© Med Sci Monit, 2012; 18(11): SC5-8
PMID: 23111752

Received: 2011.08.12
Accepted: 2012.02.23
Published: 2012.11.01

Vancomycin versus linezolid in the treatment of methicillin-resistant *Staphylococcus aureus* meningitis in an experimental rabbit model

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Source of support: Ege University

Summary

Background: The aim of this study was to compare the antibacterial efficacy of vancomycin and linezolid in a rabbit model of methicillin-resistant *Staphylococcus aureus* (MRSA) meningitis.

Material/Methods: Meningitis was induced by intracisternal inoculation of ATCC 43300 strain. After 16 h incubation time and development of meningitis, the vancomycin group received vancomycin 20 mg/kg every 12 h. The linezolid-10 and linezolid-20 groups received linezolid in 10 and 20 mg/kg dosages every 12 h, respectively. The control group did not receive any antibiotics. Cerebrospinal fluid bacterial counts were measured at the end of 16-h incubation time and at the end of 24-h treatment.

Results: Bacterial counts were similar in all groups at 16 h. At the end of treatment the decrease in bacterial counts in the vancomycin group was approximately 2 logs higher than the linezolid-20 group ($p>0.05$) and approximately 4 logs higher than in the linezolid-10 group (p: 0.037) (Vancomycin group: –2.860±4.495 versus Linezolid-20: –0.724±4.360, versus Linezolid-10: 1.39±3.37). Full or partial bacteriological response was higher in vancomycin versus linezolid-10 (p: 0.01), but not vancomycin versus linezolid-20 or linezolid-10 versus-linezolid-20 groups.

Conclusions: Our results suggest that linezolid is not statistically inferior to vancomycin in the treatment of MRSA meningitis in an experimental rabbit model in 20 mg/kg q12 h dosage; however, it is inferior in 10 mg/kg q12 h dosage. Additional data should gathered to confirm these findings in advance of clinical trials to assess efficacy in humans.

key words: **methicillin-resistant *Staphylococcus aureus* • meningitis • linezolid • rabbits • MRSA**

Full-text PDF: http://www.medscimonit.com/fulltxt.php?ICID=883528
Word count: 2002
Tables: 1
Figures: –
References: 19

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Background

Staphylococcus aureus may cause community-acquired and nosocomial bacterial meningitis and is associated with significant mortality. It is usually associated with neurosurgical interventions (including CSF shunts), staphylococcal bacteriemia or a parameningeal focus. Methicillin-resistant *S. aureus* (MRSA) is an important cause of hospital-acquired central nervous system infections [1–7].

Over the last decade, MRSA strains have become endemic in many hospitals worldwide. In addition, it is now an incipient community pathogen in many geographical regions. MRSA is important because most strains are also resistant to other antibiotics [2]. Since the available treatment options are limited, treatment of infections, particularly meningitis, is problematic [3]. The main treatment option is vancomycin. However, glycopeptides achieve relatively low cerebrospinal fluid (CSF) concentrations and treatment failure is not rare. There are a few case reports in which teicoplanin, fucidic acid and daptomycin have been used successfully [1,4,7]. To date, experience with linezolid for the treatment of meningitis is anecdotal and limited to case reports or series [2,3,6]. The aim of this study was to compare the efficacy of vancomycin and linezolid in a MRSA rabbit meningitis model.

Material and Methods

Bacterial strain

S. aureus ATCC 43300 (vancomycin MIC: 1 mg/L, linezolid MIC: 0.025 mg/L measured in duplicate using the Etest; AB BIODISK, Solna, Sweden) was used as the infecting bacteria.

Bacterial solution was prepared in 0.9% NaCl by adjusting to 0.5 McFarland standard. This solution was further diluted 1/300 to achieve a concentration of 10^6 colony-forming units (CFU)/ml [8].

Antimicrobial agents

Drugs used were vancomycin (Lilly, Indianapolis, USA) and linezolid (Pfizer Pharmaceuticals Group, New York, USA).

Rabbit meningitis model

New Zealand white rabbits weighing 2–2.5 kg were anaesthetized by intramuscular injections of ketamine (35 mg/kg) and xylazine (5 mg/kg) before each intraventricular intervention including induction of meningitis and CSF sampling [9–11]. The duration of anaesthesia was 10–15 min. Then 0.5 ml of the bacterial solution of MRSA was injected directly into the cisterna magna of each rabbit using a 22 G spinal needle (Hayat Ticaret, Istanbul, Turkey).

Animals were not anaesthetized after the primary inoculation and between the CSF sampling procedures. In addition, they were kept in their cages except for intraventricular interventions.

Sixteen hours after the inoculation, meningitis criteria were investigated. CSF white cell count more than 1000/mm^3 (counted by Thoma slide) and a bacterial count greater than 10^2 cfu/mL were accepted as the indications of meningitis [9,10]. Then rabbits were separated into 4 groups. The linezolid-10 group received 10 mg/kg linezolid every 12 h (q12h) similar with normal human dosage (at 16 h and 28 h after the induction of meningitis) [12]. The linezolid-20 group received 20 mg/kg linezolid every 12 h (q12h) (at 16 h and 28 h after the induction of meningitis) [11]. The vancomycin group received 20 mg/kg vancomycin every 12 h (q12h) (at 16 h and 28 h after the induction of meningitis) [9,10,13]. The control group did not receive any antibiotics. Vancomycin and linezolid were administered through a peripheral ear vein. At the end of the study period (24 h after the end of incubation period or start of treatment, 40 h after bacterial inoculation), animals were humanely killed by intravenous infusion of high dose Nembutal.

Measurement of bacterial concentrations

Bacterial concentrations in CSF were measured at the end of the 16th h (end of incubation period and before the first dosage of vancomycin or linezolid) and the 40th h of the study (end of treatment) by plating undiluted and serial 10-fold and 100-fold dilutions of CSF (10 µL) on 5% sheep blood agar and incubated at 37°C for 24 h. Bacterial response was evaluated in 3 categories – full response, sterilization of CSF; partial response, any decrease in bacterial count; and bacteriological failure, an increased bacterial count [10].

Statistical analysis

Data were evaluated by SPSS 11.0 package program using Mann-Whitney U test, Kruskal Wallis test and Fisher's χ^2 test. A p-value less than 0.05 was considered significant [10].

Ethics

The study was approved by the local ethics committee on animal studies (Approval no: 2005-23).

Results

At the beginning of the study, 60 rabbits were inoculated with bacteria, of which 47 were alive and had developed meningitis at the end of 16 h incubation time. These animals were separated into 4 groups. Mean bacterial concentrations of these 4 groups were similar as log10 CFU/ML at 16 h (Table 1, p>0.05). Although at 40 h the differences in mean CSF bacterial counts were significant between vancomycin and control groups (p=0.037) and between linezolid 10 or 20 and control groups (p=0.01), the mean CFU/ML in both vancomycin and linezolid-10 or -20 groups was similar (p=0.71 and 0.54) (Table 1).

At the end of treatment the decrease in bacterial counts in the vancomycin group was approximately 2 logs higher than the linezolid-20 group (p>0.05) and approximately 4 logs higher than in the linezolid-10 group (p: 0.037) (vancomycin group: –2.860±4.495 *vs.* linezolid-20: –0.724±4.360, *vs.* linezolid-10: 1.39±3.37).

During the study mortality was relatively higher in the linezolid-20 group (2/11 in vancomycin, 6/11 in linezolid-20, 3/14 in linezolid-10 and 5/11 in control group, p>0.05),

Table 1. Results of bacterial counts.

Group	Bacterial count (log^{10} CFU/ML) (Number of rabbits)	
	16 h	40 h
Vancomycin	4.33±1.42 (11)	3.09±2.05 (9)
Linezolid-10	3.56±1.04 (14)	3.59±0.79 (11)
Linezolid-20	3.43±0.91 (11)	2.22±2.08 (5)
Control	3.85±0.26 (11)	5.14±0.99 (6)

but this difference was not statistically significant. At the end of the study, rates of full (2/9 in vancomycin group, 0/11 in linezolid-10 and 2/5 in linezolid-20 group, p>0.05) and partial success rates were similar (5/9 in the vancomycin group, 1/5 in the linezolid-20 and 3/14 in the linezolid-10 group, p> 0.05). Full or partial bacteriological response was higher in vancomycin *vs.* linezolid-10 (p: 0.01) but not vancomycin *vs.* linezolid-20 or linezolid-10 *vs.* linezolid-20 groups (7/9 in vancomycin group, 3/5 in linezolid-20 and 3/11 in linezolid-10 group).

Discussion

Despite developments in intensive care facilities and antibiotic agents, meningitis is still associated with significant mortality and morbidity. *S. aureus* is an important pathogen, causing both community-acquired and hospital-acquired meningitis. The main option for the treatment of MRSA meningitis is vancomycin, but teicoplanin, linezolid, fucidic acid and daptomycin may be used as salvage therapy [1,2,4,6,7]. This decision is based primarily on published series, but not controlled human studies. Despite available treatment modalities, prognosis of MRSA meningitis is still not favorable [1,2,6].

Recent guidelines for treatment of meningitis from the European Federation of Neurological Sciences suggest linezolid as the main therapy option for methicillin-resistant staphylococcal meningitis [14]. Recently, Sipahi et al. reported microbiological eradication of MRSA in 7 of 8 MRSA meningitis cases. However, to our knowledge, there is no human or animal study comparing vancomycin and linezolid in the treatment of MRSA meningitis. This study was performed to compare antibacterial activity of vancomycin (20 mg/kg) and linezolid (10 or 20 mg/kg) against MRSA in a rabbit meningitis model. The vancomycin dosage of the study was adopted from previous meningitis studies in rabbit models [9,10,13]. There was only 1 meningitis study related to linezolid in a rabbit model using 20 mg/kg [11]. Hence, we used 2 different dosages of linezolid – 10 mg/kg q 12 h (the usual dosage in humans) [12] and 20 mg/kg q 12 h.

Our findings showed that linezolid was not statistically inferior to vancomycin in the treatment of MRSA meningitis at 20 mg/kg dosage, but was inferior at 10 mg/kg. However, the rate of full bacteriological response was very low in each treatment group. The low full bacteriological success with vancomycin is probably due to the low CSF penetration of the drug and is in concordance with previous published data. Sipahi et al [10] compared vancomycin (20 mg/kg-same dosage used in this study) and teicoplanin (6 mg/kg) in a MRSA (the same strain used in this study) rabbit meningitis model. Although they found teicoplanin as effective as vancomycin, the rates of full (2 in 11 rabbits) or partial (2 in 11 rabbits) bacteriological response rates were very low, in concordance with the present study. They analyzed trough antibiotic levels and reported that only 4 rabbits in the teicoplanin group and 2 rabbits in the vancomycin group had antibiotic levels higher than the lowest drug detection limit of the bioassay (>1 mg/L). Lee et al. [13] also analyzed the vancomycin levels in the rabbit CSF after the same vancomycin dosage used in our study. They reported that peak and trough vancomycin levels were 0.4±0.1 and 0.3±0.1 mg/l, respectively, while peak serum concentration was 36.8±8.0 mg/l and trough serum concentration was 4.2±1.0 mg/l. Both studies confirm that penetration of vancomycin in the CSF is very poor.

In the present study, at the end of treatment bacterial counts were significantly lower linezolid group in comparison to untreated controls. However, although not statistically significant, the mean decrease in bacterial counts in the vancomycin group was approximately 2 log CFU/ml more than in the linezolid-20 group and approximately 4 log CFU/ml more than linezolid-10 group (p<0.05). In the linezolid-10 group there was partial bacterial response in only 3 of 14 rabbits at 24 h. Our data are in concordance with the study of Cottagnoud et al. [11] in which they compared linezolid with ceftriaxone in penicillin-susceptible *Streptococcus pneumoniae* and with ceftriaxone+vancomycin in penicillin-resistant *S. pneumoniae* meningitis of rabbits, and reported that linezolid had lower anti-bacterial activity (more than 2 log 10 CFU difference at 8 h) than both comparators. The relatively low full bacteriological response rate with linezolid in both studies is probably due to the fact that it is mainly a bacteriostatic agent [15–17]. Although mortality was somewhat higher in the linezolid-20 group, the difference was not statistically significant.

Linezolid shows good penetration into the CNS, achieving levels in CSF 30% to 70% of those present of serum in humans [15,17]. There are not sufficient data in rabbits. The only study that analyzed penetration of linezolid into rabbit CSF was by Cottognaud et al, who reported that linezolid showed 38±4% CSF penetration with a dosage 20 mg/kg via high performance liquid chromatography technique. Linezolid CSF levels peaked at 9.5 mg/L and troughed to 1.8 mg/L [11].

In the present study, a total of 13 rabbits (21.6% of total) died during the incubation period. This rate is somewhat higher than the 13.3% mortality in a previous study performed with the same bacteria and inoculum [10], possibly due to inadequate surgical intervention. In previous studies, linezolid levels were determined in body fluids by high performance liquid chromatography technique [11,15,17]. Main limitation of our study is the lack of pharmacokinetic data, which were not analyzed since the main aim was to compare antibacterial efficacy of the drugs, the presence already existing data related to the distribution of vancomycin and linezolid in rabbits (which are summarized above), and economic reasons. Another limitation is the duration

of the treatment. Since antibacterial efficacy of glycopeptides are rather time dependent and linezolid's efficacy is slow, it would have been of major interest to have examined antibiotic efficacy over a longer period of time and to have tested different dosages, but this was not possible because of economic reasons. In addition, previous studies that analyzed antibacterial efficacy in a rabbit meningitis model lasted only 24 h or less [9–11]. We did not evaluate efficacy of vancomycin+linezolid combination, but previous reports of this combination was reported as indifferent or slightly antagonistic [18,19]. Finally, the fact that animal experiments, in particular with rabbits, do not permit large cohorts, limits the power of statistical analysis.

Conclusions

According to the US Food and Drug Administration, linezolid is not yet indicated for the treatment of meningitis [16]. Experience with linezolid is limited to single case reports or small series for the treatment of meningitis caused by gram-positive pathogens [2,3,5–7]. Our study is the first comparing vancomycin and linezolid in meningitis. Our results suggest that linezolid is not statistically inferior to vancomycin in the treatment of MRSA meningitis in an experimental rabbit model in 20 mg/kg q12 h dosage, but it is inferior in 10 mg/kg q12 h dosage. Additional data should be gathered to confirm these findings in advance of clinical trials to assess efficacy in humans. Our data also suggest that neither vancomycin nor linezolid are optimum in meningitis treatment. Hence, the medical community should continue seeking better alternatives.

Transparency declaration

None to declare.

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