Chapter 3
Pharmacokinetics and Pharmacodynamics of Antibiotics in Bone

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Chronic osteomyelitis requires prolonged antibiotic treatment, has a high recurrence rate, and can cause irreversible damage. Also, the number of orthopedic device–related infections continues to increase [1]. Therefore, adequate antibiotic treatment and surgical prophylaxis are critical. Therapeutic success is primarily determined by the antimicrobial activity against the infecting pathogen and the rate and extent of antibiotic penetration into bone. Adequate bone penetration has to be ensured as antibiotics need to reach effective concentrations at the infection site to kill bacterial pathogens. Therefore studying the time course and extent of bone penetration before launching a clinical effectiveness trial is important. The aim of this chapter is to review the pharmacokinetics (PK) and pharmacodynamics (PD) of antibiotics in bone and present methods that support optimized evidence-based selection of antibiotic dosage regimens.

Pharmacokinetics

Time course and magnitude of drug concentrations in the body and particularly at the site of action determine the drug effects. Therefore it is important to study PK, which describes the relationship between the dose of a drug and the resulting time course of drug concentrations at various spaces in the body [2, 3]. PK processes include drug absorption from the site of administration into the systemic circulation (except if administered directly into the bloodstream), distribution from the systemic circulation into tissues, and elimination via metabolism, renal excretion, or both. Most frequently PK is characterized based on drug concentrations measured in plasma or serum. However, in treating bone infections, adequate antibiotic concentrations need to be achieved at the site of infection in bone. Numerous clinical studies have been conducted to quantify antibiotic concentrations in bone.
Bone is a heterogeneous tissue, where the organic bone matrix represents 30–35% of total bone mass and includes collagen fibrils (~90%), glycoproteins, proteoglycans, and extracellular fluid. Blood vessels in bone are located in Haversian and Volkmann's canals that transverse the bone matrix. Bone cells represent only 1–2% of total bone mass and in their most mature form as osteocytes are trapped inside the bone matrix. The inorganic matrix (65–70%) consists of calcium phosphate crystals (hydroxyapatite) deposited inside the organic matrix. Due to this heterogeneous composition, most likely neither bacteria nor antibiotics distribute evenly throughout the bone tissue.

The site of the pathogens in bone is not well-known. Based on their size (e.g., ~1 µm for *Staphylococcus aureus*), bacteria are expected to distribute through the Haversian and Volkmann canals (~70-µm diameter) in bone, but not into the hydroxyapatite crystals. *S. aureus* can enter into and survive in osteoblasts, which may explain relapses. In addition, it adheres to components of the bone matrix such as collagen [4]. Techniques to separate the different components of bone and measure concentrations in each are lacking. Therefore the vast majority of published studies are based on homogenized bone samples, and the total drug concentrations in bone homogenate are reported. For the interpretation of bone penetration results, it is important to note that only free drug is microbiologically active. However, total drug concentrations in bone homogenate, provided they are reliably determined and analyzed by population modeling and Monte Carlo simulations, may be more predictive of therapeutic success than serum concentrations.

**Bone Sample Preparation and Analysis**

In contrast to plasma or serum, there is no specific guidance available for drug analysis in bone or other tissues. However, validated and reproducible sample preparation and drug determination procedures are undoubtedly critical. It is important to consider the techniques used in published studies, when interpreting the results of these trials.

After bone resection, adhering blood and soft tissue is often removed from the sample. Excess blood due to intraoperative soaking can result in biased results, for example, artificially high bone concentrations for a drug with low bone penetration but high blood concentrations. Samples are usually separated into cancellous bone (the inner part of the long bones) and cortical bone. Cancellous bone has a higher degree of vascularization, a higher percentage of extravascular fluid, and a lower percentage of inorganic matrix than cortical bone, which can cause differences in antibiotic penetration.

For efficient extraction of the antibiotic, bone samples need to be homogenized. When bone samples are pulverized under liquid nitrogen in a cryogenic mill, this provides a very fine powder, is highly reproducible, and is applicable to thermally unstable drugs (e.g., β-lactam antibiotics) that are prone to degradation during grinding without freezing. Therefore, this method is preferable to slicing, grinding by mortar and pestle, or using mixers without cooling, as frequently applied in earlier studies before more recent technology was developed. During drug extraction from the homogenized sample, sufficient recovery and stability of the drug need to be ensured.

Calibration standards and quality control samples are necessary for accurate drug determination and should be prepared in drug-free bone powder instead of plasma, serum, or buffer. An internal calibration standard should be added to each sample to improve the analytical accuracy and precision. Older studies frequently determined drug
concentrations by bioassay. Newer studies have mainly employed high-performance liquid chromatography (HPLC) and recently also liquid chromatography–tandem mass spectrometry (LC–MS/MS), offering improved sensitivity and specificity. HPLC was shown to be generally superior to bioassays when analyzing bone samples [5]. Bone penetration studies should report details on the chosen methods for sample preparation and analysis, and the recovery, bias, and precision.

Concentrations in bone are typically reported as mg/kg of total bone mass. Some studies report concentrations in relation to bone volume, organic bone mass, or interstitial fluid or correct for blood content. Potential differences in reporting need to be taken into account when comparing results between studies.

**Pharmacokinetic Sampling and Data Analysis**

Usually, only one bone sample can be taken per patient, and a blood sample is taken at the same time. Most studies report bone penetration as the concentration ratio between bone and serum or plasma at one time point. However, due to different kinetics of drug concentrations in plasma and bone, the concentration ratios change over time until eventually an equilibrium has been reached during the terminal phase. This phenomenon (system hysteresis) hampers the interpretation of results and comparison between drugs and studies when samples are taken at different times post dosing.

A better measure for the extent of bone penetration is to calculate the area under the concentration–time curve (AUC) in bone and compare it to the AUC in plasma or serum. This takes into account the full time course of the concentration profiles in bone, plasma, and serum. Instead of collecting the bone and blood samples at the same time point after the dose for all patients, samples should be spread out over a time period to support PK modeling. Based on such study designs, investigators have averaged the concentrations at each time point and derived the average AUCs in bone and plasma (naive averaging) [6–8]. Alternatively, one PK function was fit to the concentration–time data from all patients (naive pooling) and the AUC integrated [9, 10]. While these approaches remove the issue of time-dependent concentration ratios, they only consider the average concentration–time profile and ignore the true biological variability between patients.

Population PK analysis is the most powerful approach for the analysis of sparse data (e.g., one bone sample per patient) and accounts for the average rate and extent of bone penetration and interpatient variability [11, 12]. By fitting each patient’s data in the perspective of the concentrations from the other patients, the most likely concentration–time course in bone and serum and the AUC can be predicted for each patient. Estimating the rate of bone penetration enables recommendations on the administration time of antibiotic prophylaxis before surgery. An existing population PK model can also be used to identify the optimal timing of bone and plasma samples in future bone penetration studies.

Bone penetration is usually studied in joint replacement patients with uninfected bone as such patients are more easily recruited than osteomyelitis patients. The condition of the bone samples is likely more homogeneous among joint replacement patients than patients with various stages and locations of bone infections; therefore, results of different studies can be more readily compared. However, antibiotic concentrations might differ between infected and uninfected bone. Reactive hyperemia could increase the blood flow into bone, whereas pus or sequesters might limit the distribution of
antibiotics into bone. To date, few studies have been performed in patients with bone infections, which does not enable a systematic comparison of penetration between infected and uninfected bone. Presence of ischemic, calcified, or arthritic tissues, bone cysts, or fat in the cancellous bone may affect antibiotic distribution. Different types of bone (e.g., hip, knee, sternum) and influences on blood circulation, for example, tourniquet application or internal mammary artery harvesting, may also affect antibiotic bone concentrations.

**Penetration of Antibiotics into Bone**

Figure 3.1 presents an overview of the extent of bone penetration by antibiotic group. Each symbol represents the median bone-to-serum (or plasma) concentration ratio from one clinical study, and the lines indicate the median per antibiotic group. In total, 126 studies (until July 2013) were included. Most concentration ratios were reported directly in the published studies; sometimes they were calculated from the reported concentrations or read from plots. A comprehensive reference list can be found in Ref. [13]. Tables 3.1 and 3.2 list the range of average concentration ratios for various antibiotics. Concentration ratios are average ± standard deviation and based on total concentrations in bone homogenate from at least five samples, unless indicated otherwise. AUC ratios are reported in the text where available. Studies published since the previous review [13] are discussed here in more detail.

Systematic differences can be observed between antibiotic groups, which may be due to different physicochemical and binding characteristics. Median bone-to-serum concentration ratios were 0.48 for quinolones and 0.40 for linezolid. Macrolides span a

![Figure 3.1](image-url)

**Figure 3.1.** Bone penetration for different antibiotic groups [13]. Each symbol represents the median or average bone-to-serum or bone-to-plasma concentration ratio from one clinical trial. The lines represent the group medians.
wide range of concentration ratios. Despite large differences in chemical structure, clindamycin, rifampicin, glycopeptides, fosfomycin, and fusidic acid had comparable median concentration ratios of 0.23–0.35. Penicillins, cephalosporins, and β-lactamase inhibitors showed median concentration ratios of 0.16, 0.18, and 0.22. Figure 3.1 also demonstrates a large variability between antibiotics within each group, which may in part be caused by different bioanalytical methodologies and sampling times (as described earlier).

**Fluroquinolones**

Fluroquinolones are frequently used in bone infections and show one of the highest median extents of bone penetration of all antibiotic groups with bone-to-serum concentration ratios mostly between 0.3 and 1.2 (Figure 3.1). The high penetration may be partly due to binding of quinolones to the calcium in bone. As only free antibiotic is considered microbiologically active, the quinolone concentrations available for antimicrobial action are likely lower than the total bone concentrations. The concentration ratios of most quinolones tend to increase with time since the last dose, indicating slow redistribution from bone back into the bloodstream. Quinolones generally penetrate well into cells. This could be advantageous for treatment of *S. aureus* osteomyelitis, since *S. aureus* was shown to penetrate into and survive in osteoblasts in vitro [4, 34].

Multiple studies in different patient groups have examined the bone penetration of ciprofloxacin (Table 3.1). Massias *et al.* [8] took cortical bone of the mastoid process plus serum samples at five different time points from 21 patients suffering from chronic otitis.

### Table 3.1. Bone penetration of quinolones and macrolides.

<table>
<thead>
<tr>
<th>Antibiotic and bone condition</th>
<th>Range of time since last dose</th>
<th>Range of average bone/serum concentration ratios</th>
<th>Bone or surgery type</th>
<th>Bio-analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uninfected</td>
<td>0.5–13 h</td>
<td>0.27–1.2</td>
<td>Hip, knee, skull, debridement surgery</td>
<td>HPLC [8, 14, 15]</td>
</tr>
<tr>
<td>osteomyelitis</td>
<td>2–4.5 h</td>
<td>0.42</td>
<td></td>
<td>HPLC [14]</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uninfected</td>
<td>0.7–2 h</td>
<td>0.36–1.0</td>
<td>Hip, other</td>
<td>HPLC [16–18]</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uninfected</td>
<td>0.5–12 h</td>
<td>0.09–1.04</td>
<td>Hip, nasal bone, mastoid process</td>
<td>HPLC [19–21]</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uninfected</td>
<td>1.5–5 h</td>
<td>0.33–1.05</td>
<td>Hip, knee, sternum, manubrium</td>
<td>HPLC [11, 18, 22, 23]</td>
</tr>
<tr>
<td>Azithromycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>uninfected</td>
<td>0.5–6.5 days</td>
<td>2.5–6.3</td>
<td>Alveolar bone</td>
<td>Bioassay [24, 25]</td>
</tr>
<tr>
<td>Telithromycin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>uninfected</td>
<td>3.3–24 h</td>
<td>1.5–2.6</td>
<td>Ethmoid bone</td>
<td>HPLC [6]</td>
</tr>
</tbody>
</table>

HPLC, high-performance liquid chromatography.
The AUCs in bone and serum were calculated by naive averaging and the trapezoidal rule and the bone-to-serum AUC ratio was 0.63. Average bone-to-serum concentration ratios increased from 0.27 to 1.2 between 1 and 12 h after the dose, suggesting slow redistribution from bone to blood. Fong et al. [14] compared ciprofloxacin concentrations in cortical bone from patients without (n = 18, hip or knee replacement or osteotomy) and with (n = 10) osteomyelitis. Concentrations in infected bone were 30–100% higher than in uninfected bone. As serum concentrations were also higher in osteomyelitis patients, the average bone-to-serum concentration ratios were approximately 0.4 in both patient groups. Studies in various bone types indicate a penetration of 0.40 or higher for ciprofloxacin.

Bone penetration of levofloxacin was evaluated by three relatively recent studies (Table 3.1). In patients undergoing bone surgery (n = 9) or decubitus ulcer debridement (n = 12) the bone-to-serum concentration ratios were 0.36 for cortical (n = 6) and 0.85 ± 0.40 for cancellous (n = 14) bone [16]. In 12 hip replacement patients, ratios of

### Table 3.2. Bone penetration of beta-lactams.

<table>
<thead>
<tr>
<th>Antibiotic and bone condition</th>
<th>Range of time since last dose</th>
<th>Range of average bone/serum concentration ratios</th>
<th>Bone or surgery type</th>
<th>Bio-analytical method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amoxicillin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>0.5–6 h</td>
<td>0.03–0.31</td>
<td>Hip, jaw</td>
<td>bioassay [9, 26, 27], LC-MS/MS [12]</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>0.5–6 h</td>
<td>0.01–0.14</td>
<td>Hip</td>
<td>bioassay [9, 26], LC-MS/MS [12]</td>
</tr>
<tr>
<td>Ampicillin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>0.25–1 h</td>
<td>0.11–0.20</td>
<td>Hip, knee, vertebrae</td>
<td>Bioassay [10, 28]</td>
</tr>
<tr>
<td>Uninfected (with blood washing)</td>
<td>1–4 h</td>
<td>0.44–0.71</td>
<td>orthopedic surgery</td>
<td>Bioassay [29]</td>
</tr>
<tr>
<td>Sulbactam</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>0.25–1 h</td>
<td>0.17–0.58</td>
<td>Hip, knee, vertebrae</td>
<td>Gas chromatography [10, 28]</td>
</tr>
<tr>
<td>Uninfected (with blood washing)</td>
<td>1–4 h</td>
<td>0.58–0.71</td>
<td>orthopedic surgery</td>
<td>Gas chromatography [29]</td>
</tr>
<tr>
<td>Cefotiam</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected (with blood washing)</td>
<td>1–4 h</td>
<td>0.27–0.44</td>
<td>Orthopedic surgery</td>
<td>HPLC [29]</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninfected</td>
<td>1–2 h</td>
<td>0.46–0.76a</td>
<td>Hip</td>
<td>HPLC [30]</td>
</tr>
<tr>
<td>Ischemic bone</td>
<td>1–2 h</td>
<td>0.04–0.08</td>
<td>Cardiac surgery</td>
<td>Bioassay [31]</td>
</tr>
</tbody>
</table>

HPLC, high-performance liquid chromatography; LC–MS/MS, liquid chromatography–mass spectrometry.

aAssuming a bone density of 1 kg/l.
1.0 ± 0.4 for cortical and 0.5 ± 0.1 for cancellous bone were reported [17]. Concentration ratios of 0.42 ± 0.04 in cortical and 0.54 ± 0.05 in cancellous bone were found in eight hip replacement patients [18]. The differences in penetration to cortical versus cancellous bone might be partly due to relatively small sample sizes. Moxifloxacin was studied in four trials, which showed consistently high penetration considering the range of different bone types (Table 3.1) and methods for sample homogenization (hand mincing, sonication, cryogenic mill). In all moxifloxacin studies, the penetration into cancellous and cortical bone was similar. Utilizing a cryogenic mill and population PK analysis, the bone-to-serum AUC ratios in 24 hip replacement patients were 0.80 (10th–90th percentile for between-patient variability: 0.51–1.26) for cortical and 0.78 (0.42–1.44) for cancellous bone [11].

**Macrolides and Telithromycin**

Macrolides demonstrate the largest range of penetration of all antibiotic groups (Figure 3.1). All studies utilized bioassays, and most were performed decades ago when contemporary bioanalytical technology was not yet available. The analytical recovery from bone samples was often low, for example, for erythromycin. In two more recent studies [24, 25], patients received 500 mg azithromycin once daily for 3 days before periodontal surgery. In both studies, the average concentration ratios increased slightly from 12 h to 2.5 days, when they reached greater than 6.0, and then slowly decreased to approximately 2.5 at 6.5 days. Azithromycin bone concentrations decreased from 1.61 ± 0.22 mg/kg at 12 h to 0.44 ± 0.05 mg/kg at 6.5 days [25]. The rate of azithromycin penetration into bone is not well-known as the first samples were taken at 12 h. Azithromycin is known to accumulate in cells, for example, macrophages. However, bone cells represent only 1–2% of total bone weight. Depending on the bone type, differences in the content of red bone marrow as part of a cancellous bone sample might potentially lead to variations in macrolide concentrations due to their accumulation in leukocytes.

Telithromycin penetration into ethmoid bone was studied in 29 patients [6]. Using naive averaging, the average AUC in bone was 6730 mg·h/l and the AUC in plasma was 4230 mg·h/l, indicating a bone-to-serum AUC ratio of 1.6. This suggests one of the highest extents of penetration of all studied antibiotics. The concentration ratio increased between 3 and 24 h, indicating slow equilibrium that may favor administration at least approximately 12 h ahead of surgery.

**Clindamycin**

Clindamycin is often referred to as possessing exceptionally high bone penetration. The median bone-to-serum concentration ratio of 0.35 from four clindamycin studies, however, appears to be lower than for quinolones (median 0.50) and linezolid (median 0.40) (Figure 3.1). As most clindamycin studies were performed in the 1970s, that is, before the introduction of fluoroquinolones, linezolid, and azithromycin, the bone penetration of clindamycin was higher than that of other available antibiotics at that time. A more recent study reports bone concentrations between 3.4 and ~0.2 mg/l in 13 maxillofacial surgery patients at 0.5–8 h after a 600 mg dose. Concentration ratios were not reported; however, based on plots the bone concentrations were less than 50% of plasma concentrations at all times [35]. All available clindamycin studies used bioassays, which have the potential
to be confounded by active metabolites of clindamycin. Results from multiple studies suggest an extent of bone penetration of clindamycin of 0.21–0.45, similar to or slightly higher than cephalosporins.

**Rifampicin**

A wide range of bone-to-serum concentration ratios (0.08–0.56 at 2–14 h after the dose) was found for rifampicin in four studies in uninfected bone from the 1970s/1980s. One of the trials also investigated infected bone, and concentration ratios were similar to those for uninfected bone (0.57 versus 0.46). All studies utilized bioassays and had high inter-patient variability [13].

**Tetracyclines and Tigecycline**

Few studies are available for tetracyclines. Results vary despite the high binding affinity of tetracyclines to calcium. For tigecycline, initially a bone-to-serum AUC ratio of 0.41 (by naïve averaging) or 0.28 (calculated from median concentrations) over 24 h was found in 25 uninfected surgical patients [7]. Concentration ratios increased from 4 to 24 h. Re-analysis of the samples by a new LC–MS/MS assay, including a stabilizing agent, resulted in bone concentrations that were on an average 9.5-fold higher as compared to the previous method [36]. These results are also consistent with radiolabeling studies in animals. This study highlights the importance of bioanalytical methods and of taking into account the full concentration–time course.

**Cephalosporins**

Numerous studies were performed with cephalosporins, most frequently cefuroxime. Its overall average bone-to-serum concentration ratio was 0.32 (range 0.09–0.55, 10 min to 6.5 h post dose) in five studies that reported concentrations in serum and uninfected bone and in which the majority of samples were above the detection limit [13]. One study found very low penetration into sternum due to a high detection limit [37]. Median concentration ratios at 1 h after the dose were approximately 0.18 in 14 trauma surgery patients and approximately 0.06 in 7 osteomyelitis patients [38].

Ceftriaxone and cefamandole were evaluated in the same study in hip replacement patients [39]. At 10–30 min after the dose, average (95% confidence interval) bone-to-serum concentration ratios were 0.156 (0.123–0.190) for ceftriaxone and 0.184 (0.156–0.212) for cefamandole. The bone-to-plasma concentration ratios based on total drug were similar despite a sixfold difference in the non-protein-bound fractions in plasma (0.05 for ceftriaxone, 0.30 for cefamandole), although only unbound drug is believed to distribute between plasma and tissues. This issue is discussed in more detail in our previous review [13].

At 8 h after the dose, ceftriaxone bone-to-serum concentration ratios were 0.142 (0.073–0.210), very similar to those at 10–30 min, suggesting a fast equilibrium between serum and bone [39]. Recently, in 11 patients undergoing debridement for septic non-union of the tibia, ceftriaxone concentrations were measured by HPLC, and average AUCs over 24 h were calculated by the trapezoidal rule [40]. Average bone-to-plasma AUC ratios were 0.093 in cortical and 0.241 in cancellous bone. This 2.6-fold difference between cortical and cancellous bone is considerably larger than in most other studies.
Average bone-to-serum concentration ratios for cefamandole in hip replacement patients were 0.227–0.249 at 10–30 min after the dose [41]. Another trial found cefamandole bone-to-serum concentration ratios increasing from 0.8 at 1 h after the dose to 2.3 at 4 h [29]. Considering the lower values at early time points [39, 41] and the short elimination half-life (0.8 h), this could indicate slow redistribution from bone to blood. However, modeling the full concentration–time course and additional data would be required to support a sound time–course analysis. Alternatively, the high ratios [29] could be partly due to low serum concentrations as intraoperative blood saving including washing of the drained blood was applied in this study. The overall range of concentration ratios reported for cefamandole was 0.12–2.3 at 10 min to 4 h [13].

Two recent studies investigated cefazolin. The median bone-to-serum concentration ratios in eight infected patients were 0.25 (range 0.06–0.41) during continuous cefazolin infusion, with concentrations determined by bioassay [42]. Yamada et al. [43] utilized HPLC and found cancellous bone concentrations of 22.4 ± 14.8 mg/kg at 63 ± 25 min and serum concentrations of 170.3 ± 51.3 mg/l at 49 ± 13 min in 42 patients. Bone concentrations in knee replacement (16.0 mg/kg) tended to be lower than those in hip replacement (32.3 mg/kg) during a similar sampling time period. In an earlier study, the average bone-to-serum concentration ratio was 0.18 in 20 hip replacement patients at 0.9 h after the dose [44]. Additional results for several β-lactams are presented in Table 3.2.

Overall cephalosporins achieved concentration ratios of 0.1-0.5. Penetration was higher into cancellous bone than into cortical bone in all studies that analyzed both, potentially due to the higher proportion of extracellular fluid in cancellous bone [13]. β-Lactams, including cephalosporins, are assumed to distribute mainly into extracellular fluid and were found to exhibit limited binding to the inorganic bone matrix.

**Penicillins and β-Lactamase Inhibitors**

The most frequently studied β-lactams/β-lactamase inhibitors are amoxicillin/clavulanic acid, ampicillin/sulbactam, and piperacillin/tazobactam.

Two studies by different groups from 1994 and 2001 evaluated piperacillin/tazobactam penetration into uninfected hip bone in 12 patients each, used the same sample preparation methods and analysis by HPLC, and found consistent results. Bone-to-plasma concentration ratios were 0.2–0.3 for piperacillin and tazobactam in cortical and cancellous bone at 1–1.5 h after the dose [45, 46]. More recently, penetration of piperacillin/tazobactam into uninfected jaw (n = 7) and hip (n = 2) bone was studied [47]. Sample preparation was similar to the previous studies and concentrations were analyzed by LC–MS/MS. At an average of 3 h (range 1–7 h) after the start of the infusion, bone-to-plasma concentration ratios were 0.15 for piperacillin and 0.13 for tazobactam. These results were slightly lower and more variable than those from the previous studies, potentially due to different bone types and the wider range of sampling times.

A wide range of average amoxicillin bone-to-serum concentration ratios was reported in studies utilizing bioassays (Table 3.2). In a study in 20 hip replacement patients analyzed by LC–MS/MS and population PK analysis, the bone-to-serum AUC ratios were 0.20 (10th–90th percentile for between-patient variability 0.16–0.25) for cortical and 0.18 (0.11–0.29) for cancellous bone [12]. In the same study, the bone-to-serum AUC ratios of clavulanic acid were 0.15 (0.11–0.21) for cortical and 0.10 (0.051–0.21) for cancellous bone. Penetration of clavulanic acid tended to be slightly lower in most studies, although
it is the smaller molecule and might be expected to distribute more freely. However, lipophilicity information is not available, which may also play an important role.

**Linezolid**

Linezolid is comparatively stable, as opposed to many β-lactams, and the available studies were performed utilizing HPLC. Average (95% confidence interval) bone-to-serum concentration ratios were 0.51 (0.43–0.75) in 12 hip replacement patients at 30–50 min after the start of the infusion [41]. Relatively high penetration (0.40 ± 0.24) was also found in 12 elderly patients during knee replacement at 1.5 h [48]. At the same dose as the two joint replacement studies [41, 48], and 0.5–1.5 h after the dose, lower linezolid concentrations were found in 11 patients with implant-associated infections. The average bone-to-plasma concentration ratio was approximately 0.23 [49]. Inflammation-related decreased blood supply or differences in sample preparation were considered as potential reasons for the differences between uninfected and infected bone [49].

Recently, linezolid in bone was also analyzed by microdialysis, which allows determination of unbound drug concentrations in interstitial fluid, serial sampling, and calculation of individual AUCs in bone. For insertion of the microdialysis catheter, a hole is drilled into the bone, which results in a dead space that fills with blood clots and extracellular fluid exudations [50]. Measured concentrations are therefore assumed to represent concentrations in the dead space and the interstitial fluid of the adjacent bone tissue [50]. The ratio of unbound AUCs ($f_{\text{AUC}}$) in vital cancellous bone/plasma over 12 h was 1.09 ± 0.11 in three diabetic patients with severe foot infections, suggesting similar exposure to microbiologically active linezolid for pathogens in interstitial bone fluid and in the bloodstream [51]. The higher AUC ratio from microdialysis, as compared to concentration ratios based on bone homogenate, is in keeping with a low propensity of linezolid to form chelate complexes with the inorganic bone matrix.

**Daptomycin**

Bone penetration of daptomycin was evaluated in diabetic foot infections [52]. Serial microdialysis samples at the steady state were collected from 0 to 8 h after the dose in five patients and from 8 to 16 h in another four patients. The average ratio of the $f_{\text{AUC}}$ (0–16h) in interstitial fluid of metatarsal bone/plasma was 1.08. This ratio of unbound bone-to-unbound plasma concentrations suggests high penetration of daptomycin into interstitial fluid, that is, the most likely site of infection, and was achieved for a drug with high plasma protein binding (~90%) and a high molecular weight. The authors stated that the effect of drilling a hole into the bone for insertion of the microdialysis catheter on bone concentrations is not yet clarified [52]. Thus, a comparison with total bone-to-plasma concentrations would be interesting to compare the results of different methods.

**Fosfomycin**

Bone homogenate-to-serum concentration ratios of 0.13–0.45 were reported in three trials from 1980 to 1983 utilizing bioassays (Figure 3.1). Fosfomycin binds to hydroxyapatite in bone, suggesting that not all fosfomycin in bone homogenate is microbiologically active. However, a recent microdialysis study found $f_{\text{AUC}}$ ratios of 0.43 ± 0.04 in nine osteomyelitis patients with diabetic foot infection, which is higher than or similar to the
reported bone homogenate-to-plasma concentration ratios [53]. Considering the very
limited or no binding of fosfomycin to plasma proteins, this would indicate that the
average concentration bound to various components of bone tissue is lower than or sim-
ilar to the interstitial fluid concentrations. However, comparison among studies is ham-
pered by differences in study designs and methodologies.

**Glycopeptides**

A wide range of average concentration ratios, mostly between 0.1 and 0.6, has been
reported for glycopeptides in hip, knee, or sternal bone (Figure 3.1). A recent study inves-
tigated both glycopeptides in septic pseudoarthritis of the tibia [54]. Validated HPLC
assays were used. However, samples were ground without cooling, and the PK methods
to calculate individual AUCs were reported in limited detail. Average bone-to-plasma
AUC ratios for vancomycin were 0.21 for cortical and 1.04 for cancellous bone. Average
bone-to-plasma AUC ratios for teicoplanin were 0.12 for cortical (n = 17 patients) and
0.56 for cancellous bone (n = 15). Such large differences between cortical and cancellous
bone were also seen in a ceftriaxone study by the same authors (described earlier), who
suggest that this may be due to infected bone or different methods of drug extraction or
analysis. Glycopeptide bone concentrations increased with inflammatory marker concen-
trations, potentially due to increased tissue vascularization. The results from this study
fall into the range of previous reports; however, drawing overall conclusions on glycopep-
tide bone penetration remains difficult.

**Pharmacodynamics and Monte Carlo Simulations**

PD describes the relationship between drug concentrations in plasma or at the target
(i.e., infection) site and the time course of drug effect(s). For β-Lactams the time during
which the unbound antibiotic concentration remains above the minimum inhibitory
concentration (fT$_{\geq}$MIC) of the pathogen has been shown to be predictive of the extent of
antibiotic effect. For other antibiotics, such as quinolones, the fAUC/MIC best corre-
lates with effect.

For bone penetration studies that used the same sampling time for all samples, it is not
feasible to perform a PD analysis because comparing the bone concentration at a specific
time to the MIC provides limited information. Irrespective of the type of data analysis
used, adequate reporting of the methods and assumptions is important.

Naive pooling or averaging approaches (as described earlier) can calculate the average
AUC/MIC and time above MIC. This indicates whether an “average” patient would
attain the PK/PD target. However, these naive methods have the disadvantage that they
do not consider the true variability between patients, which tends to be large for bone
penetration.

Population modeling accounts for both the average penetration and its variability bet-
ween subjects (Figure 3.2). Once a population PK model for plasma and bone has been
developed, it can be employed in Monte Carlo simulations to predict the expected
concentration time profiles for other than the studied dosage regimens. This includes pre-
dicting the variability in concentration time profiles between patients. Thereby, the proba-
bility of achieving a PK/PD target can be predicted and recommendations be made on
how to dose an antibiotic to maximize the probability of successful therapeutic outcome.
The PK/PD target values for plasma and bone concentrations to successfully treat bone infections are most often unknown, and the target values for other types of infections can likely not be used. For moxifloxacin, no published clinical studies in osteomyelitis were available.

**Moxifloxacin:** To address the lack of known PK/PD target values for bone, a reverse engineering approach [55] was applied for moxifloxacin to identify the most likely PK/PD target required to achieve clinical and microbiological cure of osteomyelitis [11]. This approach combined effectiveness data from clinical studies with ciprofloxacin in osteomyelitis, the expected plasma AUCs from these studies, the AUCbone-to-AUCplasma ratio for ciprofloxacin [8], and bacterial susceptibility data from the time of the clinical studies. Reverse engineering suggested a fAUC/MIC of 40 in serum and an AUC/MIC of 33 in bone as the most likely PK/PD targets for successful clinical and microbiological outcome. No assumptions are made regarding the numerical value of the free fraction of moxifloxacin in bone. It is assumed that binding and distribution within the bone tissue is similar for moxifloxacin and ciprofloxacin, two quinolones with the same essential chemical structure that are expected to be responsible for binding characteristics. The population PK model for moxifloxacin in serum and bone was utilized to predict likely probabilities of target attainment.

A ≥90% probability of successful clinical and microbiological outcome was predicted for 400 mg moxifloxacin once daily up to an MIC of 0.375 mg/l (mg/kg) in serum and
cancellous bone and 0.5 mg/l in cortical bone (Figure 3.3). Compared to, for example, an MIC\textsubscript{90} of 0.125 mg/l for \textit{S. aureus}, these are favorable results and suggest clinical trials are warranted. The antibiotic susceptibility of the local hospital should be considered when published probabilities of target attainment are used to decide about antibiotic therapy in patients. The population PK and Monte Carlo simulation approach described for bone is applicable to other matrices, for example, synovial fluid.

As an additional complexity, no methods are currently available to measure concentrations in different compartments of bone. Also, assumptions for binding (e.g., to calcium) and distribution in bone have to be made until techniques that reliably determine free antibiotic concentrations in bone become available. While microdialysis methods have been applied for bone, it is unknown whether drilling a hole into bone affects the measured unbound concentrations in bone [52].

\textit{Amoxicillin/clavulanic acid}: To address this situation, various scenarios for distribution of amoxicillin and bacteria into interstitial fluid, total bone fluid, bone cells, and organic and inorganic matrix were considered and volumes of the bone compartments were taken from literature. Thereby the likely PK/PD breakpoints, that is, the MICs up to which a 90\% probability for successful treatment is expected, could be predicted for various scenarios [12]. As clinical effectiveness studies were not available for amoxicillin/clavulanic acid, breakpoints were calculated for potential targets ranging from
For a target of $f_{T > MIC} ≥ 50\%$, corresponding to the plasma target for near-maximal bactericidal effect of β-lactams, the PK/PD breakpoint in cortical bone was 28 µg/g, if drug and bacteria distributed through the vascular space and interstitial fluid, and it was 7.5 µg/g if distribution was throughout total bone fluid [12].

For antibiotics where clinical effectiveness trials are not available, population PK, the reverse engineering approach utilizing effectiveness data from literature as described earlier, and Monte Carlo simulations appear to be the best available approach currently to derive PK/PD targets for successful treatment of bone infections and suggest dosage regimens to be studied in clinical effectiveness trials. While sufficient bone penetration is an important factor, bone concentrations alone provide limited information to draw conclusions on the effectiveness of an antibiotic. Therefore clinical recommendations should not be made exclusively based on bone penetration studies. An antibiotic also needs to have adequate antibacterial activity against the infecting pathogen. Well-controlled PK/PD studies in osteomyelitis patients would be required to further quantitatively elucidate the PK/PD relationship between antibiotic bone concentrations and clinical outcomes. However, such studies are currently scarce.

**Conclusions**

Trends in the extent of bone penetration among different groups of antibiotics have been found from a review of greater than 120 literature studies, such as a high average penetration of 0.3–1.2 for quinolones, 0.3–0.4 for linezolid, 0.1–0.3 for penicillins, and 0.1–0.5 for cephalosporins. These differences are most likely due to different physicochemical characteristics of the antibiotic groups. High variability between studies for a particular antibiotic group is likely partly due to a lack of standardization of bioanalytical methods and study design. The variability between patients within a study needs to be taken into account, and this can be achieved by population PK modeling. Developing approaches that provide insights into distribution and binding of antibiotics in bone is warranted. In 20 of 25 antibiotics, the measured concentrations were slightly higher in cancellous bone than those in cortical bone. More data are needed to characterize the effect of infected versus uninfected bone, the presence of an implant, and the type of bone (e.g., hip, knee, sternum) on the PK. Future clinical trials should focus on validated bioanalytical methods, as well as study designs, and apply PK/PD analyses that take into account the time course of bone concentrations to contribute to evidence-based care for patients with bone infections.

**Key Points**

- There are differences in the extent of bone penetration among various antibiotic groups, such as high median bone-to-serum concentration ratios of 0.3–1.2 for quinolones and lower ratios of 0.1–0.5 for β-lactams. These trends are likely related to different physicochemical and pharmacokinetic characteristics.
- The variability within antibiotic groups and between different studies for the same agent is high. Therefore utilizing standardized, validated methods for sample preparation and bioassay as well as calculating the bone-to-serum AUC ratios instead of concentration ratios would be advantageous.
Based on the currently available data, population PK modeling and Monte Carlo simulations appear to be the most promising approach to elucidate the extent and time course of bone penetration and its relationship with likely clinical outcomes. Well-controlled PK/PD studies in osteomyelitis patients are required to directly identify PK/PD targets.

References


