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Glycopeptide Bone Penetration in Patients with Septic Pseudoarthrosis of the Tibia

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Abstract

Background and objective: In the treatment of bone infections, a major determinant of the clinical response is the active drug concentration at the infected site. Because of the high prevalence of meticillin (methicillin)-resistant staphylococci and enterococci, glycopeptides are widely used for the treatment of bone and joint infections, but data on their penetration into human bone are lacking. The aim of our study was to measure vancomycin and teicoplanin concentrations in infected human bone under steady-state conditions and verify their relationship with inflammatory markers, patient demographic characteristics and pharmacodynamic microbiological markers.

Methods and patients: Twenty-seven adult orthopaedic patients undergoing surgical debridement for septic pseudoarthrosis of the tibia and receiving either intravenous vancomycin (Vancocina® 1 g twice daily) or teicoplanin (Targosid® 10 mg/kg/day) were studied from January 2004 to January 2008. Plasma and bone specimens were simultaneously collected during surgery for pharmacokinetic and microbiological assays at a variable interval after antimicrobial administration. Bone samples were dissected into cortical and cancellous bone, cleaned of soft tissues, crushed and eluted into phosphate buffer. Necrotic samples and sequestra were not analysed.

Plasma and bone antimicrobial concentrations were measured by a validated method of high-performance liquid chromatography with UV detection, and bone/plasma concentration ratios were calculated. Cortical and cancellous bone area under the concentration-time curve (AUC) over 24 hours (AUC_{24}) values were measured by the linear-log trapezoidal rule, using WinNonlin® software, and were compared with the minimum inhibitory concentrations (MICs) of the infecting agents.

Results: For vancomycin, the mean ± SD concentrations were 2.66 ± 1.2 mg/L in cortical bone and 11.53 ± 7.8 mg/L in cancellous bone (corresponding to 20.67% and 89.39% of intraoperative plasma concentrations), and the mean ± SD tissue AUC_{24} values were 55.15 ± 25.26 h • mg/L for cortical bone and 299.16 ± 299.54 h • mg/L for cancellous bone. For teicoplanin, the mean ± SD concentrations were 2.01 ± 1.7 and 7.51 ± 7.0 mg/L in cortical and cancellous bone, respectively (12.35% and 48.6% of intraoperative plasma concentrations), and the mean ± SD teicoplanin tissue AUC_{24} values were 34.08 ± 23.6 h • mg/L and 155.17 ± 132.8 h • mg/L for cortical bone and cancellous bone, respectively. The mean vancomycin AUC_{24}/MIC ratios were 215.02 for plasma, 47.14 for cortical bone and 268.95 for cancellous bone. The mean teicoplanin AUC_{24}/MIC ratios were 336.48, 36.27 and 197.21 for plasma, cortical bone and cancellous bone, respectively.

Conclusions: Bone penetration of both glycopeptides ranged from poor (<15%) to satisfactory (15–30%) in the cortical compartment, while it was far higher into the highly vascularized cancellous tissue. Vancomycin bone penetration was slightly higher than with teicoplanin, but the difference was not statistically significant. Higher

bone concentrations were observed with higher inflammatory markers, possibly as a result of increased vascularization and vascular permeability under inflammatory conditions. Bone concentrations over the MIC and AUC/MIC ratios suggested that both glycopeptides achieve a satisfactory pharmacokinetic exposure in the cancellous bone, as far as Gram-positive pathogens are concerned. On the other hand, cortical bone exposure was suboptimal in most patients. Furthermore, as antimicrobial penetration may be affected by impaired blood supply, the role of radical surgical removal of purulent and necrotic tissues appears to be essential in order to shorten treatment duration and to reduce the risk of treatment failure.

Background

Osteomyelitis continues to be listed among the major problems in the setting of orthopaedic surgery, as is also evidenced by the remarkable proportion of cases evolving towards chronic forms. The majority of cases are caused by staphylococci, Gram-negative bacteria and anaerobes.[1] Osteosynthesis implant devices are at high risk of infection by bacteria with multiple antibacterial resistance, such as meticillin (methicillin)-resistant *Staphylococcus aureus* (MRSA), meticillin-resistant *S. epidermidis* (MRSE) or vancomycin-resistant enterococci (VRE). While both systemic and local factors have been found to be associated with lower rates of successful treatment outcome, the concentration of antibacterials in the affected bone is considered to be a major determinant of the treatment response, in association with radical surgical debridement.[2-4] Therefore, the choice of an antimicrobial agent or regimen depends not only on the pathogen's sensitivity to it, but also on the ability of the drug to reach and maintain effective concentrations at the site of infection. The penetration of an antimicrobial agent into infected bone and joint tissue depends on its intrinsic pharmacokinetic and physicochemical properties but can be influenced by the degree of vascularization, soft tissue coverage and the presence of necrotic sequesters or foreign bodies. As a consequence, the rate of bone penetration of a given drug may vary significantly depending upon the presence of infection, associated inflammation and related changes in vascular permeability.

Because of the high prevalence of MRSA, MRSE and enterococci, glycopeptides are widely used for treatment of bone and joint infections.[5,6] However, data on their penetration into human bone are limited, and most of the available data come from studies of prophylaxis rather than studies of therapy. In most of the former, antimicrobial penetration into non-infected human bone has been evaluated on samples collected during knee or hip arthroplasty after a single preoperative antimicrobial dose. Furthermore, differences in terms of general methodology, drug extraction and measurement make these studies difficult to compare. The study of antimicrobial penetration into infected bone after multiple dosing better estimates the *in vivo* situation under infection and may provide useful information for optimizing the dosage and the choice of antimicrobials. We undertook a clinical pharmacokinetic study of patients with septic pseudoarthrosis receiving either vancomycin or teicoplanin, in order to measure bone drug penetration at steady state and verify the relationship between tissue concentrations and the minimum inhibitory concentrations (MICs) of the infecting agents.

Patients and Methods

Patients

The study included patients with septic pseudoarthrosis of the tibia who were undergoing surgical debridement with resection of infected and necrotic tissue, and were treated intravenously with either vancomycin (Vancocina®;[1] Eli Lilly Italia SpA, Sesto Fiorentino, Italy; 1 g twice daily over a 1-hour infusion) or teicoplanin (Targosid®; Gruppo Lepetit SpA, Lainate, Italy; 10 mg/kg/day over a 1-hour infusion) for more than 1 week. The inclusion criteria were as follows:

1. The patient should have clinical and radiological evidence of septic pseudoarthrosis, defined as the presence of inflammation or fistula in the area of a previous bone fracture, radiological non-union of the bone involved and/or the presence of a biological inflammatory syndrome. A biological inflammatory syndrome included an erythrocyte sedimentation rate (ESR) of >50 mm/h and elevated levels (>10 mg/dL) of C-reactive protein (CRP).
2. Indication for surgical debridement of infected/necrotic bone and external fixation treatment.
3. The patients should be on current intravenous antimicrobial treatment at the time of surgical intervention – for at least 7 days for vancomycin and 18 days for teicoplanin – to guarantee attainment of steady state; antimicrobial treatment should conform to

1 The use of trade names is for product identification purposes only and does not imply endorsement.

'best practice and standard of care guidelines' as to the indication and dosage.[6]

The exclusion criteria were moderate to severe impairment of renal and hepatic function, and intolerance of or contraindication to the use of glycopeptides. At the time of inclusion, demographic, clinical and radiological data were registered, including co-morbidities, concomitant treatments, bodyweight and height; laboratory data obtained both prior to and after surgery, including a full blood count with differential counts, kidney and liver function tests, ESR, CRP, total proteins and albumin levels.

The study period started in January 2004 and ended in January 2008. The study was performed according to the current revised version of the Declaration of Helsinki, and written informed consent was obtained from each patient.

Inflammatory Markers

A full blood count with differential counts were obtained via fluorescence flow citometry using the Sysmex XE-2100™ system (TOA Medical Electronics, Kobe, Japan). The Test-1™ automated analyser (Alifax SpA, Polverara, Italy) was used for assessment of the ESR (normal value <35 mm); CRP was measured using the Olympus AU2700™ Chemistry Immuno-Analyzer (Olympus Diagnostics GmbH, Hamburg, Germany) [normal value <0.5 mg/dL].

Sample Collection

Bone specimens were collected during surgical debridement of necrotic and infected tissue at a variable interval from the start of antimicrobial infusion, depending upon surgical timing. At the same time as bone resection, peripheral venous blood samples were collected using 7 mL lithium-heparin-containing BD Vacutainer™ system vials (Becton Dickinson Italia SpA, Buccinasco, Italy). An intraoperative radiographic examination was performed on bone samples to determine the presence of resorption areas, markers of osteolytic process and infection. The amount of tissue vascularization and the eventual presence of avascular bone were determined by histopathological analysis of a fragment of collected bone; on the basis of the results, necrotic and avascular tissues, as well as sequesters, were excluded from evaluation. Another bone sample underwent microbiological assays for aetiological determination.

Serum was obtained by blood centrifugation at 3000 rpm for 10 minutes and stored at −20°C prior to assay. Bone samples for pharmacokinetic analysis were dissected into cortical and cancellous bone, washed for 10 seconds with 10 mL of sterile saline to remove blood and coagules, blotted dry and stored at −80°C. Bone marrow was separated from cancellous bone and was not included in the analysis.

At the time of analysis, cortical specimens were defrosted, cleaned of soft tissue and crushed into powder with an analytic mill (IKA® A11 Basic; IKAWerke GmbH, Staufen, Germany). Sequesters and necrotic-appearing bone were excluded from the analysis. Cancellous bone samples were thawed and ground up with a porcelain mortar and pestle. The crushing procedures consisted of 15-second grinding cycles at intervals of 10 seconds each to avoid temperature increases above the thermal instability threshold of glycopeptides. For both cortical and cancellous bone, several samples of 500 mg were weighed with a precision electronic balance and transferred into 2.5 mL plastic vials. To quantify teicoplanin and vancomycin in plasma and bone, fully validated high-performance liquid chromatography (HPLC) assays were used.

Stock Solutions, Plasma Standards and Quality Controls

Teicoplanin and vancomycin stock solutions were prepared in water at the concentration of 1 mg/mL. Standard samples were prepared by serial dilutions of the highest standard prepared after addition of determined volumes of stock solutions to blank plasma. Calibration curves ranged from 150 mg/L to 4.68 mg/L for teicoplanin and from 80 mg/L to 0.625 mg/L for vancomycin. To improve accuracy and precision of the method, three levels of quality control (high, medium and low) were prepared by successive dilutions of stock solutions with blank plasma.

Bone Standards and Quality Controls

Similarly to plasma, the unknown bone sample concentrations were calculated from a linear calibration curve. To obtain standard bone samples, the stock solution of each drug was diluted in blank weighed bone. Calibration curves of five points, including blank bone, were obtained from standard samples and ranged from 100 μg/g to 2 μg/g for teicoplanin and from 20 μg/g to 1 μg/g for vancomycin. Three levels of bone quality control were prepared: high, medium and low.

Chromatography Equipment

The chromatographic apparatus was a Merck-Hitachi LaChrom® (Tokyo, Japan) with an L-7100 pump model, an L-7200 autosampler, an L-7400 UV detector and a D-7000 interface. HPLC System Manager version 4.1 software (Merck-Hitachi) was used to manage the HPLC system. Chromatographic

separation was performed using an Atlantis® 3 μm C18 column (150 × 4.6 mm internal diameter [I.D.]; Waters Corporation, Milan, Italy) protected by a C18 SecurityGuard™ (4.0 × 3.0 mm I.D.; Phenomenex, Torrance, CA, USA) at 35°C, using an L-7350 Merck-Hitachi LaChrom® column thermostat.

For teicoplanin, assay separation was achieved by gradient elution; the mobile phase was composed of buffer A (KH_2PO_4 50 mmol/L adjusted to pH 3.23 with concentrated ortho-phosphoric acid) and acetonitrile as buffer B. For vancomycin, assay separation was achieved by gradient elution and the mobile phase was composed of buffer A (water and formic acid 0.05%) and acetonitrile as buffer B. The flow rate was set at 1 mL/min for both methods.

Vancomycin Assay

Plasma samples were prepared by mixing 250 μL of plasma with 1500 μL of acetonitrile/trifluoroacetic acid (99/1, v/v) and 50 μL of internal standard (50 mg/L of thymidine, from Sigma-Aldrich [Milan, Italy] in water, prepared daily). After centrifugation at 12 000 rpm for 10 minutes at 4°C, samples were dried at 50°C for 2 hours and reconstituted with 125 μL of water and acetonitrile (80/20, v/v); 50 μL of sample was injected into the HPLC system. Bone samples were mixed with 50 μL of internal standard and with 1 mL of KH_2PO_4 0.1 mol/L adjusted to pH 6 with KOH. Samples were tumbled for 24 hours at 25 rpm at 4°C. After centrifugation at 12 000 rpm for 5 minutes at 4°C, 10 μL of supernatant was injected into the HPLC apparatus. The UV detector was set at 280 nm. The calculation method was based on peak areas and the internal standard ratio; the kind of calibration curve selected was 'linear and forced through zero'. The retention time of vancomycin and internal standard was 10.0 (± 0.2) and 5.9 (± 0.2) minutes, respectively. The accuracy, intra- and inter-day variability, expressed as coefficients of variation (CVs), were 4.2%, 3.24% and 6.53%, respectively, for plasma samples and 8.2%, 8.5% and 11.2%, respectively, for bone samples. The recovery rates were 92% (CV = 3%) and 85% (CV = 8%) for bone and plasma samples, respectively.

Teicoplanin Assay

Plasma samples were prepared by mixing 500 μL of samples with 1000 μL of acetonitrile. Samples were vortexed for 10 seconds and centrifuged at 12 000 rpm for 10 minutes at 4°C. The supernatant was then dried and reconstituted with 250 μL of water and acetonitrile (95/5 v/v). 50 μL of sample was injected into the HPLC. Cortical and cancellous bone samples were prepared as described above, and 500 mg of sample was mixed with 1000 μL of phosphate buffer saline at pH 7.4. To allow teicoplanin extraction, samples were vortexed for 10 seconds and tumbled for 15 minutes at room temperature at 35 rpm. After centrifugation at 12 000 rpm for 5 minutes at 4°C, 50 μL of the supernatant was injected into the HPLC apparatus. The retention time of teicoplanin was 13 (± 0.2) minutes. The accuracy, intra- and inter-day variability, expressed as CVs, were 0.6%, 2.4% and 5.4%, respectively, for plasma samples and 7.2%, 8.3% and 10.1%, respectively, for bone samples. The recovery rates were >90% (CV = 5%) for plasma samples and 87% (CV = 2%) for bone samples. The stationary and mobile phases used to quantify teicoplanin in plasma and bone samples were the same as described above. The UV detector was set at 220 nm. The calculation and the calibration curve, using an external standard method, were based on peak areas. The kind of calibration curve selected was 'linear and forced through zero'.

The amount of antimicrobial in bone samples attributable to blood contamination was calculated as previously described,[7,8] using the following formula (equation 1):

$$\text{Blood contamination (\%)} = \frac{\text{Hb in supernatant}}{\text{Hb in blood}} \bullet K \bullet (100 - \text{Hct}) \quad \text{(Eq. 1)}$$

where Hb is haemoglobin (g/dL), K is the dilution factor corresponding to the mean volume of water displaced by 1 g of bone (1/bone density) and measuring 0.83 mL for cortical bone and 2 mL for cancellous bone,[9] and Hct is the haematocrit (%).

Antimicrobial concentrations were measured in duplicate in adjacent bone samples and then averaged to improve the accuracy of the assay.

Pharmacokinetic Analysis

Noncompartmental pharmacokinetic analysis of the data was performed using WinNonlin® version 5.2 software (Pharsight Corporation, Mountain View, CA, USA). The first-order approximation was used to derive population pharmacokinetic parameters, such as the drug elimination half-life ($t_{1/2}$); the maximum concentration (C_{max}) and minimum concentration (C_{min}) in plasma and bone were subsequently obtained from the known data. The area under the concentration-time curve (AUC) over 24 hours (AUC_{24}) in plasma and bone was calculated by the linear-log trapezoidal rule.

Statistical Analysis

Statistical analysis was performed using WINKS SDA 6th edition software (TexaSoft, Cedar Hill, TX, USA). Statistical decisions were made at p = 0.05. All p-values were two-tailed. To compare the two study groups, a non-parametric test was performed. To evaluate linear relationships, the Pearson and Spearman correlation tests were used. Multiple regression analysis was performed using the Newman-Keuls procedure; an α adjustment was not used.

Results

Twenty-seven patients (24 males and 3 females) met all inclusion criteria and were eligible for the study. Their mean age was 41.6 years (range 18–72 years). All patients received intravenous antimicrobial treatment, including teicoplanin or vancomycin, for post-traumatic septic pseudoarthrosis of the diaphyseal tibia. The demographic characteristics of the study population are described in table I.

Ten patients (mean age 39.7 years, range 20–54 years) received intravenous vancomycin 1 g twice daily, administered as a 1-hour infusion; the mean duration of treatment before sampling was 22.2 days (table I). Bone samples were extracted at a mean of 4.2 hours (range 1.75–9.3 hours) after the start of the vancomycin infusion. Bone cultures grew MRSA in four cases and *Enterococcus faecalis* and MRSE in three cases each (vancomycin MIC <2 mg/L). The mean ± SD plasma concentration at the time of osteotomy was 15.95 ± 6.8 mg/L. The mean ± SD bone concentration was 2.66 ± 1.2 mg/L in cortical bone and 11.53 ± 7.8 mg/L in cancellous bone. The distribution of plasma and tissue vancomycin concentrations and of bone/plasma concentration ratios over time are illustrated in figures 1 and 2. The mean bone/plasma concentration ratios were 20.67% and 89.39% for cortical and cancellous bone, respectively (table II). The mean plasma C_{max}, derived by a population pharmacokinetic analysis, was 25.96 mg/L and the mean plasma volume of distribution at steady state (V_{ss}) was 28.4 L/kg. The plasma $t_{1/2}$ was 4.2 hours and the elimination rate constant was 0.1649 h^{-1}. The mean ± SD plasma AUC_{24} was 293.44 ± 101.87 h • mg/L. The mean ± SD bone AUC_{24} values were 55.16 ± 25.26 h • mg/L for cortical bone and 299.16 ± 299.54 h • mg/L for cancellous bone. The bone $t_{1/2}$ was 7.23 hours in cortical bone and 8.7 hours in cancellous bone. The mean ± SD values for the vancomycin AUC_{24} bone/plasma concentration ratios were 21.20 ± 13.14% for cortical bone and 103.54 ± 77.36% for cancellous bone (table III). The mean overall drug exposure in cancellous bone was 4.97-fold higher than in cortical bone. The mean vancomycin concentrations over the MICs of the infecting agents (*S. aureus*, *S. epidermidis* and *E. faecalis*) were 2.20 and 9.91 mg/L for cortical and cancellous bone, respectively. The mean AUC_{24}/MIC ratios were 215.02, 47.14 and 268.95 for plasma, cortical and cancellous bone, respectively. The plasma and cancellous bone concentrations were higher than the MIC of the infecting agent for the entire dosing interval, and the mean time above the MIC (T>MIC) for cortical bone was 14.44 hours, corresponding to 60.2% of the dosing interval (table IV).

Seventeen patients were treated with teicoplanin 10 mg/kg/day, administered over a 1-hour infusion and preceded by the recommended loading dose during the first 2 days (table V). Bone samples were collected at a mean of 6.8 hours after the start of the teicoplanin infusion. Bone cultures grew MRSE and MRSA in four cases each and *Corynebacterium striatum*, meticillin-sensitive *S. aureus* (MSSA) and *E. faecalis* in three cases each (teicoplanin MIC <2 mg/L). The mean ± SD plasma concentration at the time of osteotomy was 17.0 ± 8.0 mg/L. The mean ± SD cortical bone concentration was 2.01 ± 1.7 mg/L. Cancellous bone was available in 15 patients: in those samples, the mean ± SD concentration was 7.51 ± 7.0 mg/L. The mean bone/plasma concentration ratios of teicoplanin were 12.35% and 48.6% for cortical and cancellous bone, respectively. The distribution of plasma and tissue teicoplanin concentrations and of bone/plasma concentration ratios over time is illustrated in figures 3 and 4. According to the population pharmacokinetic analysis, the mean derived plasma C_{max} was 23.76 mg/L and the mean plasma V_{ss} was 37.18 L/kg. The plasma $t_{1/2}$ was 10.36 hours and the elimination rate constant was 0.0669 h^{-1}. The mean ± SD plasma AUC_{24} was 293.34 ± 101.8 h • mg/L. The mean ± SD bone AUC_{24} values were 34.08 ± 23.6 h • mg/L for cortical bone and 155.17 ± 132.8 h • mg/L for cancellous bone. The bone $t_{1/2}$ was 9.96 hours in cortical bone and 18.38 hours in cancellous bone. The mean ± SD teicoplanin AUC_{24} bone/plasma concentration ratios were 12.14 ± 6.7% for cortical bone and 55.96 ± 48.2% for cancellous bone (table VI). The mean overall drug exposure in cancellous bone was 4.73-fold higher than in cortical bone. The mean teicoplanin concentrations over the MICs of the infecting agents (*S. aureus*, *S. epidermidis*, *C. striatum* and *E. faecalis*) were 2.08 and 9.54 for cortical and cancellous bone, respectively (table VII). The mean AUC_{24}/MIC ratios were 336.48, 36.26 and 197.2 for plasma, cortical and cancellous bone, respectively. The plasma and cancellous bone concentrations were higher than the MIC of the infecting agent for the entire dosing interval, and the mean T>MIC for cortical bone was 13.03 hours, corresponding to 54.3% of the dosing interval.

Table I. Demographic characteristics of the study population

Patient no.	Age (y)	Bodyweight (kg)	Height (m)	BMI (kg/m2)	Days of treatment[a]
Vancomycin-treated patients					
1	46	82	1.75	26.78	30
2	22	100	1.68	35.43	60
3	54	65	1.69	22.76	11
4	33	70	1.73	23.39	16
5	46	82	1.75	26.78	41
6	20	55	1.70	19.03	10
7	41	70	1.72	23.66	14
8	50	86	1.75	28.08	12
9	49	88	1.75	28.73	15
10	36	80	1.75	26.12	13
Mean (SD)	**39.7 (11.7)**	**77.8 (13)**	**1.73 (0.03)**	**26.08 (4.39)**	**22.2 (16.5)**
Teicoplanin-treated patients					
11	72	59	1.68	20.90	18
12	35	100	1.78	31.56	27
13	22	88	1.80	27.16	60
14	29	100	1.85	29.22	30
15	65	70	1.80	21.60	42
16	29	74	1.72	25.01	23
17	30	74	1.70	25.61	22
18	34	84	1.80	25.93	35
19	68	80	1.75	26.12	64
20	42	80	1.78	25.25	42
21	52	80	1.65	29.38	28
22	18	65	1.65	23.88	39
23	37	78	1.80	24.07	23
24	52	70	1.75	22.86	24
25	28	46	1.80	14.20	26
26	64	70	1.60	27.34	31
27	50	60	1.60	23.44	24
Mean (SD)	**42.7 (17)**	**75.17 (13.9)**	**1.73 (0.08)**	**24.91 (3.93)**	**32.83 (13.04)**

a Duration of antimicrobial treatment with vancomycin or teicoplanin before sampling.

BMI = body mass index.

Statistical Analysis

The two study groups were not statistically different regarding age, bodyweight, height, time of bone collection and inflammatory indices (ESR, CRP and white blood cell [WBC] counts).

The results of the correlation analysis are listed in the supplementary material ('ArticlePlus') at http://pharmacokinetics.adis-online.com

For both vancomycin and teicoplanin, Pearson's correlation coefficient indicated a statistically significant linear relationship between cortical and cancellous bone concentrations, and between the cortical bone AUC_{24} and the cancellous bone AUC_{24}.

Univariate analysis showed that the cortical and cancellous bone penetration rates of vancomycin were inversely related to blood concentrations, and multivariate analysis showed that vancomycin cancellous bone concentrations were inversely related to

the time of collection and directly related to vancomycin blood concentrations, the ESR, CRP and WBC count.

As for teicoplanin, univariate analysis showed that plasma concentrations were an independent predictor of cortical bone concentrations. Multivariate analysis showed that cortical bone concentrations and the cortical bone AUC_{24} were directly related to the time of collection, WBC count, ESR, CRP and plasma AUC_{24}; the cortical penetration rates were directly related to all inflammatory indices.

The bone penetration rates of vancomycin were numerically higher than those of teicoplanin (mean 20.67% and 89.39% vs 12.35% and 48.6%, respectively), but the difference was not statistically significant ($p = 0.071$).

Discussion

Despite medical progress, the treatment of osteo-articular infections is still characterized by a high failure rate. Although drug penetration in infected tissues is critical for efficacy, the knowledge of the pharmacokinetics of antimicrobials in the bone compartment is rather scanty. As a result of the increasing incidence of osteomyelitis due to drug-resistant Gram-positive bacteria, glycopeptides are now widely used in the treatment of bone infections, both as monotherapy or in association with other antibacterials; however, data on their penetration into infected human bone are lacking. There are several studies concerning bone penetration of vancomycin administered for surgical prophylaxis.[10-13] In two studies analysing non-infected bone, the rate of vancomycin bone penetration was found to vary between 7% and 30% for cortical bone and between 13.2% and 21% for cancellous bone.[14,15] Desplaces et al.[16] found a higher vancomycin penetration in infected bone (111–207%) after continuous infusion. Teicoplanin bone penetration has been described in three conference reports, where it was found to vary between 17% and 290% in cancellous bone and between 14% and 171% in cortical bone.[17-19] Teicoplanin concentrations during mediastinal and cardiac surgery were 0.8–1.9 mg/g in sternal bone and 6.3 mg/L in bone washing.[20,21] Two studies compared teicoplanin concentrations in bone after local and systemic prophylaxis, reporting values of 1.96–12.7 mg/L and 1.3–2.5 mg/L.[22,23] Nehrer et al.[24] reported mean concentrations of 6.2 mg/kg in cancellous bone and 7.1 mg/kg in cortical bone after a single preoperative dose of teicoplanin. No studies have investigated teicoplanin penetration into infected bone. The heterogeneity of these few studies, the low number of patients studied and the different methodologies adopted do not allow definitive conclusions to be drawn on glycopeptide pharmacokinetics in bone.

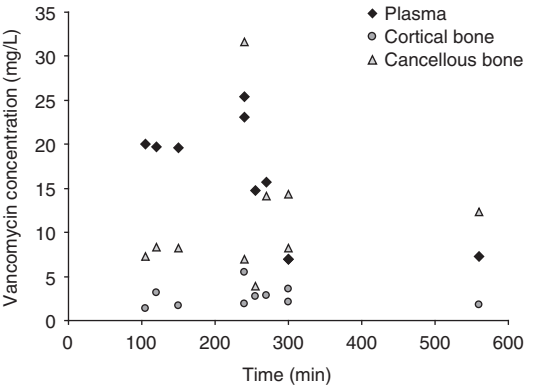

Fig. 1. Vancomycin concentrations in plasma and bone versus time.

In the present study, 27 patients with rather comparable forms of septic pseudoarthrosis of the tibia were investigated. At the time of pharmacokinetic sampling, all patients were receiving a glycopeptide and were pharmacologically classified as being at steady state. We analysed concentrations of vancomycin and teicoplanin in both cortical and cancellous infected bone using a standard validated HPLC-UV method. As tissue drug delivery may be affected by impaired blood supply (e.g. peripheral ischaemia), necrotic bone samples and sequesters were not analysed.

For both glycopeptides, the mean bone/plasma concentration ratio varied according to the bone compartment being considered. In particular, the cancellous bone/plasma concentration and AUC ratios were far higher (3- to 5-fold) than those in cortical bone. The anatomical discrepancy in tissue vascularization between the two compartments may partially explain the difference observed; secondly, a mechanism of drug accumulation or delayed clearance in the cancellous compartment may have been present in those patients whose cancellous bone concentrations were higher than their plasma concentrations. Previously published studies did not report

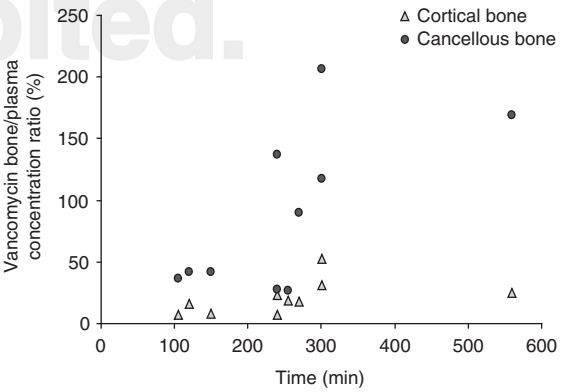

Fig. 2. Vancomycin bone/plasma concentration ratios versus time.

Table II. Vancomycin concentrations in plasma, cortical bone and cancellous bone

Patient no.	Time of sampling (min)[a]	Concentration (mg/L)			Concentration ratio (%)		Blood contamination (%)	
		plasma	cortical bone	cancellous bone	cortical bone/plasma	cancellous bone/plasma	cortical bone	cancellous bone
1	105	20.01	1.35	7.32	6.76	36.55	1.47	7.12
2	120	19.72	3.17	8.31	16.06	42.16	0.79	6.63
3	150	19.61	1.65	8.18	8.41	41.72	0.56	2.34
4	240	25.38	1.86	6.97	7.33	27.45	0.49	2.37
5	240	23.07	5.44	31.58	23.57	136.87	1.45	10.55
6	255	14.79	2.75	3.91	18.56	26.44	0.39	7.57
7	270	15.68	2.82	14.11	17.96	90.02	0.69	3.98
8	300	6.98	2.16	8.22	30.92	117.80	0.80	1.94
9	300	6.99	3.63	14.39	52.06	206.19	0.82	1.89
10	560	7.30	1.83	12.31	25.04	168.78	1.46	7.02
Mean (SD)	**254 (129)**	**15.95 (6.84)**	**2.66 (1.21)**	**11.53 (7.79)**	**20.67 (13.63)**	**89.39 (65.06)**	**0.89 (0.41)**	**5.14 (3.03)**
Median	**247.5**	**17.64**	**2.45**	**8.27**	**18.26**	**66.09**	**0.79**	**5.31**

a Time past start of infusion (duration of infusion = 1 h).

such a great difference between cortical and cancellous bone concentrations, but they had mostly analysed uninfected bone, where differences in vascular permeability and turnover processes between the two compartments may be less marked. In addition, different methods of drug extraction and dose measurements were often used.

A necessary consideration in the analysis of drug bone penetration is that single-point measurements of bone/plasma concentration ratios may yield significantly different results depending upon the time of sampling. After intravenous administration, glycopeptides distribute in tissues; however, the shape of the concentration-time curves of plasma and tissue compartments may differ substantially due to delayed equilibrium between the two compartments.[25] Secondly, the difference in sampling times between the two study groups, although not statistically significant, may pose problems in comparing the single-point bone/plasma concentration ratios of two drugs with different pharmacokinetic properties. To better estimate the bone drug exposure of vancomycin and

Table III. Vancomycin pharmacokinetic parameters in plasma, cortical bone and cancellous bone

Patient no.	Plasma V_{ss} (L/kg)	AUC_{24} (h • mg/L)			AUC_{24} ratio (%)	
		plasma	cortical bone	cancellous bone	cortical bone/plasma	cancellous bone/plasma
1	29.93	256.19	21.85	131.45	8.53	51.31
2	36.5	259.93	52.52	151.37	20.2	58.23
3	23.72	284.86	28.43	149.65	9.98	52.54
4	25.55	470.93	37.01	144.56	7.89	30.70
5	29.93	428.02	108.71	1098.21	25.40	256.58
6	20.08	286.03	56.15	85.76	19.63	29.98
7	25.55	315.99	58.92	309.45	18.65	97.93
8	31.39	152.76	47.32	184.08	30.98	120.50
9	32.12	152.96	79.80	322.24	52.17	210.67
10	29.2	326.73	60.83	414.82	18.62	126.96
Mean (SD)	**28.40 (4.73)**	**293.44 (101.87)**	**55.16 (25.26)**	**299.16 (299.54)**	**21.20 (13.14)**	**103.54 (77.36)**
Median	**29.56**	**285.44**	**54.34**	**167.72**	**19.14**	**78.08**

AUC_{24} = area under the concentration-time curve over 24 hours; **V_{ss}** = volume of distribution at steady state.

Table IV. Vancomycin bone concentrations and area under the concentration-time curve (AUC)/minimum inhibitory concentration (MIC) ratios for Gram-positive cocci

Patient no.	Pathogen	MIC	Concentration/MIC		AUC/MIC			T>MIC (h)	
			cortical bone	cancellous bone	plasma	cortical bone	cancellous bone	cortical bone	cancellous bone
1	MRSA	2	0.68	3.66	128.1	10.93	65.73	0	24
2	*E. faecalis*	2	1.59	4.16	129.97	26.26	75.68	13.06	24
3	*E. faecalis*	2	0.83	4.09	142.43	14.21	74.83	0	24
4	MRSE	2	0.93	3.49	235.46	18.51	72.28	5.08	24
5	MRSE	1	5.44	31.58	428.02	108.71	1098.21	24	24
6	*E. faecalis*	2	1.38	1.96	143.01	28.08	42.88	14.6	24
7	MRSE	2	1.41	7.06	157.99	29.46	154.72	15.64	24
8	MRSA	0.5	4.32	16.44	305.52	94.64	368.15	24	24
9	MRSA	1	3.63	14.39	152.96	79.8	322.24	24	24
10	MRSA	1	1.83	12.31	326.73	60.83	414.82	24	24
Mean (SD)			**2.20 (1.65)**	**9.91 (9.17)**	**215.02 (104.78)**	**47.14 (35.95)**	**268.95 (323.32)**	**14.44 (9.85)**	**24 (0)**

E. faecalis = *Enterococcus faecalis*; **MRSA** = meticillin (methicillin)-resistant *Staphylococcus aureus*; **MRSE** = meticillin-resistant *S. epidermidis*; **T>MIC** = time above the MIC.

teicoplanin, we therefore performed a population pharmacokinetic analysis. Starting with plasma and tissue concentrations measured at different intervals from drug administration, we inferred the AUC_{24} values for plasma and for the two bone compartments, and we estimated bone penetration via the respective AUC_{24} ratios.

Analysing our data according to the classification proposed by Boselli and Allaouchiche,[26] vancomycin can be classified as an antimicrobial with good tissue penetration (penetration rates of >30%) when considering cancellous bone, and as an antimicrobial with satisfactory tissue penetration (penetration rates between 15% and 30%) when considering cortical bone. Teicoplanin displayed similar tissue penetration (>30%) into cancellous bone but poor tissue penetration (<15%) into cortical bone. Boselli and Allaouchiche[26] attributed good tissue penetration to teicoplanin and satisfactory tissue penetration to vancomycin; however, heterogeneous studies were analysed, and cortical and cancellous bone were rarely differentiated.

In our study, interindividual variability was high for both cortical and cancellous bone concentrations: this is probably accounted for by the different intensity of the infecting inflammatory process and the different timing of sample extraction.

Bone concentrations were found to have no association with individual variables such as age, bodyweight, height and body mass index, while an association was found in some cases between bone and plasma concentrations, a result suggesting that the latter might serve as an indirect indicator of bone exposure. On multivariate analysis, inflammatory markers were found to be associated with higher bone concentrations, possibly as a result of increased vascularization and vascular permeability under inflammatory conditions.

Tissue penetration is an estimate of the capacity of an antimicrobial to reach the site of infection, but it does not give information about its antimicrobial properties *per se*. In the choice of the optimal antimicrobial treatment, it is also necessary to consider the bacterial sensitivity to antimicrobials, which is expressed by parameters such as the MIC and the inhibitory quotient (IQ), the latter being the ratio between the concentration and the MIC. The IQ gives an indication of antimicrobial activity at a precise site of infection against a particular micro-organism; as increases in IQ values tend to be associated with an increase in efficacy, the IQ might provide indications about the probability of therapeutic success.[27] Vancomycin (and, similarly, teicoplanin) displays concentration-independent killing of Gram-positive bacteria; maximum killing is achieved at concentrations 4–5 times the MIC of the infecting pathogen, and maintenance of concentrations at or above these levels for the entire dosing interval will likely produce an adequate antimicrobial effect.[28-30] However, the IQ is a rough estimate of antimicrobial efficacy, and it may vary according to the time at which the tissue concentration is measured. The best pharmacokinetic/pharmacodynamic index for glycopeptides is the AUC/MIC.[31] Hyatt et al.[32] demonstrated that a vancomycin AUC_{24}/MIC of <125 was associated with a high probability of

Table V. Teicoplanin concentrations in plasma, cortical bone and cancellous bone

Patient no.	Time of sampling (min)[a]	Concentration (mg/L)			Concentration ratio (%)		Blood contamination (%)	
		plasma	cortical bone	cancellous bone	cortical bone/plasma	cancellous bone/plasma	cortical bone	cancellous bone
11	90	31.23	7.64	21.77	24.47	69.72	1.57	13.23
12	150	25.68	1.45	4.20	5.64	16.36	1.06	0.96
13	240	8.91	0.77	NA	8.68	NA	0.34	NA
14	240	17.3	2.87	25.91	16.56	149.72	1.22	13.20
15	240	18.26	2.71	NA	14.82	NA	2.00	NA
16	240	26.05	1.53	6.06	5.88	23.26	0.91	2.18
17	240	26.04	1.47	1.98	5.65	7.59	0.88	1.97
18	240	10.0	1.32	3.04	13.15	30.31	0.96	7.70
19	240	22.69	1.00	4.82	4.42	21.22	1.15	9.67
20	270	18.25	2.14	8.98	11.73	49.22	0.92	2.40
21	300	21.48	4.00	6.07	18.63	28.25	1.03	9.95
22	300	8.0	0.89	3.90	11.17	48.69	1.18	9.94
23	540	21.2	1.05	3.72	4.94	17.54	2.58	3.73
24	600	8.31	1.21	8.53	14.55	102.61	1.30	3.14
25	600	8.46	2.01	5.08	23.72	60.09	1.04	8.76
26	1200	8.34	1.56	6.32	18.75	75.74	0.22	8.90
27	1200	7.61	0.55	2.21	7.16	29.04	1.64	3.95
Mean (SD)	**407.6 (331.03)**	**17.0 (7.99)**	**2.01 (1.69)**	**7.51 (6.97)**	**12.35 (6.5)**	**48.60 (38.42)**	**1.18 (0.56)**	**6.64 (4.17)**
Median	**240**	**18.24**	**1.47**	**5.08**	**11.73**	**30.31**	**1.06**	**7.70**

a Time past start of infusion (duration of infusion = 1 h).

NA = not available.

treatment failure in patients with enterococcal infection. Moise-Broder et al.[33] identified the AUC_{24}/MIC as the best predictor of the therapeutic response of vancomycin in acute lower respiratory tract infections caused by *S. aureus*, setting 350 as the threshold for clinical success and 400 as the threshold for bacterial eradication; no relationship between the T>MIC and the clinical/bacteriological outcome was found. It is very likely that the same pharmacokinetic/pharmacodynamic indices also apply to bone infections, even if pharmacokinetic/pharmacodynamic targets have not yet been identified. In our study, the cancellous bone concentrations over the MIC ratios for both vancomycin and teicoplanin were ≥1 in all patients, with 68% of patients having values of >4 (mean values of 9.91 and 9.54 for vancomycin and teicoplanin, respectively), the mean AUC/MIC ratios were 268.95 for vancomycin and 197.21 for teicoplanin, and the T>MIC values corresponded to 100% of the dosing interval. These data suggested that both glycopeptides achieve satisfactory exposure in cancellous bone tissue, as far as Gram-positive and glycopeptide-susceptible pathogens are concerned. On the other hand, in cortical bone, glycopeptide concentrations were in some cases under the MICs of the infecting agents, with a mean AUC/MIC ratio of <125 (47.14 and 36.27 for vancomycin and teicoplanin, respectively) and a T>MIC of <60%, suggesting possible suboptimal exposure in this compartment. In this setting, newer anti-Gram-positive agents with better tissue penetration (i.e. linezolid, tigecyclin) may be an alternative to glycopeptides in the treatment of bone infections.

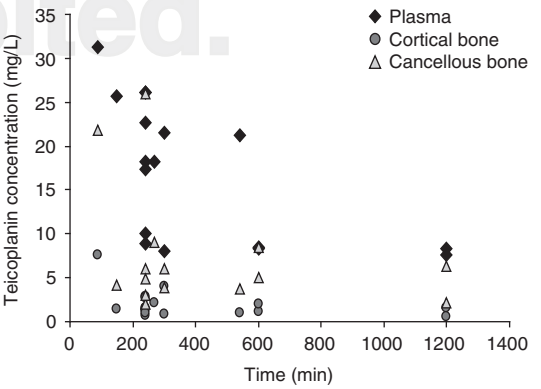

Fig. 3. Teicoplanin concentrations in plasma and bone versus time.

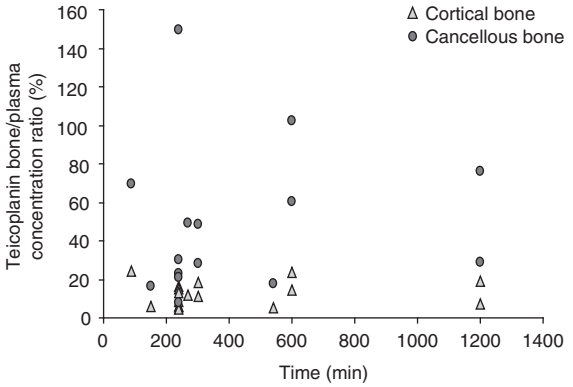

Fig. 4. Teicoplanin bone/plasma concentration ratios versus time.

Our study had several limitations. Firstly, the study lacked a control group of non-infected human bone. Secondly, although all samples were extracted from the same bone segment (the tibia), differences in the local degree of vascularization and inflammation could account for some interindividual variability in glycopeptide bone concentrations. Finally, the method of drug extraction from bone samples had intrinsic limitations and did not differentiate between intracellular and extracellular compartments, thus incomplete extraction and consequent underestimation could not be excluded. Drug removal during washing of samples was minimized by the short duration of the procedure; drug concentrations in the washing fluid were in all cases under the level of detectability. During grinding without cooling, drug degradation may occur; we therefore used short crushing cycles to avoid raising the temperature to the thermal instability threshold of glycopeptides. The size of the study population appears to be clinically relevant, considering the small case series reported in the literature to date.

In some cases, statistical analysis demonstrated a correlation between blood and bone concentrations of glycopeptides: this observation supports the hypothesis that antimicrobial plasma concentrations can have an indirect influence on the clinical outcome. Our findings provide pharmacomicrobiological confirmation of the results described in some previous clinical reports. As cortical bone concentrations correspond to <20% of plasma concentrations, high systemic dosages of glycopeptides are required to reach and maintain adequate tissue exposure and the pharmacokinetic/pharmacodynamic targets. In a retrospective study, MacGowan et al.[34,35] reported that cure rates were directly

Table VI. Teicoplanin pharmacokinetic parameters in plasma, cortical bone and cancellous bone

Patient no.	Plasma V_{ss} (L/kg)	AUC_{24} (h • mg/L)			AUC_{24} ratio (%)	
		plasma	cortical bone	cancellous bone	cortical bone/plasma	cancellous bone/plasma
11	22.52	398.65	95.81	393.33	24.03	98.66
12	34.14	350.44	19.34	72.12	5.52	20.58
13	66.80	134.49	11.43	NA	8.50	NA
14	45.85	261.09	42.63	514.36	16.33	197.00
15	32.57	275.54	40.24	NA	14.60	NA
16	30.44	393.02	22.76	110.76	5.79	28.18
17	30.46	392.92	21.94	35.91	5.58	9.14
18	39.64	150.84	19.67	57.83	13.04	38.33
19	34.96	342.34	14.99	93.00	4.38	27.17
20	42.02	284.76	32.94	165.06	11.57	57.96
21	25.90	346.48	63.97	122.56	18.46	35.37
22	69.48	129.02	14.25	78.42	11.04	60.78
23	26.77	447.00	11.54	82.84	2.58	18.53
24	47.92	187.39	27.34	195.81	14.59	104.49
25	31.35	190.67	45.40	122.22	23.81	64.10
26	24.44	367.12	70.63	209.48	19.24	57.06
27	26.78	335.01	24.49	73.82	7.31	22.04
Mean (SD)	**37.18 (13.74)**	**293.34 (101.84)**	**34.08 (23.58)**	**155.17 (132.76)**	**12.14 (6.69)**	**55.96 (48.18)**
Median	**32.57**	**335.01**	**24.45**	**110.76**	**11.57**	**38.33**

AUC_{24} = area under the concentration-time curve over 24 hours; **NA** = not available; **V_{ss}** = volume of distribution at steady state.

Table VII. Teicoplanin bone concentrations and area under the concentration-time curve (AUC)/minimum inhibitory concentration (MIC) ratios for Gram-positive cocci

Patient no.	Pathogen	Teicoplanin MIC	Concentration/MIC		AUC/MIC			T>MIC (h)	
			cortical bone	cancellous bone	plasma	cortical bone	cancellous bone	cortical bone	cancellous bone
11	MRSA	2	3.82	10.89	199.33	47.90	196.66	20.73	24
12	*C. striatum*	2	0.73	2.1	175.22	9.67	36.06	0	24
13	MSSA	0.5	1.54	NA	268.99	22.86	NA	9.76	NA
14	MRSA	0.5	5.74	51.82	522.18	85.27	1028.71	24	24
15	*C. striatum*	2	1.35	NA	137.77	20.12	NA	7.85	NA
16	MRSA	0.5	3.06	12.12	786.05	45.52	221.53	20	24
17	MRSA	2	0.74	0.99	196.46	10.97	17.95	0	24
18	*E. faecalis*	1	1.32	3.04	150.84	19.67	57.82	7.5	24
19	MRSE	1	1.00	4.82	342.34	15.0	93.00	3.39	24
20	*E. faecalis*	0.5	4.28	17.96	569.52	65.88	330.11	24	24
21	MRSE	1	4.00	6.07	346.48	63.97	122.55	24	24
22	MSSA	0.5	1.78	7.8	258.05	28.49	156.85	13.1	24
23	*E. faecalis*	1	1.05	3.72	447.0	11.54	82.84	0	24
24	MRSE	1	1.21	8.53	187.4	27.34	195.81	12.46	24
25	*C. striatum*	2	1.00	2.54	95.33	22.70	61.11	9.66	24
26	MRSE	1	1.56	6.32	367.12	70.63	209.48	24	24
27	MSSA	0.5	1.10	4.42	670.01	48.97	147.65	21.1	24
Mean (SD)			**2.08 (1.51)**	**9.54 (12.52)**	**336.48 (200.76)**	**36.27 (23.8)**	**197.21 (244.87)**	**13.03 (9.13)**	**24 (0)**

C. striatum = *Corynebacterium striatum*; ***E. faecalis*** = *Enterococcus faecalis*; **MRSA** = meticillin (methicillin)-resistant *Staphylococcus aureus*; **MRSE** = meticillin-resistant *S. epidermidis*; **MSSA** = meticillin-sensitive *S. aureus*; **T>MIC** = time above the MIC.

related to teicoplanin trough concentrations, and indicated a concentration of 10–20 mg/L as the optimal teicoplanin concentration range at the end of the dosing interval. For this reason, therapeutic drug monitoring might have a role in the treatment of bone infections as an additional item on which to base individually tailored therapy. In order to better meet the pharmacokinetic requirements of osteomyelitis, another related pharmacological issue of importance deserves attention in this treatment setting. Administration of vancomycin as a continuous infusion has been reported to allow bone penetration rates between 100% and 200%,[16] and a clinical study has demonstrated the superiority of vancomycin in continuous infusion (40 mg/kg/day) compared with the standard twice-daily regimen in terms of both efficacy and plasma exposure in the treatment of chronic osteomyelitis.[36]

Conclusion

The analysis of the degree of penetration of antimicrobials into infected tissues aims to give clinicians additional information on which to base the choice of the optimal antibacterial treatment. In our study, glycopeptides displayed poor to satisfactory penetration into septic cortical bone, while penetration into the highly vascularized cancellous bone was far higher. As antimicrobial exposure can be suboptimal in the infected cortical compartment, and drug penetration may be impaired into necrotic bone and sequesters, the role of radical surgical removal of purulent and necrotic tissues appears to be essential in order to shorten the treatment duration and reduce the risk of treatment failure.

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