Journal of Antimicrobial Chemotherapy (2002) **50**, 73–77
DOI: 10.1093/jac/dkf066

JAC

Penetration of linezolid into bone, fat, muscle and haematoma of patients undergoing routine hip replacement

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Received 22 October 2001; returned 22 February 2002; revised 4 March 2002; accepted 21 March 2002

Twelve patients undergoing total hip replacement were given 600 mg of linezolid as a 20 min iv infusion along with conventional prophylaxis of 1 g of cefamandole immediately before surgery. Routine total hip arthroplasty was carried out, and at timed intervals during surgery samples of bone, fat, muscle and blood were collected for assay by high-performance liquid chromatography analysis. Samples of the haematoma fluid that formed around the operation site and further blood samples for assay were also collected at timed intervals following the operation. The penetration of linezolid into bone was rapid, with mean concentrations of 9.1 mg/L (95% CI 7.7–10.6 mg/L) achieved at 10 min after the infusion, decreasing to 6.3 mg/L (95% CI 3.9–8.6 mg/L) at 30 min. Correction for the simultaneous blood concentrations gave mean values for bone penetration of 51% at 10 min, 60% at 20 min and 47% at 30 min. Although the penetration of linezolid into fat was also rapid, mean concentrations and degree of penetration were *c.* 60% of those in bone; at 10 min they were 4.5 mg/L (95% CI 3.0–6.1 mg/L; penetration 27%); at 20 min they were 5.2 mg/L (95% CI 4.0–6.4 mg/L; penetration 37%); and at 30 min, 4.1 mg/L (95% CI 3.3–4.8 mg/L; penetration 31%). For muscle the corresponding values were 10.4 mg/L (95% CI 8.1–12.7 mg/L; penetration 58%) at 10 min, 13.4 mg/L (95% CI 10.2–16.5 mg/L; penetration 94%) at 20 min and 12.0 mg/L (95% CI 9.2–14.8 mg/L; penetration 93%) at 30 min. Mean concentrations of linezolid in the haematoma fluid drained from around the operation site were 8.2 mg/L at 6–8 h and 5.6 mg/L at 10–12 h after the infusion, and 7.0 mg/L at 2–4 h following a second 600 mg infusion given 12 h post-operatively. We conclude that linezolid exhibits rapid penetration into bone, fat and muscle of patients undergoing hip arthroplasty, to achieve levels in excess of its MIC for susceptible organisms (≤4 mg/L); therapeutic concentrations were maintained in the haematoma fluid that surrounds the operation site for >16 h.

Introduction

Linezolid is the first member of a new synthetic class of antimicrobials, oxazolidinones, to enter clinical practice. Linezolid is characterized by a broad spectrum of activity against Gram-positive organisms and acts by inhibition of an early stage in the protein synthesis pathway.[1–3] This mode of action, although not fully characterized, is not common to other classes of antibacterial and cross-resistance associated with the known mechanisms responsible for acquired resistance does not occur with linezolid.[1] As a result, linezolid retains activity against the clinically important strains of methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide-intermediate *S. aureus* (GISA) and glycopeptide-resistant enterococci (GRE).

Linezolid is rapidly absorbed following an oral dose to give concentrations similar to those following parenteral administration, with bioavailability *c.* 100%.[4–7] Linezolid has a relatively high volume of distribution (*c.* 50 L in adults), and achieves levels in inflammatory fluid similar to those in

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blood, suggesting good penetration into extra-vascular sites and a possible role in the treatment of infections involving a wide range of soft tissue sites.[8] Linezolid is cleared primarily through the renal route with a half-life of *c*. 5 h, which permits a twice daily dosing regimen.[4–6]

MRSA is an established problem in many hospitals across the world, causing problems of both colonization and nosocomial infection.[9] When these infections involve either bone or joint prostheses they present a particularly difficult problem in patient management, frequently resulting in prolonged periods of hospitalization for parenteral therapy, and often only modest therapeutic success. As linezolid has both a microbiological and pharmacokinetic profile, which suggests a potential use in the treatment of such infections, we have evaluated the penetration of linezolid, after a single 600 mg iv dose, into the bone and associated fat and muscle of 12 patients undergoing routine total hip replacement and receiving standard prophylaxis with cefamandole.

Materials and methods

Study protocol

This was approved by the local medical research ethics committee and all patients gave written informed consent. Twelve patients who had not received antimicrobials in the preceding 72 h and were to undergo routine hip replacement were enrolled into the study. Immediately before the induction of anaesthesia a 20 min infusion of 600 mg of linezolid followed by a single bolus of 1 g of cefamandole were administered through a forearm vein. The operation began immediately at the end of the infusion and all timings are expressed relative to this time point. Further doses of cefamandole were administered 8 and 16 h after the start of the operation and a further dose of linezolid was given 12 h after the start of the operation. Routine total hip replacement was carried out on all patients, and samples of bone, fat, muscle and blood were collected 10, 20 and 30 min after the start of the operation. Samples of the haematoma fluid draining from the operation site were collected over the periods 6–8, 10–12 and 14–16 h after the start of the operation, and samples of blood were collected at 8, 12 and 16 h immediately before the administration of further doses of cefamandole or linezolid. Blood and haematoma samples were centrifuged, and the supernatant removed and stored at –70°C along with the bone, fat and muscle samples until assayed.

HPLC assay procedures

Samples were assayed for the presence of cefamandole and linezolid by a high-performance liquid chromatography (HPLC) method that permitted the simultaneous assay of both agents, adapted from Tobin *et al*.[10] Chromatography was performed on a Hypersil 5ODS column (HPLC Technology Ltd, Macclesfield, UK) using a mobile phase of methanol/water/phosphoric acid (30:69:1) with the addition of 2 g/L heptane sulphonic acid (Sigma Chemical Co.) and the pH adjusted to 4.5. Detection of both compounds was by UV absorbance at 254 nm with quantification by the external standard method. Serum and haematoma samples were diluted with an equal volume of acetonitrile, centrifuged at 5000**g** and 10 μL of the supernatant was injected into the chromatograph. Bone, fat and muscle samples were processed as previously described and the two antibiotics were extracted in phosphate-buffered saline (PBS) at 4°C for 5 h.[11,12] In brief, bone samples (*c*. 0.5 g) were crushed, a volume of PBS equal to twice the weight of bone added (1 g ≅ 1 mL) and the antimicrobial agents extracted at 4°C for 5 h. Any samples with visible evidence of blood contamination were discarded and, where possible, the total sample collected was processed. After extraction the samples were centrifuged at 5000**g** and an aliquot of the aqueous layer was treated with acetonitrile, as described for serum samples. Samples of fat and muscle were treated as described for bone, but were finely sliced rather than crushed.

The assay response was linear over the concentration range 0.5–100 mg/L, with a lower limit of detection of 0.1 mg/L for linezolid and 0.5 mg/L for cefamandole. Recovery of the two agents from spiked bone and fat samples was in the range 95–110%, depending on drug, concentration and tissue, and for the purposes of this study was taken as 100%. Intra- and inter-day accuracy and precision were assessed by the use of quality control standards with limits for accuracy of 10% and coefficient of variability for precision of 5% used.

Results

Following a 600 mg infusion of linezolid, mean serum concentrations for the 12 patients were 19.2 mg/L (median 16.9 mg/L; range 10.7–38.2 mg/L) at 10 min after the infusion, dropping to 14.3 mg/L (median 13.2; range 5.4–21.3 mg/L) at 30 min after the infusion (Table 1). By 8 h after the infusion mean levels had fallen to 5.1 mg/L (median 4.1 mg/L; range 3.4–12.0 mg/L), further decreasing to 2.8 mg/L (median 2.2 mg/L; range 0.5–9.2 mg/L) at 12 h (Table 1). Although too few samples were taken to make a conventional assessment of the serum half-life of linezolid, a review of the data indicates that these results are consistent with a half-life of *c*. 4–5 h.

The penetration of linezolid into bone was rapid, with mean concentrations of 9.1 mg/L (range 4.3–13.0 mg/L), 8.6 mg/L (range 5.2–23.7 mg/L) and 6.3 mg/L (2.6–17.3 mg/L) found at 10, 20 and 30 min, respectively, after the end of the infusion (Table 1). Although there was considerable variation in concentrations (range 2.6–23.7 mg/L), in only one of the 36 samples assayed was the concentration of linezolid <4 mg/L; a concentration of 2.6 mg/L was measured in the 30 min sample from patient six, compared with 7.4 and 5.2 mg/L at 10 and

Table 1. Concentrations of linezolid and cefamandole in bone, fat, muscle and haematoma in 12 patients undergoing total hip replacement

	Linezolid			Cefamandole		
Parameter/time	median (mg/L)	mean (mg/L)	95% CI (mg/L)	median (mg/L)	mean (mg/L)	95% CI (mg/L)
Blood concentration						
10 min	16.9	19.2	15.5–22.8	88.0	87.0	72.0–102.1
20 min	14.4	15.8	12.5–19.1	74.7	67.7	54.1–81.4
30 min	13.2	14.3	11.3–17.2	58.2	58.9	44.9–72.8
8 h	4.1	5.1	3.8–6.5	1.4	1.7	1.0–2.5
12 h	2.2	2.8	1.4–4.3	4.0	5.2	2.6–7.8
16 h[a]	11.3	11.6	8.8–14.4	1.2	1.5	0.8–2.3
Bone concentration						
10 min	8.8	9.1	7.7–10.6	17.6	17.5	12.6–22.5
20 min	7.1	8.6	5.6–11.6	13.9	13.1	10.8–15.5
30 min	5.7	6.3	3.9–8.6	9.7	11.5	8.2–14.8
Fat concentration						
10 min	4.4	4.5	3.0–6.1	10.6	10.4	6.9–14.0
20 min	4.2	5.2	4.0–6.4	9.7	10.9	8.8–13.0
30 min	3.9	4.1	3.3–4.8	8.4	8.7	6.8–10.6
Muscle concentration						
10 min	10.5	10.4	8.1–12.7	16.3	14.9	11.0–18.8
20 min	11.8	13.4	10.2–16.5	17.9	17.0	13.7–20.3
30 min	10.1	12.0	9.2–14.8	14.5	15.9	12.6–19.2
Drain fluid concentration						
6–8 h drain	7.1	8.2	6.3–10.1	5.6	7.2	3.8–10.7
10–12 h drain	4.8	5.6	4.2–6.9	14.8	15.0	9.4–20.6
14–16h drain[a]	5.4	7.0	3.5–10.5	10.1	10.4	8.0–12.8

[a]Further dose of linezolid given at 12 h.

20 min, respectively. The lowest bone concentration of cefamandole (5.3 mg/L) was also measured in this sample. When compared with simultaneous blood concentrations, the mean bone penetration of linezolid was 51.0% at 10 min, 60.0% at 20 min and 47% at 30 min (Table 2); overall penetration was 51% (95% CI 43–75%).

Penetration of linezolid into the muscle surrounding the operation site was also rapid, achieving mean concentrations of 10.4 mg/L at 10 min after the infusion and a peak concentration of 13.4 mg/L by 20 min after the end of the infusion (Table 1). When compared with simultaneous blood concentrations these values represented penetration of 58.3% at 10 min, 94.3% at 20 min and 93.0% at 30 min (Table 2). As with bone and muscle, penetration of linezolid into fat was also rapid, with mean peak concentrations of 5.2 mg/L achieved 20 min after the infusion. However, both the concentrations and degree of penetration into fat relative to serum levels were approximately half the values obtained for bone (Tables 1 and 2). The good penetration and levels of linezolid observed in the soft tissues surrounding the operation site at the time of surgery were also seen in the drainage from the wound site. Mean concentrations of linezolid in drainage fluid were 8.2 mg/L 6–8 h and 5.6 mg/L 10–12 h after the first dose (Table 1) and 7.0 mg/L 2–4 h after the second dose (Table 2).

For all tissue samples the concentrations of cefamandole were significantly higher (Welch's t-test, $P < 0.01$) than those of linezolid, but when corrected for the simultaneous blood concentrations the penetration of linezolid into these tissues was significantly higher ($P < 0.05$).

Discussion

In this study we have attempted to characterize the tissue distribution and penetration profile of linezolid during routine orthopaedic surgery. Although the role of antibiotic administration in this procedure has been for prophylaxis, the study objective was to gain data to help support decisions on the role of linezolid in the treatment of bone and associated soft tissue infections. In designing the study we have used a two-drug administration protocol to ensure adequate prophylaxis whilst at the same time providing comparative data for one of the major classes of antimicrobial used in the treatment and prophylaxis of such infections, namely the β-lactams. Although

Table 2. Degree of penetration of linezolid and cefamandole into bone, fat and muscle in 12 patients undergoing total hip replacement

	Linezolid percentage penetration			Cefamandole percentage penetration		
Parameter/time	median (mg/L)	mean (mg/L)	95% CI (mg/L)	median (mg/L)	mean (mg/L)	95% CI (mg/L)
Bone penetration (%)						
10 min	52.0	51.0	41.4–60.5	19.4	24.9	15.6–34.2
20 min	48.0	60.0	37.7–82.3	19.9	24.7	12.9–36.6
30 min	35.9	46.4	31.2–61.6	17.8	22.7	13.3–32.2
Fat penetration (%)						
10 min	21.4	26.5	15.8–37.1	12.6	14.5	9.4–19.7
20 min	29.4	36.9	24.8–49.0	14.5	19.4	12.3–26.6
30 min	27.8	31.4	23.5–39.2	13.3	16.2	12.6–19.8
Muscle penetration (%)						
10 min	56.5	58.3	44.9–71.6	21.3	19.5	15.1–24.0
20 min	73.6	94.3	66.2–122.4	27.7	31.3	18.9–43.7
30 min	74.3	93.0	64.5–121.5	27.5	31.3	21.9–40.7

the use of such a protocol avoids much of the variability seen in conventional two-group study designs, a central assumption is the lack of a pharmacodynamic interaction between the two agents being co-administered.[11,12] The decision to use a β-lactam was based on the apparent lack of interaction between the pharmacokinetics of β-lactams and linezolid, and from similarities in both PK/PD parameters (time above MIC) and the MICs of the two agents for susceptible pathogens.[7,13] However, the limitations of comparison between groups of agents as dissimilar as the β-lactams and the oxazolidinones must be recognized.

Following a 600 mg infusion in patients undergoing routine hip arthroplasty, linezolid penetrated rapidly into bone, fat and muscle at the operation site, reaching peak concentrations at between 10 and 20 min after the end of the infusion. For bone, these concentrations were *c.* 50% of the simultaneous blood levels, whereas for muscle and fat they were *c.* 90% and 30%, respectively. In all but one bone sample the concentration of linezolid exceeded the MIC for susceptible pathogens (≤4 mg/L);[1,3] concentrations in the other two samples from the same patient exceeded the MIC, but this sample also had a significantly lower cefamandole concentration than other samples. Although we are unable to offer an explanation for the apparently lower levels of both agents in this sample, it does highlight the heterogeneous nature and variable penetration of antimicrobials into bone, and further illustrates the complexity of such studies.[11,12]

With the soft tissue samples, fat concentrations of linezolid at the time of operation were similar to its MIC for susceptible pathogens, whereas muscle concentrations exceeded the MIC by a factor of three or four, suggesting good penetration into the tissues surrounding the bone. This is supported by the concentrations of linezolid found in the drainage from these tissues, which exceeded simultaneous blood levels for up to 12 h after dosing and were above the MIC for susceptible pathogens throughout the dosing interval.

The concentrations of cefamandole found in this study are in agreement with the findings of our earlier studies,[11,12] and we found broad similarities in the bone and soft tissue disposition of linezolid and cefamandole. Both agents exhibit rapid penetration into the bone and soft tissues, with concentrations similar to or exceeding their MICs for susceptible pathogens at the time of operation. Inhibitory levels of both agents were also maintained in the haematoma around the operation site for the entire dosing interval. However, in making such comparisons it must be recognized that the two agents differ in their mode of action and other aspects, and that similar pharmacokinetic disposition in bone may not necessarily predict similarities in efficacy. Although these data have been obtained for healthy, well perfused bone the values for linezolid are similar to the single reported level of 9.0 mg/L for necrotic bone from a patient with osteomyelitis,[14] suggesting that our findings may be relevant to treatment of infected tissue.

In conclusion, we find that linezolid has a similar distribution and penetration profile in bone and associated soft tissues to cefamandole, an agent that may be used in the treatment of infections in these tissues. This would indicate a potential role for linezolid in the management of such patients, in line with an encouraging clinical report,[14] but further clinical evaluation is needed.

Acknowledgements

This study was supported by a grant from Pharmacia Ltd, Milton Keynes, UK.

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