



■ INFECTION

Flucloxacillin bone and soft tissue concentrations assessed by microdialysis in pigs after intravenous and oral administration

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Aims

Flucloxacillin is commonly administered intravenously for perioperative antimicrobial prophylaxis, while oral administration is typical for prophylaxis following smaller traumatic wounds. We assessed the time, for which the free flucloxacillin concentration was maintained above the minimum inhibitory concentration ($fT > MIC$) for methicillin-susceptible *Staphylococcus aureus* in soft and bone tissue, after intravenous and oral administration, using microdialysis in a porcine model.

Methods

A total of 16 pigs were randomly allocated to either intravenous (Group IV) or oral (Group PO) flucloxacillin 1 g every six hours during a 24-hour period. Microdialysis was used for sampling in cancellous and cortical bone, subcutaneous tissue, and the knee joint. In addition, plasma was sampled. The flucloxacillin $fT > MIC$ was evaluated using a low MIC target (0.5 µg/ml) and a high MIC target (2.0 µg/ml).

Results

Intravenous administration resulted in longer $fT > MIC$ (0.5 µg/ml) compared to oral administration, except for cortical bone. In Group IV, all pigs reached a concentration of 0.5 µg/ml in all compartments. The mean $fT > MIC$ (0.5 µg/ml) was 149 minutes (95% confidence interval (CI) 119 to 179; range 68 to 323) in subcutaneous tissue and 61 minutes (95% CI 29 to 94; range 0 to 121) to 106 minutes (95% CI 76 to 136; range 71 to 154) in bone tissue. In Group PO, 0/8 pigs reached a concentration of 0.5 µg/ml in all compartments. For the high MIC target (2.0 µg/ml), $fT > MIC$ was close to zero minutes in both groups across compartments.

Conclusion

Although intravenous administration of flucloxacillin 1 g provided higher $fT > MIC$ for the low MIC target compared to oral administration, concentrations were surprisingly low, particularly for bone tissue. Achievement of sufficient bone and soft tissue flucloxacillin concentrations may require a dose increase or continuous administration.

Cite this article: *Bone Joint Res* 2021;10(1):60–67.

Keywords: Flucloxacillin, Bone, Infection, Osteomyelitis, Animal model

Article focus

- What is the best choice: intravenous or oral administration of flucloxacillin for bone and soft tissue targets?
- Do routine dosing regimens for prophylaxis reach the minimum inhibitory concentration in bone?

- Longer time above minimum inhibitory concentration in all tissues except cortical bone with intravenous versus oral administration.
- The time above minimum inhibitory concentration probably needs to be increased by changing the dosage or the mode of administration in order to be clinically effective.

Key messages

- This is the first assessment of free flucloxacillin in bone.

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doi: 10.1302/2046-3758.101.BJR-2020-0250.R1

Bone Joint Res 2021;10(1):60–67.

Strengths and limitations

- The main limitation of the present study is that results from the experimental porcine model mandate verification in clinical studies.
- Microdialysis allows for serial and simultaneous sampling from multiple compartments of the unbound, active fraction of drug.

Introduction

Staphylococcus aureus is the main pathogen causing soft tissue and bone infections.¹ Flucloxacillin is among the first-choice antibiotics for prophylaxis in orthopaedic surgery because of its narrow gram-positive antibacterial spectrum.² The efficacy is related to the time of free drug concentration above the minimum inhibitory concentration ($fT > MIC$). For flucloxacillin, most methicillin-susceptible *Staphylococcus aureus* (MSSA) isolates display a MIC ($\leq 0.5 \mu\text{g/ml}$).³ However, the clinical breakpoint MIC for MSSA, defined by the Clinical and Laboratory Standards Institute, is $2.0 \mu\text{g/ml}$.⁴ Clinical breakpoint MIC refers to the highest MIC (i.e. the lowest susceptibility) at which the chance of microbiological eradication and a positive clinical outcome is greater than failure. It represents a worst-case scenario regarding bacterial susceptibility, which is important to consider in empirical antibiotic treatment. For perioperative prophylaxis, flucloxacillin is conventionally administered intravenously. Oral administration is typical for bacterial inoculation prophylaxis following smaller traumatic wounds.^{2,5} While it is generally recommended that both plasma and tissue concentrations exceed the MIC of relevant pathogens throughout the surgical procedure for perioperative prophylaxis, the target for bacterial inoculation prophylaxis following smaller traumatic wound infections is undefined.⁶

Flucloxacillin has a high degree of protein-binding, approximately 96%,⁷ which necessitates a sampling technique that exclusively allows measuring the unbound fraction. Microdialysis allows dynamic in situ assessment of microbiologically active concentrations of unbound antibiotics simultaneously from multiple compartments.⁸⁻¹¹

We aimed to compare bone and soft tissue $fT > MIC$ following intravenous and oral administration of flucloxacillin, using microdialysis in a healthy porcine model.

Methods

Animal model, experimental intervention, and randomization. A total of 16 female Danish Landrace Breed pigs (weight 78 kg to 82 kg, aged five months) were included. All pigs had flucloxacillin 1 g every six hours, for a total time of 24 hours (4 g of flucloxacillin). The pigs were allocated to two study groups by computer-generated block-randomization in blocks of two: administration of flucloxacillin was intravenous in Group IV (Staphylex; Puren, Munich, Germany); and oral in Group PO (Heracillin; Mylan, Ballerup, Denmark) via a percutaneous endoscopic gastrostomy tube (PEG-tube)

directly into the duodenum. Pylorus was visually located and the position of the PEG-tube in the duodenum was verified by palpation of the balloon distal to the pylorus. No PEG-tube was found misplaced at the post-mortem.

Microdialysis. Catheter-based microdialysis allows continuous sampling of unbound water-soluble molecules (e.g. antibiotics) in the interstitial space of virtually all tissues.¹²⁻¹⁵ Solutes diffuse across a semipermeable membrane at the tip of the catheter along the concentration gradient. As the catheter is continuously perfused, equilibrium never occurs. Therefore, the concentration in the dialysate is a fraction of the actual tissue concentration. This fraction is the relative recovery. Consequently, calibration is imperative to determining total tissue concentrations. In the present study, individual in vivo catheter calibration was performed for all catheters according to retro-dialysis by the calibrator method,¹⁶⁻¹⁹ using a microdialysis system (M Dialysis AB, Stockholm, Sweden) with CMA 107 precision pumps (CMA Microdialysis AB, Stockholm, Sweden) and CMA 63 catheters (membrane lengths 10 mm and 30 mm, 20 kDa molecule cut-off).

General anaesthesia. All pigs were kept in general anaesthesia maintained by titrated continuous infusion of propofol (400 mg/hr to 1,000 mg/hr) and fentanyl (0.6 mg/hr to 1.2 mg/hr) (propofol/fentanyl; B. Braun, Frederiksberg, Denmark). After endotracheal intubation, arterial blood pH was maintained in normal range (7.36 to 7.47) using volume-controlled positive pressure ventilation. Core body temperature was continuously monitored and maintained within normal porcine body temperature range (36.2°C to 39.0°C) using cooling and insulation.

Microdialysis catheter placement. After induction of general anaesthesia and skin incision, microdialysis catheters were placed in holes drilled in cortical and cancellous bone. The depth of cortical bone drill holes was 15 mm (membrane length: 10 mm) and placed in the anterior tibial margin. The depth of the cancellous bone holes was 35 mm (membrane length: 30 mm) and placed 10 mm distal from the epiphyseal line in the tibial condyles. Microdialysis catheters were placed in the knee joint (membrane length: 30 mm) and in subcutaneous tissue (membrane length: 30 mm) using splitable introducers. After placement of the microdialysis catheters, they were perfused with isotonic saline 0.9% with a flow rate of $1 \mu\text{l/min}$. The positions of the bone catheters were confirmed by fluoroscopy and correct location of the drill holes was assessed by post-mortem CT scans.

Sampling of dialysate and plasma, and calibration. Flucloxacillin was administered after 20 minutes of tissue-equilibrium (time 0). Dialysates were collected during the first (zero to six hours) and fourth (18 to 24 hours) dosing intervals. From zero to four hours and 18 to 22 hours, dialysates were collected every 40 minutes. From four to six hours and 22 to 24 hours, dialysates

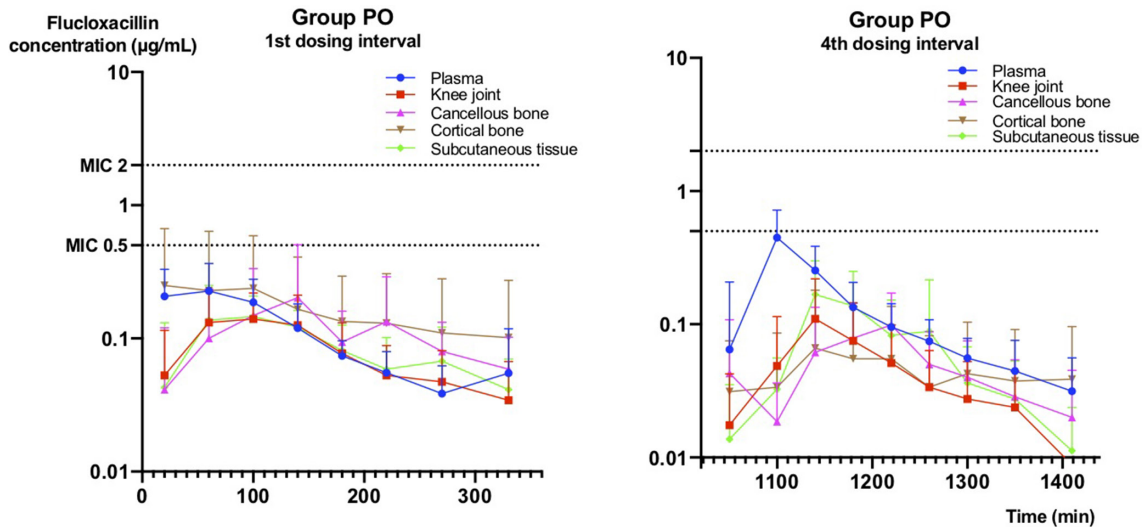


Fig. 1

Mean concentration-time profiles in Group PO for plasma, subcutaneous tissue, knee joint, and cancellous and cortical bone. The error bars represent upper 95% confidence intervals (CIs). The figure displays the profiles of the first and the fourth dosing intervals. Minimum inhibitory concentrations (MICs: 0.5 µg/ml and 2.0 µg/ml) are indicated by the dotted horizontal lines (y-axis is log-scaled).

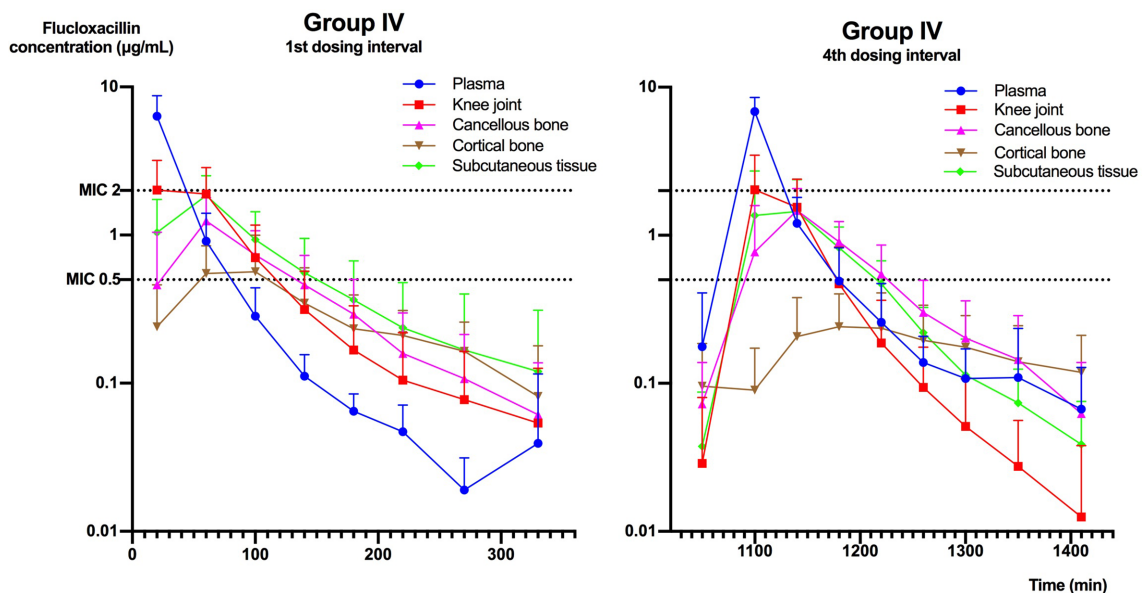


Fig. 2

Mean concentration-time profiles in Group IV for plasma, subcutaneous tissue, knee joint, and cancellous and cortical bone. The error bars represent upper 95% confidence intervals (CIs). The figure displays the profiles of the first and the fourth dosing intervals. Minimum inhibitory concentrations (MICs) of 0.5 µg/ml and 2.0 µg/ml are indicated by the dotted horizontal lines (y-axis is log-scaled).

were collected every 60 minutes. The total number of samples per compartment was 18 in each pig, including samples at time 0. Plasma samples were drawn at the midpoint of each dialysate sampling interval from a central venous catheter. When the last dialysate was collected, the perfusate was replaced with isotonic saline 0.9% containing 100 µg/ml flucloxacillin for individual calibration of all catheters by collecting two samples over 40 minutes. After the last calibration sample was collected, the animals were euthanized per protocol.

Plasma was extracted by centrifuging the blood samples (3,000 rpm, ten minutes, 4°C) after keeping the blood samples at 5°C for a maximum of two hours. The dialysate and plasma samples were stored at -80°C until biochemical analysis.

Quantification of flucloxacillin concentrations in microdialysates and plasma by liquid chromatography-tandem mass spectrometry. A 10 µl microdialysate sample was transferred to a 700 µl 96-well microplate (Waters Corporation, Milford, Massachusetts, USA), then a 190 µl

Table 1. Mean times the free flucloxacillin concentration was maintained above the minimum inhibitory concentration ($fT > MIC$), expressed as percentages and minutes, for the first and fourth dosing intervals.

Parameter	First dosing interval		Fourth dosing interval	
	Intravenous flucloxacillin (Group IV)	Oral flucloxacillin (Group PO)	Intravenous flucloxacillin (Group IV)	Oral flucloxacillin (Group PO)
Mean $fT > MIC$ (mins), MIC = 0.50 $\mu\text{g/ml}$ (95% CI; range)				
Plasma	77 (47 to 106; 57 to 105)	1 (-29 to 31; 0 to 8)	100 (82 to 119; 70 to 155)	11 (-8 to 29; 0 to 46)
SCT	149 (119 to 179; 68 to 323)	0 (-28 to 28; 0 to 0)	108 (89 to 126; 0 to 159)	0 (-18 to 18; 0 to 0)
Knee joint	114 (81 to 146; 97 to 184)	0 (-34 to 34; 0 to 0)	76 (57 to 95; 0 to 113)	0 (-18 to 18; 0 to 0)
Cancellous bone	106 (76 to 136; 71 to 154)	10 (-19 to 38; 0 to 70)	126 (108 to 144; 72 to 166)	0 (-19 to 19; 0 to 0)
Cortical bone	61 (29 to 94; 0 to 121)	26 (-14 to 46; 0 to 162)	7 (-25 to 38; 0 to 31)	0 (-18 to 18; 0 to 0)
Mean $fT > MIC$ (mins), MIC = 2.00 $\mu\text{g/ml}$ (95% CI; range)				
Plasma	44 (35 to 52; 35 to 54)	0 (-8 to 8; 0 to 0)	49 (40 to 58; 40 to 68)	0 (-9 to 9; 0 to 0)
SCT	16 (8 to 24; 0 to 55)	0 (-8 to 8; 0 to 28)	15 (6.4 to 24; 0 to 62)	0 (-9 to 9; 0 to 0)
Knee joint	39 (29 to 47; 0 to 80)	0 (-9 to 9; 0 to 0)	33 (23 to 42; 0 to 60)	0 (-9 to 9; 0 to 0)
Cancellous bone	0 (-8 to 8; 0 to 0)	0 (-8 to 8; 0 to 0)	9 (0.4 to 18; 0 to 39)	0 (-9 to 9; 0 to 0)
Cortical bone	0.5 (-8 to 9; 0 to 76)	0 (-8 to 8; 0 to 0)	0 (-9 to 9; 0 to 0)	0 (-9 to 9; 0 to 0)
Mean $fT > MIC$ (%), MIC = 0.50 $\mu\text{g/ml}$ (95% CI; range)				
Plasma	23 (10 to 36; 17 to 32)	0.3 (-9 to 9; 0 to 2)	30 (21 to 40; 21 to 47)	3 (-6.4 to 13; 0 to 14)
SCT	45 (32 to 58; 21 to 98)	0 (-8 to 8; 0 to 0)	33 (23 to 42; 0 to 48)	0 (-9.6 to 9.6; 0 to 0)
Knee joint	35 (25 to 44; 29 to 56)	0 (-10 to 10; 0 to 0)	23 (17 to 29; 0 to 34)	0 (-9.6 to 9.6; 0 to 0)
Cancellous bone	32 (23 to 41; 22 to 47)	3 (-6 to 12; 0 to 21)	38 (29 to 48; 22 to 50)	0 (-9.6 to 9.6; 0 to 0)
Cortical bone	18 (9 to 28; 0 to 37)	8 (-4 to 14; 0 to 49)	2 (-7.6 to 12; 0 to 9)	0 (-9.6 to 9.6; 0 to 0)
Mean $fT > MIC$ (%), MIC = 2.00 $\mu\text{g/ml}$ (95% CI; range)				
Plasma	13 (10 to 16; 11 to 16)	0 (-3.2 to 3.2; 0 to 0)	15 (12 to 18; 12 to 21)	0 (-2.7 to 2.7; 0 to 0)
SCT	5 (2.4 to 7.3; 0 to 17)	0 (-3.2 to 3.3; 0 to 0)	5 (1.9 to 7.4; 0 to 19)	0 (-2.7 to 2.7; 0 to 0)
Knee joint	12 (9 to 14; 0 to 24)	0 (-3.2 to 3.2; 0 to 0)	10 (7 to 13; 0 to 18)	0 (-2.7 to 2.7; 0 to 0)
Cancellous bone	0 (-3.2 to 3.2; 0 to 0)	0 (-3.2 to 3.2; 0 to 0)	3 (0.04 to 5.5; 0 to 12)	0 (-2.7 to 2.7; 0 to 0)
Cortical bone	0.2 (-0.2 to 0.3; 0 to 23)	0 (-3.2 to 3.2; 0 to 0)	0 (-2.7 to 2.7; 0 to 0)	0 (-2.7 to 2.7; 0 to 0)

CI, confidence interval; MIC, minimum inhibitory concentration; SCT, subcutaneous tissue.

internal standard solution was added (0.01 $\mu\text{g/ml}$ of oxacillin; Merck, Darmstadt, Germany) in water:methanol (95:5) with 0.1% formic acid, and mixed. Separate calibrator samples were prepared using a flucloxacillin reference compound (Merck analytical standard, eight different calibrator solutions in range 0.0045 $\mu\text{g/ml}$ to 10 $\mu\text{g/ml}$). Quantification of the free (non-protein bound) concentration of flucloxacillin in plasma was performed after ultrafiltration of plasma as described previously²⁰ using the Centrifree ultrafiltration device with Ultracel PL membrane with a nominal molecular weight limit of 30 kDa (Merck Millipore, Darmstadt, Germany). A 400 μl plasma sample was transferred to the ultrafiltration device and allowed to equilibrate for 20 minutes at 37°C followed by centrifugation for 20 minutes at 1,500 g at 37°C. A 10 μl aliquot of the filtrate sample was then processed and analyzed as described for microdialysate samples. A 7.5 μl sample was analyzed with an ultra-high performance liquid chromatography system (Acquity UPLC with HSS-C18, 1.8 μm ,

100 \times 2.1 mm column; Waters Corporation). The flow rate was 0.4 ml/minute and the initial condition was 95% mobile phase A (water with 0.2% formic acid) and 5% mobile phase B (methanol with 0.1% formic acid), which was changed through a linear gradient to 95% B over 2.5 minutes. After 3.2 minutes, the gradient was returned to 95% A and allowed to equilibrate for 1.5 minutes. Electrospray ionization in the positive mode was used for MS analysis (Xevo TQ-S; Waters Corporation) with a capillary voltage of 2.5 kV. The analytes were detected in the multiple reaction monitoring mode with the following transitions (m/z) and cone voltage/collision energies (CV/CE): flucloxacillin (quant ion m/z: 454.1 > 160 and qualifier ion 454.1 > 295, CV/CE = 25 V/11 eV) and oxacillin (m/z: 402.1 > 160, CV/CE = 25 V/10 eV). Calibration curves were constructed by linear regression of the peak area ratio (analyte/internal standard) versus the nominal analyte concentrations. The method was validated and showed acceptable levels of precision (CV < 15%) and bias (CV < 15%) as evaluated at 0.5 $\mu\text{g/}$

Table II. Flucloxacillin pharmacokinetic parameters for plasma, subcutaneous tissue, knee joint, cancellous bone, and cortical bone, for the first and fourth dosing intervals.

Parameter	First dosing interval		Fourth dosing interval	
	Intravenous flucloxacillin (Group IV)	Oral flucloxacillin (Group PO)	Intravenous flucloxacillin (Group IV)	Oral flucloxacillin (Group PO)
Median AUC, min µg/ml (95% CI)				
Plasma	250 (206 to 293)	37 (-6.7 to 80)	311 (268 to 355)	44 (0.12 to 87)
SCT	203 (160 to 247)	26 (-18 to 69)	174 (130 to 217)	26 (-20 to 72)
Knee joint	222 (176 to 269)	33 (-17 to 83)	180 (134 to 226)	25 (-30 to 80)
Cancellous bone	138 (95 to 182)	40 (-6.7 to 86)	171 (128 to 215)	16 (-29 to 62)
Cortical bone	98 (51 to 144)	62 (14 to 111)	58 (12 to 105)	25 (-36 to 87)
Median C_{max}, µg/ml (95% CI)				
Plasma	6.4 (5.8 to 7.0)	0.3 (-0.3 to 0.9)	7 (6.4 to 7.6)	0.5 (-0.12 to 1.09)
SCT	1.9 (1.3 to 2.5)	0.2 (-0.4 to 0.8)	1.9 (1.3 to 2.5)	0.2 (-0.45 to 0.85)
Knee joint	2.5 (1.8 to 3.1)	0.2 (-0.5 to 0.9)	2.6 (1.9 to 3.2)	0.2 (-0.59 to 0.95)
Cancellous bone	1.3 (0.66 to 1.9)	0.2 (-0.4 to 0.9)	1.6 (0.96 to 2.17)	0.3 (-0.32 to 0.89)
Cortical bone	0.7 (0.04 to 1.3)	0.3 (-0.5 to 1.1)	0.3 (-0.38 to 0.92)	0.1 (-0.72 to 1.0)
Median T_{max}, mins (95% CI)				
Plasma	20 (-11 to 51)	55 (24 to 86)	20 (-11 to 51)	25 (-6.3 to 56)
SCT	60 (29 to 91)	126 (95 to 158)	55 (24 to 86)	83 (49 to 116)
Knee joint	37 (3.6 to 71)	100 (67 to 136)	31 (-2.1 to 65)	68 (28 to 108)
Cancellous bone	65 (34 to 96)	130 (96 to 164)	65 (34 to 96)	95 (61 to 128)
Cortical bone	54 (21 to 88)	118 (82 to 154)	154 (121 to 188)	75 (30 to 120)
Median AUC_{tissue}/AUC_{plasma} (95% CI)				
SCT	0.9 (0.4 to 1.3)	0.8 (0.33 to 1.2)	0.6 (0.13 to 1.0)	0.6 (0.12 to 1.1)
Knee joint	0.9 (0.44 to 1.4)	0.9 (0.41 to 1.5)	0.6 (0.15 to 1.1)	0.7 (0.09 to 1.2)
Cancellous bone	0.6 (0.2 to 1.1)	1 (0.50 to 1.5)	0.6 (0.13 to 1.0)	0.4 (0 to 0.8)
Cortical bone	0.5 (-0.02 to 0.93)	1.7 (1.2 to 2.1)	0.2 (-0.29 to 0.66)	0.6 (-0.01 to 1.3)

AUC_{0-last}, area under the concentration–time curve from t = 0 to t = 6 hrs (AUC₀₋₆) and AUC from t = 18 to t = 24 hrs (AUC₁₈₋₂₄); C_{max}, peak drug concentration; T_{max}, time to C_{max}; AUC_{tissue}/AUC_{plasma}, tissue penetration expressed as the ratio of AUC_{tissue}/AUC_{plasma}. CI, confidence interval; SCT, subcutaneous tissue.

ml and 0.05 µg/ml. Lower limit of quantification was 0.015 µg/ml. The measurement range was 0.015 µg/ml to 10 µg/ml. Selectivity and carryover effects were investigated and found to be satisfactory.

Pharmacokinetic data analysis and statistics. Non-compartmental analysis (NCA) of pharmacokinetic data was performed using Stata v.15.1 (StataCorp, College Station, Texas, USA). Flucloxacillin plasma and dialysate concentrations were plotted as a function of time. These curves were employed to estimate the following parameters separately for each animal in every compartment, for both dosing intervals: the area under the concentration–time curve from t = 0 to t = 6 hrs (AUC₀₋₆) and AUC from t = 18 to t = 24 hrs (AUC₁₈₋₂₄), using the trapezoidal rule; peak drug concentration (C_{max}); time to peak drug concentration (T_{max}); and tissue penetration (AUC_{tissue}/AUC_{plasma}). Pharmacokinetic parameters were estimated for each dosing interval, pig, and compartment. Using linear interpolation (Microsoft Excel v.16.35; Microsoft, Redmond, Washington, USA) in each compartment for each pig, the flucloxacillin *fT* > MIC was determined for MIC (0.5 µg/ml) and MIC (2.0 µg/ml) for each dosing interval, respectively.

Pharmacokinetic parameters were analyzed with repeated measurements of analysis of variance (ANOVA). Normal distribution of residuals and error terms was assessed with quantile-quantile (QQ) plots. Homogeneity of the variance of error terms was assessed with ‘residual versus fits’ plot. Log-transformation of the observed raw data of pharmacokinetic values was required to satisfy the assumption about normality of distribution. Data for AUC₀₋₆, AUC₁₈₋₂₄, C_{max} and T_{max} were log-transformed, analyzed, back-transformed to the original scale, and summarized as medians and 95% confidence intervals (CIs). The Kenward-Roger approximation method was used to correct degrees of freedom, mandated by the small sample size. Linear combination of estimates was employed for pairwise comparisons. The level of significance was 0.05. Incomplete tissue penetration was defined as a ratio AUC_{tissue}/AUC_{plasma} < 1, where the 95% CI did not overlap the value of 1.

Results

All 16 pigs completed the study. All drill holes and microdialysis catheters were correctly placed and verified by CT scans. Due to membrane malfunction, the following catheters were excluded: one knee, one cancellous bone, and one cortical bone in Group PO; and one cortical bone and one knee joint in Group IV. All samples from all other catheters were included in the analysis.

The mean (SD) relative recoveries were 67% (SD 4%), 65% (SD 6%), 39% (SD 9%), and 71% (SD 6%) for the subcutaneous tissue, knee joint, cortical bone, and cancellous bone, respectively. Mean concentration-time profiles for all compartments during both dosing intervals are shown in Figure 1 (Group PO) and Figure 2 (Group IV).

In plasma, subcutaneous tissue, knee joint, and cancellous bone, mean values of $fT > MIC$ were comparable in the first and fourth dosing intervals for both the low and high MIC targets in both groups (Table I). For cortical bone, $fT > MIC$ was longer in Group IV in the first dosing interval compared to the fourth dosing interval. Intravenous administration resulted in longer $fT > MIC$ (0.5 µg/ml) compared to oral administration ($p < 0.05$, ANOVA), except for cortical bone ($p = 0.135$, ANOVA). In Group IV, all pigs reached a concentration of 0.5 µg/ml in all compartments. The mean time above 0.5 µg/ml was 149 minutes (119 to 179) in subcutaneous tissue, and 61 minutes (29 to 94) and 106 minutes (76 to 136) in cortical bone and cancellous bone, respectively, in Group IV. In Group PO, no pigs (0/8) reached a concentration of 0.5 µg/ml in all compartments. For MIC (2.0 µg/ml), $fT > MIC$ was close to zero minutes in both groups (Table I).

Pharmacokinetic parameters. The pharmacokinetic parameters between the first and the fourth dosing intervals in plasma, subcutaneous tissue, knee joint, cancellous bone, and cortical bone were similar. For all the investigated compartments and in both dosing intervals, tissue penetration was complete across groups and dosing intervals, except for cortical bone in Group IV (Table II). The C_{max} was higher after intravenous compared to oral administration in all compartments.

Discussion

We assessed the unbound flucloxacillin bone concentrations after intravenous and oral administration in a porcine model. Conventional dosing used for perioperative prophylaxis and bacterial inoculation prophylaxis following smaller traumatic wound infections was investigated.^{2,5} For the low MIC target (0.5 µg/ml), intravenous administration of flucloxacillin provided superior $fT > MIC$ compared to oral administration in all the investigated compartments for both dosing intervals, except for cortical bone. The $fT > MIC$ data for the high MIC target (2 µg/ml) were too low to have an expected clinical relevance. Flucloxacillin 1 g demonstrated complete penetration from plasma to subcutaneous tissue, cancellous bone, and the knee joint, after intravenous as well as oral administration.

Interpretations of $fT > MIC$. Oral and intravenous administration resulted in comparable tissue penetration ratios. However, tissue concentrations and $fT > MIC$ s (0.5 µg/ml) were higher after IV compared to oral administration. This supports perioperative flucloxacillin being administered intravenously rather than orally. However, in Group IV, the observed mean of $fT > MIC$ (0.5 µg/ml) was only 149 minutes (95% CI 119 to 179; range 68 to 323) in subcutaneous tissue and 61 minutes (95% CI 29 to 94; range 0 to 121) to 106 minutes (95% CI 76 to 136; range 71 to 154) in bone tissue. For flucloxacillin, *S. aureus* exhibits MIC values in the range of 0.12 µg/ml to 2 µg/ml,³ and it remains a matter of further research to decide which target to employ.^{21,22} Moreover, the definition of sufficient $fT > MIC$ remains a matter of dispute. For perioperative prophylaxis, the conventional recommendation is to achieve 100% $fT > MIC$ throughout surgery, whereas for antibiotic therapy of, for example, chronic osteomyelitis, the literature suggests that 50% $fT > MIC$ may be sufficient for β-lactam antibiotic therapeutic effect.²³ Our findings suggest that flucloxacillin 1 g intravenously provides concentrations above 0.5 µg/ml for short orthopaedic procedures, while higher doses may be necessary to achieve a concentration above 2 µg/ml.

Considering the target of 50% $fT > MIC$, none of the compartments in either group reached a mean $fT > MIC$ above 50%. For other β-lactam antibiotics, such as piperacillin, cefuroxime, and meropenem, continuous infusion has been found to provide superior $fT > MIC$ in comparison to intermittent bolus infusion.^{13,24,25} Further research is warranted to investigate if this is also the case for flucloxacillin.

Intravenous versus oral administration. In the management of orthopaedic infections, intravenous therapy is generally believed to be superior compared to oral administration.²⁶ However, this view has been challenged by the Oral Versus Intravenous Antibiotics for Bone and Joint Infection (OVIVA) trial, which compared oral and intravenous antibiotic treatment for bone and joint infections.²⁷ There was no difference in clinical outcome between the two treatment groups, defined as treatment failure in the management of bone and joint infections within one year. Of note, all patients initially received equal intravenous antibiotics until antibiotic susceptibility was determined.²⁷ Obviously, drug penetration is a complex phenomenon as it is affected by variations of multiple factors such as patient comorbidity and pathophysiology, clearance, and volume of distribution. The present experimental study was limited to 24 hours of sampling in healthy tissue, without surgical debridement, and therefore translational comparison to the OVIVA trial is not possible.

However, our findings raise an interesting discussion regarding the optimal mode of flucloxacillin administration in the treatment of osteomyelitis. We demonstrated superior pharmacokinetic parameters, including $fT > MIC$, in Group IV compared to Group PO. However, as previously mentioned, the $fT > MIC$ was limited in both groups. Regarding the efficacy of different flucloxacillin doses (i.e. 1 g or 2 g) for perioperative prophylaxis, the evidence is sparse, despite

the fact that it has been employed for surgical perioperative prophylaxis for decades.²⁸ A large prospective study concerning perioperative prophylaxis on 26,849 patients showed combination treatment of intravenous flucloxacillin 2 g with gentamicin 3 mg/kg to have the lowest surgical site infection (SSI) rate (0.72%) compared with intravenous cefuroxime 1.5 g as a single dose (0.92%) or cefuroxime 1.5 g as a triple dose (2.46%).²⁹ Furthermore, recent studies have shown that administration of postoperative oral antibiotics to patients undergoing revision surgery resulted in lower rates of infection.^{30,31} The sparse evidence that exists recommends that a broad-spectrum antibiotic should be included in the perioperative prophylaxis along with flucloxacillin.³²

Thus, future studies assessing various flucloxacillin dosing scenarios and modes of administration are needed to further investigate the association between $fT > MIC$ and clinical efficacy in bone infections.

Flucloxacillin pharmacokinetics. Flucloxacillin plasma pharmacokinetics have previously been investigated in several clinical studies.²¹⁻²³ In the study by Landersdorfer et al,²³ intravenous administration of flucloxacillin 1.5 g every six hours, administered as a 30-minute infusion, resulted in 50% $fT > MIC$ (0.5 µg/ml) in 50% of the patients, which is comparable to the present findings.

Until now, studies investigating flucloxacillin bone pharmacokinetics have employed static estimates of bone biopsies.^{33,34} These two studies reported bone penetration in the range of 5% to 20%, which is contrary to the findings of the present study.

The bone biopsy method has a number of limitations. Most importantly, only the total concentration of flucloxacillin can be measured. Moreover, bone biopsy samples can only be obtained during surgery, resulting in poor temporal resolution of data. Microdialysis allows for serial and simultaneous sampling, from multiple compartments of the unbound, active fraction of drugs. In the present study, we observed higher antibiotic concentrations in cancellous compared to cortical bone. This complies with previous studies suggesting that bone may not be considered a homogenous compartment.^{19,35-37} This may be explained by a higher fraction of extracellular fluid and more dense vascularity in cancellous bone compared to cortical bone. Drilling of holes may also affect the local flow of antibiotics into the cavities. This, however, mimics the true orthopaedic perioperative situation.

In humans, flucloxacillin is primarily cleared by renal excretion.³⁸ This leads us to believe that the primary reason for the low PO concentrations in the present study is reduced absorption from the intestine, because of the use of general anaesthesia. However, free human plasma C_{max} observed by Gardiner et al²² complies well with the observed plasma C_{max} in the present experimental porcine model and therefore, indirectly, suggests otherwise.

There were several limitations in this study. To a great extent pigs and humans resemble each other regarding physiology and anatomy. However, the main limitation of this study is that results from an experimental porcine

model do not necessarily translate well to clinical effects in patients. In both humans and pigs the metaphyseal vasculature diminish with age.³⁹ As this study was conducted in healthy juvenile pigs (aged five months), it can be speculated that our findings of low flucloxacillin concentrations in bone would be even lower in clinical practice.

Furthermore, the intestinal absorption of flucloxacillin may differ between pigs and humans, prompting caution, especially when interpreting the flucloxacillin pharmacokinetic parameters from Group PO in the present study.⁴⁰ The absolute concentrations were corrected for relative recovery. The mean (SD) relative recoveries were 67% (SD 4%), 65% (SD 6%), 39% (SD 9%), and 71% (SD 6%) for the subcutaneous tissue, knee joint, cortical bone, and cancellous bone, respectively. It is recommended that relative recovery exceeds 20% in microdialysis studies.⁴¹ Thus, the coefficients of relative recovery were within acceptable limits in the present study.

In conclusion, although intravenous administration of flucloxacillin 1 g provided higher $fT > MIC$ for the low MIC target (0.5 µg/ml) compared to oral administration, concentrations were surprisingly low in both groups, particularly for bone tissue. For the high MIC target (2 µg/ml), $fT > MIC$ was too low to have an expected clinical relevance. Achievement of sufficient bone and soft tissue flucloxacillin concentrations may require a dose increase or continuous administration.

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Funding statement:

- The authors report institutional grants (paid to Aarhus University) from the Doctor Sofus Carl Emil Friis & Wife Olga Doris Friis' Fund and the Aase and Ejnar Danielsens Fund, related to this study. These were payments for conduction of the study (animals, analyses). M. A. F. Bendtsen reports a scholarship to conduct the study from the Novo Nordisk Foundation, grant number: NNF18OC0033304. No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Acknowledgements

- We would like to acknowledge the financial support we have received, and the outstanding animal facilities and help available at the Department of Clinical Medicine at Aarhus University Hospital. We would also like to acknowledge Anette Bastrup at the Orthopaedic Research Lab, Aarhus, Denmark.

Ethical review statement

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

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