial 352 measurements can be obtained even after surgery.

353

Incomplete and uneven tissue distribution of antibiotics has been demonstrated in a number of studies(17, 25, 40-44). We found incomplete tissue penetration for SCT and cortical bone in the STI group. In the CI group, low SCT and cortical bone penetration
were also found, but in this group, the findings were not statistically significant. Notably, no difference between the groups could be detected when comparing tissue penetration. It could be speculated that incomplete SCT penetration may be related to the vasoconstrictive effect of the adrenalin injected at the end of surgery. For STI, the majority of cefuroxime is available for tissue distribution in the beginning of a dosing interval. Consequently, this potential effect would have a greater impact on STI compared to CI.

364

365 Altogether, our findings support the use of CI for a short half-lived and time-dependent 366 drug like cefuroxime. In a recent analogous porcine study, we found lower steady state 367 concentrations and substantially incomplete tissue penetration, especially for bone(16). 368 These findings suggested a risk of attaining sub-therapeutic concentrations at the site of 369 infection for the entire dosing interval using CI. However, with the present clinical data, 370 there is a considerably lower risk of encountering this pitfall. Nonetheless, very high 371 MICs may still jeopardise CI of cefuroxime, and for serious infections, more aggressive 372 dosing schemes should be considered.

373

374 Only a limited number of studies have investigated antibiotic pharmacokinetics in 375 infected bone, and the effect of infection on antibiotic bone penetration remains 376 unclear(1, 45). The picture is further blurred by the fact that studies on this issue have 377 used bone biopsies, which suffer from important methodological limitations(1-3). 378 Nevertheless, IAI and osteomyelitis are difficult to treat, and at least in sequestrated and 379 ischemic bone, antibiotic penetration is presumably substantially impaired. High dose 380 CI of time-dependent drugs may prove to be an important supplement in the 381 management of these conditions. Nevertheless, studies investigating infected bone 382 antimicrobial penetration with alternative approaches like microdialysis are warranted.

384 This study has a number of limitations. First of all, healthy males having TKR surgery 385 is a rather selected group that may not reflect the average population. The anatomical 386 area in which the measurements were obtained had been subjected to a substantial 387 surgical trauma. Additionally, local injection of adrenaline may also have affected the 388 pharmacokinetics. For practical reasons, bone measurements by means of MD can only 389 be conducted postoperatively, and as such, our findings do not reflect the 390 pre/perioperative administration of cefuroxime. Nevertheless 24-hour continuation of 391 antimicrobial prophylaxis has been shown to reduce infection rates following large joint 392 replacement, and for this situation, our findings are likely to be applicable(37). The 393 perioperative situation, infection and other pathological conditions may result in 394 different pharmacokinetic profiles.

395

In pharmacokinetic MD studies, correction for RR is required in order to obtain 396 397 absolute concentrations. This leads to a magnification of the variations associated with 398 the preanalytical sample handling and the chemical assay. We have previously found 399 comparable variations between plasma and tissue PK parameters, but variations in 400 tissue PK parameters were higher than those found in plasma in the present study. This 401 is in accordance with the findings in other clinical studies(46, 47). Given the RR-related 402 magnification of the variations, the surgical trauma, local injection of adrenalin and the 403 well-known biological variation, the sizes of the tissue variations illustrated in figure 2 404 and by the 95%-CIs of AUCs in table 2 are not surprising, and supposedly not an 405 indication of inadequate precision of the methodological setup nor a poor model 406 description of the data.

408 We used retrodialysis by drug for estimation of RR. Using this approach, one assumes 409 that RR remains constant for the entire duration of the experiment. In a previous 410 experimental study on anaesthetized pigs, we found that RR remained constant over 7 411 hours in SCT, cancellous and cortical bone(39). The participants in the present study 412 were subject to major surgery, administration of local adrenalin, and for ethical reasons, 413 no restrictions regarding mobilization were applied. Consequently local changes in 414 perfusion, and thus in RR, cannot be ruled out. The use of an internal calibrator would 415 have solved this uncertainty, and for future studies this seems to be advisable.

416

In conclusion, the findings in this study suggest that CI of cefuroxime is favourable compared to STI. Particularly for STI, complete tissue penetration cannot be taken for granted. Irrespective of mode of administration, higher-than-standard doses of cefuroxime are needed in case of high-MIC organisms. A high-dose CI approach may prove important in orthopaedic perioperative antimicrobial prophylaxis and in the management of deep-seated othopeadic infections.

423

424

425

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429

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638 Figures





652 Figure 2. Observed concentrations (dots) and modelled concentration-time profiles (solid lines).





Figure 3. Observed versus fitted individual- and population cefuroxime concentrations for free plasma,

655 SCT, cortical and cancellous bone.





Figure 4. Mean concentration-time profiles for short-term and continuous infusion of cefuroxime for





Figure 5. Probability of target attainment in the different tissues.

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697 Table 1

698 Patient characteristics

	Variable	Short-term infusion	Continuous infusion
	Number of patients	9	9
	Age (years), mean (range)	68.7 (58 - 76)	70.0 (60 - 75)
	Height (cm), mean (range)	180 (170 – 190)	176 (169 – 183)
	Weight (kg), mean (range)	99 (73 – 110)	89 (73 – 107)
	Body mass index (kg/m ²), mean (range)	30.6 (21.8 - 36.0)	28.7 (23.9 - 35.8)
	Plasma creatinine (µmol/l), mean (range)	76 (64 - 99)	87 (68 – 111)
	Plasma albumin (g/l), mean (range)	42 (38 - 47)	42 (40 - 46)
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715 Table 2

716 Comparison of AUC and tissue penetration

Parameter	STI	CI	P-value
Free plasma $AUC_{0-\infty}(min mg/L)$	5801 (4902; 7277)	5415 (4625; 6670)	P = 0.63
SCT AUC _{0-∞} (min mg/L)	3016 (1929; 4675)	3764 (2164; 6426)	P = 0.56
Cancellous bone AUC _{0 - ∞} (min mg/L)	6035 (3718; 9831)	6256 (4276; 8954)	P = 0.91
Cortical bone AUC _{0-∞} (min mg/L)	2630 (1746; 3945)	3557 (1375; 7262)	P = 0.56
SCT fAUC _{tissue} /fAUC _{plasma}	0.52 (0.32; 0.83)	0.69 (0.38; 1.21)	P = 0.48
Cancellous bone fAUC _{tissue} /fAUC _{plasma}	1.03 (0.61; 1.74)	1.15 (0.74; 1.71)	P = 0.76
Cortical bone fAUCtissue/fAUCplasma	0.35 (0.28; 0.70)	0.65 (0.25; 1.36)	P = 0.50

717 AUC_{0- ∞}, area under the concentration-time curve from 0 to infinity; *f*AUC_{tissue}/*f*AUC_{plasma}, tissue

718 penetration expressed as the ratio of free $AUC_{0-\infty}$ tissue to free $AUC_{0-\infty}$ plasma. Values are given as

- 719 mean (95%-confidence intervals).

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Declaration of co-authorship

Full name of the PhD student: Mikkel Tøttrup

This declaration concerns the following article/manuscript:

Title:	Pharmacokinetics of cefuroxime in porcine cortical and cancellous bone determined by microdialysis
Authors:	Tøttrup M, Hardlei TF, Bendtsen M, Bue M, Brock B, Fuursted K, Soballe K, Birke- Sorensen H.

The article/manuscript is: Published \boxtimes Accepted \square Submitted \square In preparation \square

If published, state full reference: Tøttrup M, Hardlei TF, Bendtsen M, Bue M, Brock B, Fuursted K, Soballe K, Birke-Sorensen H. Pharmacokinetics of cefuroxime in porcine cortical and cancellous bone determined by microdialysis. Antimicrobial Agents and Chemotherapy. 2014 Jun;58(6):3200-5. doi: 10.1128/AAC.02438-14.

If accepted or submitted, state journal: Antimicrobial Agents and Chemotherapy.

Has the article/manuscript previously been used in other PhD or doctoral dissertations?

No \boxtimes Yes \square If yes, give details:

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- C. Has contributed considerably (40-60 %)
- D. Has done most of the work (70-90 %)
- E. Has essentially done all the work

Element	Extent (A-E)
1. Formulation/identification of the scientific problem	D
2. Planning of the experiments and methodology design and development	D
3. Involvement in the experimental work/clinical studies/data collection	D
4. Interpretation of the results	D
5. Writing of the first draft of the manuscript	E
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Title:	Bone, Subcutaneous Tissue and Plasma Pharmacokinetics of Cefuroxime in Total Knee Replacement Patients – a Randomized Controlled Trial Comparing Continuous versus Short-term Infusion.
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