University Hospital of Heidelberg, Heidelberg, Germany

Bone tissue and plasma concentrations of linezolid and vancomycin in rabbits with prosthesis-related infection due to MRSA

S. Swoboda, L. Helbig, M. Kommerell, H. G. Simank, F. Kees, H. K. Geiss, T. Hoppe-Tichy, K. Schröder

Received January 14, 2009, accepted February 13, 2009

Dr. Stefanie Swoboda, University Hospital of Heidelberg, Pharmacy Department, Im Neuenheimer Feld 670, 69120 Heidelberg, Germany
stefanie.swoboda@med.uni-heidelberg.de

Pharmazie 64: 407–409 (2009) *doi: 10.1691/ph.2009.9510*

Background: Due to its safety profile and ease of oral administration, linezolid became an alternative to vancomycin in the treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections. The aim of our study was to compare bone tissue and plasma concentrations of linezolid and vancomycin in prosthesis-related MRSA infection in a rabbit model.
Material and methods: During implantation of titanium cylinders into the femurs of nine rabbits, a bacterial suspension of MRSA was added to induce infection. Antibiotic treatment was started eight hours later. Antibiotic concentrations in plasma (day one, three and seven) and bone (day seven) were determined by HPLC analysis.
Results: At steady state the mean peak and trough plasma levels of linezolid were 29.0 μg/mL and 8.2 μg/mL and for vancomycin 39.1 μg/mL and 28.2 μg/mL. On day seven the mean peak concentration of linezolid in plasma was 28.5 μg/mL and after six hours 26.3 μg/mL and for vancomycin 53.8 μg/mL and 29.1 μg/mL after six hours. Vancomycin showed a penetration into the infected bone (femur) of 53% of plasma concentration, into the uninfected 28%, linezolid 11% (for both six hours after administration).
Conclusion: In conclusion, we observed a higher rate of tissue penetration for vancomycin than for linezolid into femur bone in this animal model. As linezolid offers the option for oral treatment of gram-positive organisms, results of further studies comparing vancomycin and linezolid are keenly awaited.

1. Introduction

Osteomyelitis and prosthesis-related infections with methicillin-resistant *Staphylococcus aureus* (MRSA) continue to be major problems in orthopaedic surgery. Gram-positive organisms particularly Staphylococci and Streptococci are responsible for the majority of bone and joint infections. Treatment of these infections can be difficult, usually involving a prolonged course of antibiotics, often continued by additional surgical intervention (Darley and MacGowan 2004). MRSA infections involving either bone or joint prosthesis present a severe problem in anti-infective treatment. Antibiotics with activity against MRSA species are limited and until recently vancomycin was considered the drug of choice.
Administration of prolonged courses of intravenous antibiotic therapy is expensive and an oral antibiotic with anti-MRSA activity comparable to vancomycin was lacking. Antimicrobial resistance appears to be increasing all over Europe with a high prevalence of MRSA found in most European hospitals. Increasing antimicrobial resistance necessitates a critical appraisal of the remaining antibiotic treatment options (Goossens 2005). The emergence of *Staphylococcus aureus* strains resistant to vancomycin has been described (Rodriguez et al. 2005).
Linezolid is an important therapeutic option for the treatment of infections caused by multiresistant gram-positive bacteria. Linezolid is active against vancomycin-resistant Enterococci, MRSA and glycopeptide-intermediate *Staphylococcus aureus* (GISA) (Perry and Jarvis 2001). The bioavailability of linezolid is ~100% even after oral administration. Its plasma protein binding is approximately 31%, the volume of distribution is about 40–50 L. The drug is eliminated via renal and non-renal routes with a terminal plasma elimination half-life between 4.5 and 5.5 h (Diekema and Jones 2001).
For effective antimicrobial treatment it is extremely important to have thorough knowledge about drug concentrations at the site of action (Mueller et al. 1999). The purpose of this study was to investigate the pharmacokinetic properties of linezolid and vancomycin in plasma and bone of rabbits with prosthesis-related infections due to MRSA.

2. Investigations and results

2.1. Linezolid

To determine the peak and trough levels at steady state we measured the concentrations in plasma on day three at timepoints two and eleven hours after administration. The mean peak level of linezolid was 29.0 (SD 10.7) μg/mL and the trough level was 8.2 (SD 3.7) μg/mL. On day seven (last day of antibiotic therapy) the mean peak concentration at $t_{2\ hours}$ in plasma was 28.5 (SD 15.0) μg/mL and after $t_{6\ hours}$

Table: Bone (cancellous) concentrations of linezolid and vancomycin following administration of linezolid 300 mg p.o. bid or vancomycin 100 mg s.c. bid for seven days. Time from last dosage to sampling: six hours

	Linezolid (n = 4)			Vancomycin (n = 5)		
	Plasma (μg/mL)	Bone uninfected (μg/mL)	Bone infected (μg/mL)	Plasma (μg/mL)	Bone uninfected (μg/mL)	Bone infected (μg/mL)
	20.21	1.31	1.75	30.0	10.52	14.51
	38.21	5.71	5.18	23.6	7.08	10.84
	19.43	1.34	2.26	42.8	10.6	25.3
	27.49	2.36	3.33	24.0	5.29	9.44
				25.0	7.45	17.37
Mean	26.34	2.68	3.13	29.08	8.19	15.49
Standard deviation	8.71	2.08	1.52	8.09	2.31	6.3

the concentration was 26.3 (SD 8.7) μg/mL. On day seven (six hours after the last administration) linezolid plasma and bone concentrations showed a linear correlation with a regression coefficient of $r = 0.98$. Furthermore there was no correlation between rabbits' weight or renal function and the linezolid concentrations in plasma and bone.
Bone concentrations of linezolid were approximately 11% of the plasma concentration (12% right femur, 10% left femur). Bone concentrations of linezolid after seven days of treatment and six hours after the last administration are shown in the Table. Additionally, we determined the drug concentration from soft tissue adjacent the operation site from two animals. The concentrations were 71% and 101% of the plasma concentrations.

2.2. Vancomycin

After the first dose a mean peak plasma concentration of 24.2 (SD 5.6) μg/mL vancomycin were observed two hours after subcutaneous injection. Mean peak and trough concentrations at steady state (on day three) were 39.1 (SD 8.2) μg/mL and 28.2 (SD 17.5) μg/mL. On day seven of treatment maximum plasma levels were 53.8 (SD 17.2) μg/mL two hours after administration and after $t_{6\ hours}$ the concentration was 29.1 (SD 8.1) μg/mL. Vancomycin showed a penetration into the infected femur of 53% of plasma concentration and into the non-infected one of only 28% ($p \leq 0.05$). Bone concentrations of vancomycin after seven days of treatment and six hours after the last administration are shown in the Table. There was no correlation between rabbits' weight or renal function and the vancomycin concentrations in plasma and bone.

3. Discussion

Vancomycin has been approved for the therapy of osteomyelitis or prosthetic joint infections due to MRSA. There are limited data available on the comparison of bone tissue penetration of linezolid and vancomycin. In the present study the penetration of linezolid and vancomycin into bone was evaluated in an animal model.

3.1. Linezolid

Following the administration of linezolid 300 mg p.o. the plasma concentrations were comparable to previous published data in animals and humans (Rana et al. 2002). In contrast to human studies (Kutscha-Lissberg et al. 2003; Lovering et al. 2002; Rana et al. 2002) our data show relatively low bone concentrations of linezolid. We would like to emphasis on this finding. This discrepancy may be due to a discrepancy in animal models and human studies. Only limited data are available regarding the concentration of linezolid in animal bone tissue. Patel et al. (2000) published a rat model of chronic *Staphylococcus aureus* osteomyelitis to test antimicrobial activity against *Staphylococcus aureus*. In this animal model linezolid treatment was not different from no treatment of *Staphylococcus aureus* osteomyelitis.

3.2. Vancomycin

Our observation of significantly higher vancomycin concentrations in the infected compared to the uninfected bone is in accordance with other both human and animal studies (Beckmann et al. 2007; Graziani et al; 1988, Wilson and Mader 1984). Colwell et al. (1988) noted mean vancomycin concentrations of 5.5 μg/mL in samples of cancellous bone, 30 min following a 1 g vancomycin infusion administered over 60 min to 6 patients undergoing joint arthroplasty surgery. Two further human studies showed that vancomycin penetrated into sternal bone (Martin et al. 1994; Massias et al. 1992). Our findings are in accordance with literature data from both human and animal studies as mentioned above.
For coagulase-negative staphylococcal or MRSA infection, vancomycin is a logical choice based on its *in vitro* activity. To reduce the risk of relapse, the duration of treatment should generally exceed four (McDonald and Fitzgerald 1987) weeks to six weeks (Brause 1986).

3.3. Study limitations

A number of points of criticism is raised: (1) small number of animals examined (2) no power calculation (3) high dose of linezolid p.o. for rabbits compared to the human standard dose (we adapted the dose for rabbits of a rat model).

3.4. Conclusion

In conclusion, we observed a higher rate of tissue penetration for vancomycin than for linezolid into femur bone in this animal model. As linezolid offers the option for oral treatment of gram-positive organisms, results of further studies comparing vancomycin and linezolid are keenly awaited.

4. Experimental

4.1. Animals

Nine New Zealand white rabbits underwent titanium cylinder implantation with direct lateral approach to both distal femurs, mimicking prosthetic

surgery. The condyles were exposed and a central hole about 5 mm proximal to the articular cartilage was drilled using a diamond shaper with an outer diameter of 4 mm. The titanium cylinders (outer diameter 4.1 mm, length 5 mm, Aesculap Inc., Germany) press-fit implanted to the right femoral condyle into the cancellous bone were intra-operatively coated with 25 μL of the bacterial suspension containing 10^6 CFU (colony forming units) MRSA (ATTC 33591). The left leg was also provided with a titanium cylinder, however no infection was induced. Antibiotic treatment was started 8 h postoperatively. Five animals received vancomycin which was injected subcutaneously (s.c.) in the back of the neck 100 mg twice a day, four animals received linezolid 300 mg orally twice a day. For oral administration linezolid was prepared as a 2 mL suspension of the antibiotic and mixed with food in a syringe, which was placed in the mouth of the animal and fed. Blood samples were collected on the first day at 1, 2, 6 and 12 h, on day three at 2 and 11 h and on day seven at 1, 2 and 6 h after administration. Nine rabbits were sacrificed on day seven. Linezolid and vancomycin concentrations in the femur bone matrix (cancellous bone) of the left (uninfected) and right (infected) leg were analyzed 6 h after the last drug administration. Linezolid concentration from soft tissue adjacent the operation site from two animals was measured. All studies were conducted in accordance with standard animal experimentation guidelines. The study protocol was approved by the institutional animal welfare review board (Regierungspräsidium Karlsruhe, No 35–9185.81/G-99/03).

4.2. Analytics

Linezolid concentrations were determined in plasma and bone samples using a previously described HPLC method with UV absorbance detection (Swoboda et al. 2007). Vancomycin in plasma was analyzed in the routine lab using the fully automated fluorescence immunoassay (Centaur®, Bayer, Germany). Vancomycin out of bone was assayed adapting previously published methods (Backes et al. 1998; Luksa and Marusic 1995).

4.3. Statistics

The degree of drug penetration into bone tissue was assessed by calculating the penetration ratio (ratio of bone tissue $C_{6\ hours}$ to plasma $C_{6\ hours}$). Statistical analysis was carried out using JMP™ statistical software package (SAS Institute Inc., N.C., USA). Results are expressed as mean value ± standard deviation (SD). Student's t-test (unpaired, two-tailed) was used to determine any significant differences in concentration between infected and uninfected bone. P values of < 0.05 were considered to indicate statistical significance.

Acknowledgement: This study was supported by an unrestricted grant from Pharmacia, Pfizer Group, Germany.

Conflict of interest and funding: None to declare by the authors. Pfizer has not planned the experiment, has not took part in data collection, analyses, interpretation of data or writing of the manuscript.

References

Backes DW, Aboleneen HI, Simpson JA (1998) Quantitation of vancomycin and its crystalline degradation product (CDP-1) in human serum by high performance liquid chromatography. J Pharm Biomed Anal 16: 1281–1287.

Beckmann J, Kees F, Schaumburger J, Kalteis T, Lehn N, Grifka J, Lerch K (2007) Tissue concentrations of vancomycin and moxifloxacin in periprosthetic infection in rats. Acta Orthop 78: 766–773.

Brause BD (1986) Infections associated with prosthetic joints. Clin Rheum Dis 12: 523–536.

Colwell CW, Frey C, Morris B, Freedman SD (1988) Vancomycin distribution in patients undergoing total hip replacement. Atlanta, Georgia.

Darley ESR, MacGowan AP (2004) Antibiotic treatment of Gram-positive bone and joint infections. J Antimicrob Chemother 53: 928–935.

Diekema DJ, Jones RN (2001) Oxazolidinone antibiotics. Lancet 358: 1975–1982.

Goossens H (2005) European status of resistance in nosocomial infections. Chemotherapy 51: 177–181.

Graziani AL, Lawson LA, Gibson GA, Steinberg MA, MacGregor RR (1988) Vancomycin concentrations in infected and noninfected human bone. Antimicrob Agents Chemother 32: 1320–1322.

Kutscha-Lissberg F, Hebler U, Muhr G, Köller M (2003) Linezolid penetration into bone and joint tissues infected with methicillin-resistant Staphylococci. Antimicrob Agents Chemother 47: 3964–3966.

Lovering AM, Zhang J, Bannister GC, Lankester BJ, Brown JH, Narendra G, MacGowan AP (2002) Penetration of linezolid into bone, fat, muscle and haematoma of patients undergoing routine hip replacement. J Antimicrob Chemother 50: 73–77.

Luksa J, Marusic A (1995) Rapid high-performance liquid chromatographic determination of vancomycin in human plasma. J Chromatogr B Biomed Appl 667: 277–281.

Martin C, Alaya M, Mallet MN, Viviand X, Ennabli K, Said R, DeMicco P (1994) Penetration of vancomycin into mediastinal and cardiac tissues in humans. Antimicrob Agents Chemother 38: 396–399.

Massias L, Dubois C, de LP, Brodaty O, Fischler M, Farinotti R (1992) Penetration of vancomycin in uninfected sternal bone. Antimicrob Agents Chemother 36: 2539–2541.

McDonald DJ, Fitzgerald RH, Jr., Ilstrup DM (1989) Two-stage reconstruction of a total hip arthroplasty because of infection. J Bone Joint Surg Am 71: 828–834.

Müller E, Stass H, Brunner M, Möller JG, Lackner E, Eichler HG (1999) Penetration of moxifloxacin into peripheral compartments in humans. Antimicrob Agents Chemother 43: 2345–2349.

Patel R, Piper KE, Rouse MS, Steckelberg JM (2000) Linezolid therapy of Staphylococcus aureus experimental osteomyelitis. Antimicrob Agents Chemother 44: 3438–3440.

Perry CM, Jarvis B (2001) Linezolid: a review of its use in the management of serious gram-positive infections. Drugs 61: 525–551.

Rana B, Butcher I, Grigoris P, Murnaghan C, Seaton RA, Tobin CM (2002) Linezolid penetration into osteo-articular tissues. J Antimicrob Chemother 50: 747–750.

Rodriguez JC, Ethlvarez JM, Escribano I, Royo G (2005) In vitro activity of linezolid against Staphylococcus aureus: a population study. Chemotherapy 51: 86–88.

Swoboda S, Ober M, Anagnostakos K, Geiss HK, Weigand MA, Hoppe-Tichy T (2007) A simple isocratic HPLC assay to determine linezolid concentrations in different biomatrices for in vivo and in vitro studies. Clin Chem Lab Med 45: 1019–1022.

Wilson KJ, Mader JT (1984) Concentrations of vancomycin in bone and serum of normal rabbits and those with osteomyelitis. Antimicrob Agents Chemother 25: 140–141.