Penetration of Moxifloxacin into Bone Evaluated by Monte Carlo Simulation

Cornelia B. Landersdorfer¹, PhD; Martina Kinzig¹, PhD; Friedrich F. Hennig², MD; Jürgen B. Bulitta¹, PhD; Ulrike Holzgrabe³, PhD; George L. Drusano⁴, MD; Fritz Sörgel⁵, PhD; Johannes Gusinde², MD.

¹: IBMP – Institute for Biomedical and Pharmaceutical Research, Nürnberg-Heroldsberg, Germany
²: Department of Surgery, University of Erlangen, Germany
³: Institute of Pharmacy and Food Chemistry, University of Würzburg, Germany
⁴: Ordway Research Institute, Albany, New York, USA
⁵: Department of Pharmacology, University of Duisburg-Essen, Germany

*Address of correspondence:
Fritz Sörgel, PhD, BSc, Professor, IBMP – Institute for Biomedical and Pharmaceutical Research, Paul-Ehrlich-Str. 19, D-90562 Nürnberg-Heroldsberg, Germany; Phone: ++49-911-518290, Fax: ++49-911-5182920, e-mail: ibmp@osn.de

#Present address:
Department of Pharmaceutical Sciences, State University of New York at Buffalo, NY 14260, USA.

Running title: Moxifloxacin Pharmacokinetics in Bone

Key words:
Pharmacokinetic modeling
Bone penetration
Monte Carlo simulation
Pharmacodynamic profile
Abstract (Word count: 250)

Moxifloxacin is a fluoroquinolone with a broad spectrum of activity and good penetration into many tissues, including bone. Penetration of moxifloxacin into bone has not yet been studied using compartmental modeling techniques. Therefore, we determined rate and extent of bone penetration of moxifloxacin and evaluated its pharmacodynamic profile in bone via Monte Carlo simulation.

Twenty-four (10 males, 14 females) patients undergoing total hip replacement received 400 mg moxifloxacin orally 2-7 h prior to surgery. Blood and bone specimens were collected. Bone samples were pulverized under liquid nitrogen by a cryogenic mill, including an internal standard. Drug concentrations were analyzed by HPLC. We used ADAPT II (results reported), NONMEM and WinBUGS for pharmacokinetic analysis. Monte Carlo simulation was performed to reverse engineer the necessary area under the free concentration time curve $\text{AUC}_{\text{SERUM}}/\text{MIC}$ in serum and total $\text{AUC}_{\text{BONE}}/\text{MIC}$ in bone for successful clinical or microbiological outcome.

The median [10%-90% percentile for between subject variability] of the AUC in bone divided by the AUC in serum ($\text{AUC}_{\text{BONE}}/\text{AUC}_{\text{SERUM}}$) was 80% [51-126%] for cortical bone and 78% [42-144%] for cancellous bone. Equilibration between serum and bone was rapid. Moxifloxacin achieved robust ($\geq 90\%$) probabilities of target attainment (PTAs) in serum, cortical, and cancellous bone up to MICs $\leq 0.375$ mg/L based on the targets $\text{fAUC}_{\text{SERUM}}/\text{MIC} \geq 40$ and $\text{AUC}_{\text{BONE}}/\text{MIC} \geq 33$.

Moxifloxacin showed high bone concentrations and a fast equilibrium between bone and serum. The favorable PTAs compared to the MIC$_{90}$ of $S.$ aureus warrant future clinical trials on the effectiveness of moxifloxacin in the treatment of bone infections.
Introduction

Quinolones are established in the treatment of osteomyelitis. Most clinical experience has been gained with ciprofloxacin and ofloxacin (28). Oral administration of quinolones was efficacious in surgical prophylaxis, even after a single dose (6, 39), and also facilitates prolonged therapy. In in vitro studies S. aureus penetrates and survives in bone cells i.e. osteoblasts (25), therefore quinolones, which penetrate intracellularly, might be of advantage.

Moxifloxacin achieves high concentrations in many tissues. Good penetration into bone has been reported for several quinolones. The average bone/serum concentration ratio in humans at approximately 2 to 5 h post dose was 0.33 to 0.54 for moxifloxacin (30, 34, 35).

Resistance to the older quinolones has been emerging, and they do not show sufficient microbiological efficacy against S. aureus, coagulase-negative staphylococci and streptococci (28). Moxifloxacin has improved activity against gram-positive and anaerobic pathogens frequently encountered as causative agents in osteomyelitis (28), such as staphylococci, enterobacteriaceae, streptococci, and H. influenzae (19). Moxifloxacin has lower MICs than levofloxacin, ciprofloxacin, ofloxacin, and norfloxacin for S. aureus (51) which is the most common pathogen in osteomyelitis. The main causative bacteria for osteomyelitis are S. aureus (methicillin susceptible or resistant), coagulase-negative staphylococci, propionibacterium, streptococci, and Pseudomonas aeruginosa (27). P. aeruginosa can cause osteomyelitis due to nosocomial infections or chronic unresolved middle ear infections in children.
Studying the time course and extent of bone penetration before investigating the agent in a clinical trial is important (17, 51). Bone penetration studies most often report the ratio of concentrations in bone and serum. However, the ratio of tissue to serum concentrations of a drug changes with time, a phenomenon known as system hysteresis, and therefore the concentration ratio depends on the sampling time. Like in the present study, bone penetration of antimicrobials is most often studied in patients undergoing joint replacement, where only one bone sample can be obtained per subject. Modeling the full serum and bone concentration time course allows one to evaluate the penetration of antimicrobials into bone and to study the pharmacodynamic profile in bone. We are not aware of any reports about pharmacokinetic pharmacodynamic (PKPD) modeling of quinolones in bone in humans or animals.

The first objective of our study was to determine moxifloxacin concentrations after oral administration in cortical and cancellous bone in a controlled study in subjects undergoing hip replacement surgery. We developed a highly standardized, validated analytical method and quantified moxifloxacin in serum and bone. As second objective we intended to develop a PK model to describe the time course of moxifloxacin concentrations in bone. Our third objective was to calculate the probabilities of target attainment (PTA) for serum, cortical and cancellous bone based on PKPD targets for successful microbiological and clinical outcome.
Methods

**Study participants:** The study comprised 24 patients (10 males, 14 females) who were scheduled to undergo total hip replacement. The patients were diagnosed with coxarthrosis and had no inflammation of the joints. Their average ± SD weight was 76.8 ± 13.4 kg, height 168.3 ± 9.9 cm and age 63 ± 15 years. The study was approved by the local ethics committee and performed according to the revised version of the Declaration of Helsinki. All subjects gave their written informed consent prior to starting the study.

**Study design and drug administration:** Each patient received a single oral dose of 400 mg moxifloxacin (Avalox®, BayerVital, Germany) 2 to 7 h before surgery. Before surgery, 20 patients received amoxicillin / clavulanic acid, three patients received levofloxacin, and one patient received clindamycin, as intravenous infusions. Since there were no data on the bone penetration of moxifloxacin published prior to initiation of this study, those standard treatments for perioperative prophylaxis were given in parallel to moxifloxacin to assure antibacterial prophylaxis by an established treatment option.

**Sampling Schedule:** Blood samples were collected pre-dose and at the time of femoral bone resection. The blood samples were cooled in an ice-water bath and allowed to clot before centrifugation at 4°C. After centrifugation, serum was immediately frozen and stored at -80°C until analysis. Hip replacement involved resection of the femoral head, or both femoral head and femoral neck, prior to implantation of the prosthetic hip joint. Bone samples were immediately frozen on dry ice and stored at -80°C until analysis.
Determination of Serum and Bone Concentrations:

Some bone samples consisted only of femoral head, others included both femoral head and femoral neck. The latter specimens were separated into femoral head and femoral neck. Then the samples were separated into cortical and cancellous tissue and pulverized under liquid nitrogen by a cryogenic mill. Specified amounts of the resulting powder were shaken with buffer for 24 h. Eluates and serum samples were deproteinized by acetonitrile containing the internal standard (pifloxacin). After thorough mixing, the samples were centrifuged for 5 min at 12000 rpm, and the supernatant was past diluted with ammonium formate buffer.

Moxifloxacin concentrations in bone and serum were determined by high-performance liquid chromatography coupled with fluorometric detection (296/504 nm). All sample handling was done under daylight protection. Twenty (20) µL of each sample were chromatographed on a reversed-phase column (Spherisorb ODS II, 5 µm, 250 x 4.6 mm) eluted with a gradient elution system consisting of 0.1 M citric acid buffer containing 44 mM ammonium perchlorate (A) and acetonitrile (B) [0-2.6 min: 65% (A):35% (B) at 1.0 mL/min, 2.6-8.0 min: 40% (A):60% (B) at 1.3 mL/min, 8.0-8.1 min: 65.0% (A):35% (B) at 1.0 mL/min]. Under these conditions moxifloxacin and the internal standard were eluted after approximately 4.4 min and 3.1 min. The Turbochrom 3 software (Version 3.2, 1991, PE Nelson, Cupertino, CA, USA) was used for evaluation of chromatograms.

For analysis of bone samples, calibration standards and spiked quality control samples were prepared by adding appropriate amounts of standard
solutions to moxifloxacin-free bone tissue. Concentrations of moxifloxacin were determined using reversed-phase HPLC with gradient elution and fluorometric detection (296/504 nm). For evaluation of the calibration standards a weighted linear regression \( (1/y^2) \) was performed with theoretical concentrations of calibration standards and measured peak height ratios (peak height moxifloxacin / peak height internal standard).

No interferences were observed in serum and bone for moxifloxacin and the internal standard, including specimens of the three patients who had received a dose of levofloxacin in addition to moxifloxacin. The linearity of moxifloxacin calibration curves was demonstrated from 0.0100 to 5.00 mg/L in serum, and from 0.009 to 4.76 mg/L in bone homogenate. The inter-day precision and accuracy of the spiked quality control standards of moxifloxacin in human serum ranged from 1.8 to 5.9% and from 95.1 to 103.8%. The inter-day precision and accuracy of the spiked quality control standards of moxifloxacin in bone homogenate ranged from 3.7 to 9.2% and from 94.7 to 97.6%.

**Pharmacokinetic modeling:** Models with one or two disposition compartments in addition to bone were tested. Models with or without separate compartments for cortical and cancellous bone as well as for samples from femoral head and femoral head were considered. Informative and non-informative priors for \( ka \) were tested. The predictive performance of our final model was tested via visual predictive checks, the generalized information criterion for MAP estimation (MAP objective function), plots of observed versus predicted concentrations, and residual plots.
For the visual predictive check, the serum and bone concentration profiles were simulated for 10,000 subjects. From these data the median, the nonparametric 90% prediction interval (5% to 95% percentile), and the nonparametric 50% prediction interval (25% to 75% percentile) for the predicted profiles were calculated. These prediction interval lines were compared with the original observed data. If the model described the data adequately, 10% of the observed data points should fall outside the 90% prediction interval and 50% of the data should fall outside the interquartile range. The median predicted concentrations and the prediction intervals were compared to the observed data and it was tested, whether the median and the prediction intervals mirrored the central tendency and the variability of the raw data for the respective model.

Structural model: Moxifloxacin concentrations were determined in serum, cortical bone, and cancellous bone. Due to the relatively small number of samples from the femoral neck and to prevent making the model more complex, our model did not distinguish between samples from femoral neck and femoral head. A two compartment disposition model for moxifloxacin in serum and in the peripheral compartment plus one peripheral compartment for each bone matrix was used (Figure 1). The differential equations for the model are:

\[
\frac{dX_1}{dt} = -ka \cdot X_1
\]

\[
\frac{dX_2}{dt} = ka \cdot X_1 \left( \frac{CL + CL_{ic}}{V_{central}} + k_{24} + k_{25} \right) \cdot X_2 + \frac{CL_{ic}}{V_{Peripheral}} \cdot X_3 + k_{42} \cdot X_4 + k_{52} \cdot X_5
\]

\[
\frac{dX_3}{dt} = \frac{CL_{ic}}{V_{Central}} \cdot X_2 - \frac{CL_{ic}}{V_{Peripheral}} \cdot X_3
\]
Compartment 1 is the gut compartment, compartment 2 is the central compartment and compartment 3 the peripheral compartment. Compartment 4 is the compartment for cortical bone, and compartment 5 is the compartment for cancellous bone. \( X_1, X_2, X_3, X_4 \), and \( X_5 \) denote the amounts of drug in the respective compartment. CL is the apparent total clearance from the central compartment, \( k_a \) is the absorption rate constant, CLic is the apparent intercompartmental clearance between the central and peripheral compartment, and \( k_{24}, k_{42}, k_{25}, k_{52} \) are first-order intercompartmental transfer rate constants. \( V_{Central} \) and \( V_{Peripheral} \) are the apparent volumes of distribution of the respective compartment. For all apparent clearance and apparent volume terms the extent of absorption term \((1/F)\) is left out for a clearer presentation of the equations.

Scale terms for the concentrations in cortical and cancellous bone that describe the equilibrium concentration ratio between cortical bone and serum \( (F_{cortical}) \) and between cancellous bone and serum \( (F_{cancellous}) \) were included. \( F_{cortical} \) equal to 1 means that concentrations after a continuous infusion at steady-state are the same in cortical bone and serum, \( F_{cortical} \) smaller (greater) than 1 means that these concentrations are lower (higher) in cortical bone than in serum.

**PK modeling approach:** We had sparse serum concentration time data between 2 and 7 h post oral administration. As the moxifloxacin half-life is
approximately 12 h, these data did not allow us to estimate all PK parameters for a two compartment model. Therefore, maximum a posteriori (MAP)-Bayesian estimation based on the disposition parameters of Simon et al. (41) was used and the average clearance and its standard deviation were derived from published studies (41, 45-47, 52). Average age was 46.3 (standard deviation 10.6) years in the Simon et al. study. Based on those disposition parameters and their standard deviation, we estimated a typical half-life of absorption from our serum data via population PK in NONMEM V (5).

We had no prior information on the rate and extent of bone penetration of moxifloxacin. The raw data and initial modeling showed that the equilibrium between serum and bone was virtually achieved 2 h after dosing, indicating that the rate of equilibration ($k_{42}$ and $k_{52}$) was fast. Therefore we could not estimate $k_{42}$ and $k_{52}$ and fixed those values to an equilibration half-life of 15 min. The plausibility of this choice was assured via visual predictive checks. A sensitivity analysis was performed using the three-stage hierarchical population approach in S-Adapt (version 1.55, Monte Carlo parametric expectation maximization algorithm). Initial estimates for absorption half-life, $k_{42}$ and $k_{52}$ were systematically perturbed and re-estimated using physiologically plausible but uninformative priors.

The disposition parameters of moxifloxacin as described above have been determined in absence of a bone compartment. As we used MAP-Bayesian estimation (see below), we had to keep the amount of moxifloxacin in the bone compartments minimal so that the serum PK was not affected by the presence of
the bone compartments. This can be achieved by choosing a small volume for
the bone compartment or equivalently a small value for the rate constants \(k_{24}\) and
\(k_{25}\). Therefore, a volume of distribution of 0.5 L each for the cortical and
cancellous bone compartments which is equivalent to fixing \(k_{24}\) and \(k_{25}\) to 0.022
\(h^{-1}\) in our model was chosen.

**MAP-Bayesian estimation:** The individual PK parameters were estimated
by MAP-Bayesian estimation as implemented in ADAPT II (13). We used
informative priors with prior means and standard deviations and a log-normal
distribution to estimate the individual disposition parameters. In absence of prior
information on the bone penetration, non-informative priors (uniform distribution)
were used to estimate \(F_{cortical}\) and \(F_{cancellous}\) in the MAP-Bayesian step. The
residual unidentified variability was described by a proportional error model for
the serum and bone concentrations.

**Estimation by the three-stage hierarchical Bayesian approach:** To confirm
the results from the MAP-Bayesian method, PK parameters were estimated by
the 3-stage hierarchical Bayesian approach in WinBUGS 1.4 using PKBugs 2.1
(29, 44). For \(V_{Central}\), \(V_{Peripheral}\) and \(CLic\), priors for population means and between
subject variability were obtained from Simon et al (41). As described above, the
population mean absorption rate constant was estimated to be 1.6 \(h^{-1}\) in
NONMEM V and a between subject variability of 40% coefficient of variation
which is common for oral absorption parameters was assumed. The equilibration
between bone and serum was assumed to be rapid. Physiologically plausible but
uninformative priors were used for the population mean and variability of CL, $F_{\text{cortical}}$ and $F_{\text{cancellous}}$ based on literature data (26, 41).

**Reverse engineering method for PKPD targets:** The ratio of the free (non-protein bound) area under the plasma concentration time curve and MIC ($fAUC/MIC$) has been shown to be predictive for the microbiological and clinical outcome for fluoroquinolones (12, 15). However, there is no PKPD target for moxifloxacin in osteomyelitis patients in serum or for quinolones in bone. Therefore, we used a reverse engineering method (7) to propose a PKPD target for moxifloxacin in serum and bone based on studies in osteomyelitis patients.

The reverse engineering method uses the success rate from clinical studies in osteomyelitis patients, the expected AUCs after the doses given in these studies, and published MIC distributions from the relevant time period to derive the most likely target. The target which best predicts the observed clinical success rate is derived via Monte Carlo simulation (MCS) in an iterative process.

We used published data from four studies (21, 22, 24, 37) on the clinical or microbiological outcome of osteomyelitis caused by *S. aureus* in patients who obtained ciprofloxacin orally at 500 mg or 750 mg every 12 h. Their expected AUCs were derived based on published PK data for ciprofloxacin (2, 54) or based on the AUCs reported by the authors (37). A log-normal distribution was assumed for clearance and a 25% protein binding was used for ciprofloxacin to simulate the expected $fAUC$s for 5,000 virtual subjects for each osteomyelitis study. We combined these $fAUC$s with susceptibility data for *S. aureus* (4, 8-10, 18, 20, 23, 31, 32, 42, 43, 49, 53) from the time period of the osteomyelitis
studies to derive the PKPD target in serum which predicted the observed successful rate. This yielded the PKPD target for *S. aureus* infections of osteomyelitis patients in serum \((\text{AUC}_{\text{SERUM}}/\text{MIC})\). A protein binding of 0% was assumed in bone, because. The ratio of total concentrations in bone and serum \((\text{AUC}_{\text{BONE}}/\text{AUC}_{\text{SERUM}})\) has been reported 0.63 for ciprofloxacin (33). We derived the PKPD target in bone \((\text{AUC}_{\text{BONE}}/\text{MIC})\) based on this ratio. There are no data on protein binding of ciprofloxacin or moxifloxacin in bone. Therefore the target \((\text{AUC}_{\text{BONE}}/\text{MIC})\) refers to the total ciprofloxacin concentration in bone. As only the unbound fraction is considered active, application of this target for total concentrations derived for ciprofloxacin to moxifloxacin without further corrections assumes the same binding in bone for both drugs.

**Monte Carlo simulation:** We studied a range of MICs from 0.125 to 16 mg/L. The protein binding of moxifloxacin has been reported to range between 47% and 55% (3, 40, 45, 54). Therefore, an average protein binding of 50% was assumed for moxifloxacin in serum. Between subject variability (BSV) was not included for protein binding as the BSV for protein binding is already included in the estimated variability for total clearance and for volume of distribution. For moxifloxacin in bone, it was first assumed that it has the same binding as ciprofloxacin. Other extents of binding (free fraction of 75%, 50%, 25%, or 10% of the free fraction of ciprofloxacin in bone) were also assessed.

We simulated the serum and bone concentration time curves for 10,000 patients after an oral moxifloxacin dose of 400 mg q24h at steady-state in absence of residual error. The PTA was derived by calculating the fraction of...
subjects who attained the PKPD target at each MIC. The PKPD breakpoint was defined as the highest MIC for which the PTA was at least 90%.
Results

Concentrations of moxifloxacin in serum and cortical and cancellous bone are shown in Figure 2. Moxifloxacin concentrations in cortical and cancellous bone were similar to those found in serum. In cortical bone moxifloxacin concentrations were similar in femoral head and femoral neck, whereas in cancellous bone concentrations were slightly higher in femoral neck. Moxifloxacin was stable during the 24 h extraction period.

PK analysis: The absorption half-life (ln 2 / ka) was estimated to be 26 min in NONMEM V. A sensitivity analysis showed that absorption half-lives between 8 and 34 min yielded similar log-likelihoods (differences <1) and that the mean (individual) bone to serum AUC ratio was affected by <3% (<7%) for this range of absorption half-lives. A sensitivity analysis for $k_{42}$ and $k_{52}$ (Figure 1) showed that mean equilibration half-lives below 30 min yielded similar objective functions. The considered range of initial equilibration half-lives was 4 to 415 min. For mean equilibration half-lives between 4 and 30 min, the mean bone to serum AUC ratios differed by less than 9% under all studied conditions.

Final parameter estimates from the MAP-Bayesian estimation in Adapt, from WinBUGS and S-Adapt (results not shown) were similar, as shown in Table 1. Figure 3 shows the extent of moxifloxacin penetration into cortical and cancellous bone, and its between subject variability, calculated from the ratios of $AUC_{\text{cortical}} / AUC_{\text{serum}}$ and $AUC_{\text{cancellous}} / AUC_{\text{serum}}$ of 10,000 subjects that we simulated at steady-state. The median penetration [10% - 90% percentile] was 80% [51% - 126%] for cortical bone and 78% [42% - 144%] for cancellous bone.
The visual predictive checks showed acceptable predictive performance of the final model for all three matrices (Figure 4). The central tendency of the concentrations in bone was slightly under-predicted between 2.5 and 3.5 h, whereas it was predicted adequately in serum. The variability was well predicted for all three matrices. This supported the use of our model in the MCS.

Concentration time profiles in serum and bone predicted by the final estimates from WinBUGS (Table 1) were comparable to the predicted profiles shown in Figure 4.

**Reverse engineering method for PKPD targets:** We used data from published clinical studies with ciprofloxacin in osteomyelitis patients and assumed a protein binding of 25% for ciprofloxacin and of 50% for moxifloxacin in serum. The resulting PKPD targets for successful clinical or microbiological outcome were $f_{\text{AUC}_{\text{SERUM}}/\text{MIC}} \geq 15$ (21), $f_{\text{AUC}_{\text{SERUM}}/\text{MIC}} \geq 36$ (37), $f_{\text{AUC}_{\text{SERUM}}/\text{MIC}} \geq 43$ (24), and $f_{\text{AUC}_{\text{SERUM}}/\text{MIC}} \geq 66$ (22). The respective targets for total bone concentrations were $\text{AUC}_{\text{BONE}}/\text{MIC} \geq 13$ (21), $\text{AUC}_{\text{BONE}}/\text{MIC} \geq 30$ (37), $\text{AUC}_{\text{BONE}}/\text{MIC} \geq 36$ (24), and $\text{AUC}_{\text{BONE}}/\text{MIC} \geq 55$ (22). As the targets calculated from the studies by Nix et al. (37) and Hoogkamp-Korstanje et al. (24) were very similar, we used the average from these two studies investigating clinical or microbiological outcome, i.e. $f_{\text{AUC}_{\text{SERUM}}/\text{MIC}} \geq 40$ and $\text{AUC}_{\text{BONE}}/\text{MIC} \geq 33$ for MCS. In one of these two studies PK parameters were reported for several subjects and could be used for calculation of the AUCs. The resulting targets from these two studies ($f_{\text{AUC}_{\text{SERUM}}/\text{MIC}} \geq 40$ and $\text{AUC}_{\text{BONE}}/\text{MIC} \geq 33$) fall between the targets calculated from the other two studies (Table 2).
Monte Carlo simulation: The PTA versus MIC plots are shown in Figure 5. The PKPD breakpoints for serum, cortical bone and cancellous bone are listed in Table 2 for the assumption that the extent of moxifloxacin binding to bone matrix is the same as for ciprofloxacin binding to bone. The breakpoints were similar in serum and bone. The PKPD breakpoint was about 0.375-0.5 mg/L for the median PKPD target in serum and bone for a dosage regimen of 400 mg moxifloxacin q24h at steady-state. For a moxifloxacin binding in serum of 47%, the breakpoint is 0.375 mg/L and for 55% protein binding the breakpoint is 0.25 mg/L for the target $\text{fAUC}_{\text{SERUM}}/\text{MIC} \geq 40$. Table 3 reports breakpoints in bone for various extents of moxifloxacin binding to bone.
It is important to study the penetration properties of a drug before investigating the agent in a clinical trial. Knowledge about how fast moxifloxacin reaches effective levels in bone is important to choose the adequate time period between moxifloxacin administration for perioperative prophylaxis and surgery. The maintenance dose in treating bone infections should be selected based on the AUC in bone. Average bone / plasma concentration ratios for moxifloxacin in bone ranged from 0.3 to 0.54 between 1 h and 5 h post end of infusion in various patient groups (30)(34)(35).

The complexities of determination of drug concentrations in bone might be a reason for widely different results between studies even for the same antimicrobial agent. Malincarne et al. (30) report that bone samples were hand minced into 20 to 30 mg pieces and then extracted. Manual slicing of bone samples to small pieces resulted in a lower recovery after extraction than pulverization by a cryogenic mill (38). We developed a highly standardized, validated method for sample preparation and analysis and tested the degree of extraction over time to ensure reproducible results. Our calibration standards were prepared in moxifloxacin-free bone tissue, whereas other studies often prepared standards in buffer or serum (26)(30) or did not report the matrix (34).

Most tissue penetration studies (26), including previous studies on moxifloxacin (30, 34, 35), only report the concentration ratio between tissue and serum for PK analysis and compare concentrations in tissue to MICs of pathogens. Mouton et al. (36) criticized this method of analysis and cited a bone
penetration study as an example. The bone / serum concentration ratios may change over time and therefore ratios at a single time point are difficult to interpret. Some authors fitted the time-course of bone concentrations by naïve techniques which ignore the between patient variability. Drusano et al. analyzed the penetration of levofloxacin (17)(16) by use of population PK and MCS. This approach considers the full time course of penetration, estimates between patient variability and allows one to calculate the extent of penetration by the ratio of AUCs in tissue and serum.

A three-stage hierarchical Bayesian population PK approach additionally offers the advantage to borrow information on mean PK parameters and between patient variability with uncertainty based on previous studies. Borrowing of information from a study with frequent sampling is particularly important for analysis of very sparse data, as in this and most other bone penetration studies.

Population PK and MCS may then be used to estimate PTAs for the desired PD endpoint (e.g. successful microbiological outcome) in serum and tissue. The ratio of AUC/MIC is the most predictive surrogate for microbiological success of treatment with quinolones (1, 14). To the best of our knowledge, population PK and Monte Carlo simulation have not been used for analyzing bone penetration studies with antibiotics and a full Bayesian population PK approach has not yet been applied to tissue penetration studies with antibiotics.

A limitation of our study in only 24 patients are the rather narrow range of sampling times between 2 and 7 h and sampling of only one blood and bone sample post dosing. Optimal design methodology should be applied in future
studies to select several informative blood sampling times per patient. For bone it is usually not feasible to obtain concentrations at more than one time point and even in case this was done, the blood circulation to the bone would be impaired and this could bias the observed time course. While these limitations apply to the vast majority of published bone penetration studies, we applied latest modeling approaches to derive as much information as possible from the available data.

As most bone penetration studies (26), our study was performed in patients with non-infected bone. In osteomyelitis patients, rate and extent of bone penetration might differ. Blood flow into bone might be increased due to reactive hyperemia or decreased due to pus, ischemic regions, and sequester. Some studies (26) show higher concentrations in infected compared to non-infected bone. The PK in bone might also be influenced by a potentially decreased bone density in elderly patients.

Our study in hip replacement patients was a single dose trial. This reflects the common practice for surgical prophylaxis where usually a single dose is given before surgery, which might be followed by additional doses afterwards. As moxifloxacin displays linear PK after single and multiple doses (48), we simulated the drug concentrations after multiple dosing to predict the PKPD profile in serum and bone for treating osteomyelitis.

As another limitation of our study, we measured total concentrations after extraction of bone homogenate. Bone is not a homogenous tissue and consists of blood vessels, extracellular fluid, bone cells, organic matrix (mainly collagen fibrils), and inorganic matrix (mainly hydroxyapatite crystals). Quinolones
[Wittmann 1986 7] bind to hydroxyapatite. It seems possible that neither antibiotics nor bacteria distribute uniformly in bone tissue as discussed previously (26).

In general only the free antibiotic concentration is considered active, as molecules bound to the bone matrix might not contribute to bacterial killing. Determination of total concentrations in bone homogenate is a limitation of virtually all bone penetration studies. Analytical techniques to determine unbound drug concentrations in bone would provide further insights and may allow better predictions of the effectiveness of different antibiotics in bone infections.

To address this potential limitation we reverse engineered the required PKPD targets in serum and bone based on clinical trials in osteomyelitis patients. This reverse-engineered PKPD target for bone from clinical trials accounts for the potentially inhomogeneous distribution of bacteria and quinolones in bone. This approach assumes that ciprofloxacin and moxifloxacin have similar binding and distribution properties due to their structural similarity. Additionally, we calculated the PKPD profile of moxifloxacin in bone for various values for the free fraction.

We studied the concentrations of moxifloxacin in bone and serum between 2 and 7 h post the oral dose of moxifloxacin. The average concentration ratio between serum and bone showed no obvious change with time during our observation period. The observed data and initial modeling showed that the bone penetration in our study was faster than expected, and equilibrium between serum and bone was virtually achieved 2 h after dosing. Therefore, the bone and the central compartment were in pseudo-equilibrium during our observation
period (from 2 to 7 h post dose) and the bone concentrations declined in parallel to
the serum concentration. A fast distribution equilibrium of moxifloxacin
between plasma and bone is in agreement with the data from Malincarne et al.
(30) and Metallidis et al. (34).

Our data could be adequately described by a model with first-order
distribution. A rapid equilibrium between serum and bone might have been
carried by an active transporter from bone tissue to serum. Transporters involved
in tissue distribution have been found for quinolones in other tissues. High flow
rates of interstitial fluid of up to 600 µL/g/h have been calculated based on in vivo
studies (11). For an antimicrobial with similarly fast absorption and bone
penetration as moxifloxacin, antibacterial prophylaxis should be achieved within 2
h in both cortical and cancellous bone after an oral dose. Due to the risk of
emergence of resistance, moxifloxacin was not recommended for use in surgical
prophylaxis (50).

In addition to MAP-Bayesian estimation we performed a three-stage
hierarchical Bayesian analysis in WinBUGS. The parameter estimates from
WinBUGS were comparable to the results from MAP-Bayesian estimation (Table
1). Contrary to MAP-Bayesian estimation in Adapt, WinBUGS allows the
population PK parameter estimates and their between subject variability to
deviate from their prior values based on the uncertainty of the priors. This is
potentially the main reason for the differences in PK parameter estimates
between both methods. However, predictions from both sets of parameter
estimates were similar.
Overall, the concentrations in our hip bone samples were about twice as high as those found in the other studies in knee and sternum. Possible reasons could be that different types of bone were studied and different methods of sample preparation were employed. In knee replacement surgery, most often a tourniquet is applied which restricts blood flow to the leg that is operated on and this could influence bone concentrations. Also inflammation in and around the joint which was not present in our study could potentially affect bone penetration.

Secondary to the high extent of bone penetration for moxifloxacin, MCS showed robust (≥90%) PTAs for MICs up to 0.375 mg/L in serum and in cancellous bone for the targets $\text{fAUC}_{\text{SERUM}/\text{MIC}} \geq 40$ and $\text{AUC}_{\text{BONE}/\text{MIC}} \geq 33$, and up to 0.5 mg/L in cortical bone for 400 mg moxifloxacin q24h at steady-state (see Table 2). We used a protein binding of 50% for moxifloxacin in serum and assumed the protein binding in bone to be the same for moxifloxacin and ciprofloxacin, because of the absence of reports on protein binding in bone. Assuming twice as high (protein) binding of moxifloxacin in bone compared to ciprofloxacin, breakpoints would still be 0.125 mg/L in both cortical and cancellous bone for all calculated targets (Tables 2 and 3).

An MIC\textsubscript{90} of 0.125 mg/L has been reported for moxifloxacin against \textit{S. aureus} (51). If one simplifies the PTA vs. MIC profile by assuming a PTA of 100% for all MICs ≤0.125 mg/L and a PTA of 0% for all MICs ≥0.25 mg/L, it is possible to calculate that the overall probability of target attainment will be ≥90% for moxifloxacin against \textit{S. aureus} based on an MIC\textsubscript{90} of 0.125 mg/L. Therefore, a high (>90%) probability for successful clinical and microbiological outcome would
be predicted for *S. aureus* infections up to a target $\frac{\text{AUC}_{\text{BONE}}}{\text{MIC}} \geq 55$ and a protein binding in bone of 50%.

In conclusion, we found a good penetration of moxifloxacin into bone. Based on AUC ratios, the median penetration [10% - 90% percentile for between subject variability] was 80% [51% - 126%] for cortical bone and 78% [42% - 144%] for cancellous bone. We found a fast equilibrium half-life (< 60 min) between serum and cortical bone as well as between serum and cancellous bone. The PKPD breakpoint for moxifloxacin 400 mg q24h at steady-state was 0.375 mg/L in serum and cancellous bone, and 0.5 in cortical bone, based on the target $\frac{\text{AUC}_{\text{BONE}}}{\text{MIC}} \geq 33\ (\frac{\text{AUC}_{\text{SERUM}}}{\text{MIC}} \geq 40)$ for successful microbiological outcome and assuming a protein binding of 50% for moxifloxacin in serum and the same extent of binding as ciprofloxacin in bone. As the MIC$_{90}$ of moxifloxacin is 0.125 mg/L against *S. aureus*, moxifloxacin was predicted to have a high probability (≥90%) for successful microbiological outcome. This provides the required basis for a larger study on the clinical effectiveness of moxifloxacin against bone infections.

**Acknowledgement**

We thank Stephen B. Duffull, Ph.D. and Venkata V. Pavan Kumar for helpful comments about the use of WinBUGS.
References:


15. Drusano, G. L., S. L. Preston, C. Fowler, M. Corrado, B. Weisinger, and J. Kahn. 2004. Relationship between fluoroquinolone area under the curve: minimum inhibitory concentration ratio and the probability of


Table 1  Median parameter estimates (coefficient of variation) and range of individual PK parameter estimates

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>MAP- Bayesian (ADAPT II) Median (% CV) [range]</th>
<th>3 stage hierarchical Bayesian (WinBUGS) Median (% CV) [range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>L h(^{-1})</td>
<td>10.8 [9.85-11.5]</td>
<td>10.7 [7.80-27.4]</td>
</tr>
<tr>
<td>(V_{\text{Central}})</td>
<td>L</td>
<td>62.0 [58.5-65.4]</td>
<td>45.2 [41.1-45.6]</td>
</tr>
<tr>
<td>(V_{\text{Peripheral}})</td>
<td>L</td>
<td>59.5 [48.0-71.6]</td>
<td>58.6 [49.0-76.7]</td>
</tr>
<tr>
<td>CLic</td>
<td>L h(^{-1})</td>
<td>18.9 [15.3-23.2]</td>
<td>20.0 [17.3-22.8]</td>
</tr>
<tr>
<td>(F_{\text{Cortical}})</td>
<td>-</td>
<td>0.803 (35%) [0.185-1.71]</td>
<td>1.01 (17%) [0.761-1.42]</td>
</tr>
<tr>
<td>(F_{\text{Cancellous}})</td>
<td>-</td>
<td>0.775 (48%) [0.278-1.56]</td>
<td>0.953 (26%) [0.592-1.49]</td>
</tr>
</tbody>
</table>

CL is the apparent total clearance (CL/F) from the central compartment. \(V_{\text{Central}}\) and \(V_{\text{Peripheral}}\) are the apparent volumes of distribution of the central and peripheral compartment, and CLic is the apparent intercompartmental clearance. \(F_{\text{Cortical}}\) and \(F_{\text{Cancellous}}\) describe the equilibrium concentration ratio between bone and serum (see Methods for details).
Table 2  PKPD breakpoints for moxifloxacin in serum, cortical and cancellous bone, and various PKPD targets for fAUC/MIC after oral moxifloxacin doses of 400 mg q24h at steady-state (based on the estimates from Adapt II, Table 1)

<table>
<thead>
<tr>
<th>fAUC/MIC PKPD breakpoint (mg/L)</th>
<th>AUC/MIC PKPD breakpoint (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Serum*</td>
</tr>
<tr>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>0.375</td>
</tr>
<tr>
<td>66</td>
<td>0.19</td>
</tr>
<tr>
<td>Bone</td>
<td>Cortical bone* Cancellous bone*</td>
</tr>
<tr>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>33</td>
<td>0.5</td>
</tr>
<tr>
<td>55</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*: Assuming a moxifloxacin protein binding of 50% in serum and the same binding as for ciprofloxacin in bone.
Table 3  PKPD breakpoints in cortical and cancellous bone for the target AUC/MIC ≥ 33 after oral moxifloxacin doses of 400 mg q24h at steady-state depending on free fraction of moxifloxacin in bone compared to ciprofloxacin (based on the estimates from Adapt II, Table 1)

<table>
<thead>
<tr>
<th>Free fraction in bone compared to ciprofloxacin</th>
<th>PKPD breakpoint (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortical bone*</td>
</tr>
<tr>
<td>Same extent (100 %)</td>
<td>0.5</td>
</tr>
<tr>
<td>75 %</td>
<td>0.375</td>
</tr>
<tr>
<td>50 %</td>
<td>0.25</td>
</tr>
<tr>
<td>25 %</td>
<td>0.125</td>
</tr>
<tr>
<td>10 %</td>
<td>0.047</td>
</tr>
</tbody>
</table>
Figure 1 Diagram of the compartmental model

Cortical bone compartment X4

\[ k_{24} \]

\[ k_{42} \]

Central compartment X2

\[ V_{\text{Central}} \]

Cancellous bone compartment X5

\[ k_{25} \]

\[ k_{52} \]

Peripheral compartment X3

\[ V_{\text{Peripheral}} \]

Abbreviations are explained in the methods section.
Figure 2  Concentrations in serum and bone of subjects undergoing hip replacement surgery after a single oral dose of 400 mg moxifloxacin.

Panel A: Serum

Panel B: Cortical bone

Panel C: Cancellous bone
Figure 3 Penetration of moxifloxacin into cortical and cancellous bone (based on the estimates from Adapt II, Table 1), determined by the ratio of AUCs in bone and serum at steady-state.
Figure 4  Predictive check for serum and bone concentrations after 400 mg moxifloxacin po based on the estimates from Adapt II (Table 1): The plots show the raw data, the 90% prediction interval [5 - 95% percentile] and the interquartile range [25 - 75% percentile]. Ideally, 50% of the raw data points should fall inside the interquartile range at each time point and 90% of the raw data should fall inside the 90% prediction interval.
Figure 5 Probabilities of target attainment for serum, cortical and cancellous bone after 400 mg moxifloxacin po q24h at steady-state (based on the estimates from Adapt II, Table 1)

<table>
<thead>
<tr>
<th>fAUC/MIC</th>
<th>Target calculated from</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>Gentry et al. (21) for successful clinical outcome in osteomyelitis patients</td>
</tr>
<tr>
<td>Bone</td>
<td>Nix et al. (37) for bacterial eradication and Hoogkamp-Korstanje (24) for successful clinical outcome in osteomyelitis patients</td>
</tr>
<tr>
<td>Serum</td>
<td>Greenberg et al. (22) for successful clinical outcome in osteomyelitis patients</td>
</tr>
</tbody>
</table>

![Graphs showing probabilities of target attainment for serum, cortical and cancellous bone](#)