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Original article

Experimental study of the efficacy of linezolid alone and in combinations against experimental meningitis due to *Staphylococcus aureus* strains with decreased susceptibility to beta-lactams and glycopeptides

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ABSTRACT

Background: To evaluate in vitro and *in vivo* efficacies of linezolid, vancomycin, and the combination of linezolid and rifampicin against two *Staphylococcus aureus* strains with reduced susceptibility to beta-lactams and one of them also to glycopeptides.
Methods: In vitro killing curves and a rabbit model: Meningitis was induced by intracisternal inoculation of 10^8 CFU/ml of each strain. Five hours later (0 h), rabbits were randomly assigned to control or to therapeutic groups. CSF bacterial counts, lactate and protein concentrations, and pharmacokinetic parameters were determined.
Results: In vivo: linezolid and its combination with rifampicin reduced bacterial concentrations at 24 h, median cfu/mL 4.85 vs 3.87 ($p < 0.05$) for linezolid and 5.02 vs 4.21 ($p < 0.05$) for linezolid + rifampicin, against the glycopeptide intermediate *S. aureus* (GISA) strain and improved inflammatory parameters.
Conclusions: Despite the need for more experimental data, our results suggest that linezolid and its combinations could be considered as a potential alternative in difficult-to-treat CNS infections and especially in those due to GISA strains and deserve more studies.

1. Introduction

Infection caused by methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the major challenges in modern health-care systems, not only because of its ability to spread within and between hospitals and health-care systems [1–4] but because these infections cause high mortality and the effectiveness of alternative treatment regimens is limited [5,6]. Moreover, infections caused by MRSA may result in prolonged hospital stay and thus increase health-care costs [7,8]. Glycopeptides were traditionally considered the treatment of choice against these strains. However, since the first strain of *S. aureus* with reduced susceptibility to vancomycin and teicoplanin was reported in Japan in 1997 [9], there have been several reports of additional cases worldwide [10,11]. Moreover, increases in vancomycin MIC have been related to treatment failures in several infections [12,13].

For all these reasons, new effective therapies must be sought for these difficult-to-treat infections. The problem is even greater in CNS infections, where antibiotic levels are usually low due to the blood brain barrier. Strategies to find solutions to these problems include research on new drugs and also on drug combinations including new and old drugs. At present, daptomycin and linezolid appear to be promising options. Linezolid is an oxazolidinone with a single mechanism of action, and its effectiveness against MRSA or GISA has been clearly demonstrated. In patients with MRSA infections, the drug has shown comparable efficacy to vancomycin [14,15]. As linezolid is bacteriostatic but not bactericidal, it was not initially considered as a CNS infection therapy. However, reports of its good penetration in the CSF have prompted study of its usefulness in these infections [16,17]. In addition, in view of the special difficulties involved in CNS infections and the reports of the

Abreviations: CNS, central nervous system; MRSA, methicillin resistant *Staphylococcus aureus*; GISA, glycopeptide intermediate *S. aureus*; CSF, cerebrospinal fluid.

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development of resistance during linezolid therapy, the use of a combined strategy may be a good approach [18,19], even though few studies to date have considered linezolid as part of a combined regimen against non-susceptible *S. aureus*. In one study, linezolid plus β-lactams exhibited bactericidal and synergistic activity against MRSA and hGISA strains in experimental models of endocarditis and meningitis [20]. Additionally, linezolid plus rifampicin was shown to be an effective prophylactic regimen for preventing staphylococcal prosthetic vascular graft infection, although the combination did not significantly change bacterial counts compared to linezolid monotherapy [21]. Rifampicin has excellent anti-staphylococcal activity but cannot be used as monotherapy because of the strong possibility of resistance. Overall, clinical experience with linezolid as CNS infection therapy is limited, but case series of nosocomial infection and animal model reports show good activity against difficult-to-treat MRSA strains.

We created a meningitis rabbit model using two strains of *S. aureus* with reduced susceptibility to beta-lactams and glycopeptides to evaluate the effect of linezolid alone and in combination with vancomycin and rifampicin in treating CNS infection.

2. Materials and methods

2.1. Bacterial strains

Two clinical isolates of *S. aureus* with different degrees of resistance to glycopeptides were used: the MRSA COL [22] strain and a GISA strain (Mu50, ATCC 700699) reported as the first GISA strain by Hiramatsu et al. [9]. MICs of both strains were determined using the E-test and the macrodilution method and MBCs using macrodilution method following the CLSI guidelines. MICs (mg/L) for the MRSA COL strain were vancomycin, 1; linezolid, 1; rifampicin, 0.018; cloxacillin, 512; for the GISA strain they were vancomycin, 8; linezolid 1; rifampicin, 512 and cloxacillin, 128. The breakpoints for susceptibility and resistance were those defined by the Clinical and Laboratory Standards Institute.

2.2. In vitro studies

Time–kill curve assays were derived using glass tubes containing a final volume of 10 mL of cation-adjusted Mueller–Hinton broth and a final inoculum of 5×10^5 to 1×10^6 cfu/mL. Antibiotic concentrations ranging from 1/2 to 2 × MIC of vancomycin and rifampicin alone and from 1/2 to 4 × MIC of linezolid alone were studied, as were all the possible combinations of these antimicrobials. Samples were removed at 0, 6 and 24 h of incubation. The detection limit was 10 cfu/mL. Bactericidal effect was defined as a decrease in the initial inoculum of ≥3 log cfu/mL. Synergy of a combination was defined as a >2 log cfu/mL reduction compared with the most active agent alone, with one of the drugs at subinhibitory concentration.

2.3. Meningitis model

The experimental protocol obtained permission from the Ethics Committee for Animal Experiments at the University of Barcelona. The rabbit model of meningitis originally described by Dacey and Sande [23] was modified slightly. Young female New Zealand white rabbits weighing 2–2.5 kg were anaesthetized intramuscularly (im) with 35 mg/kg of ketamine (Ketolar; Parke-Davs, Prat de Ll., Spain) and 5 mg/kg of xylazine (Rompum; Bayer AG, Leverkusen, Germany). Animals were placed in the stereotaxic frame and a cerebrospinal fluid (CSF) sample was taken before bacterial inoculation to confirm its sterility. The organisms were suspended in sterile saline and diluted to 1×10^8 cfu/ml. The suspension was immediately injected into the *cisterna magna*. Once the rabbits were infected, therapy was started 5 h post-inoculation. A blood sample was collected to assess secondary bacteraemia. Animals were placed in the stereotactic frame and a baseline CSF sample was taken (0 h). Six rabbits were randomly included in one of the following therapeutic groups: Control group (saline), linezolid (20 mg/kg/4h/iv), vancomycin (25 mg/kg/4h/iv), linezolid plus rifampicin for both strains and rifampicin (15 mg/kg/24h/iv) and vancomycin plus rifampicin also against the MRSA strain. No animals were treated with rifampicin alone against the GISA strain, due to the strain's high level of resistance to this antimicrobial drug. CSF samples were taken at 0, 4, 6 and 24 h of therapy. Hydration was ensured throughout the experiment. Mortality was assessed at 24 h. Surviving animals were sacrificed using a lethal dose of thiopental sodium at the end of each experiment.

2.4. Sample processing

CSF samples were used to determine bacterial counts, lactate and protein concentrations, and antibiotic levels at peak and trough time points. Bacterial counts were determined by plating 10-fold dilutions and an undiluted 0.1 mL CSF sample on blood agar plates. The lowest bacterial concentration detectable was 10 cfu/mL. For purposes of analysis, a value of 0.99 log cfu/mL was assigned to the first sterile culture, and a value of 0 log cfu/mL was assigned to the subsequent ones. Therapeutic failure was defined as an increase in bacterial concentration of at least 1 log cfu/mL compared with a previous count. A therapy was considered bactericidal when a reduction of 3 log cfu/mL was achieved. Samples were centrifuged at 3000 rpm for 7 min, and the supernatants were stored at −70 °C. Lactate concentrations were measured using a Lactate PAP kit (Biomérieux S.A., Marcy l'Etoile, France). Protein concentrations were determined using the Bradford method (Bio-Rad Protein Assay, Bio-Rad Laboratories, Munich, Germany).

2.5. Pharmacokinetics

Pharmacokinetic data were compiled from a study of eight infected animals after a single iv dose of 20 mg/kg of linezolid and 25 mg/kg of vancomycin. Blood and CSF samples were taken at different time points, depending on the therapy. A computer-assisted method (PK functions for Microsoft Excel; J. I. Usansky, A. Desai and D. Tang-Liu, Department of Pharmacokinetics and Drug Metabolism, Allergan, Irvine, CA 92606, USA) was used to determine the following parameters in serum and CSF: maximum concentration (C_{max}), area under the concentration–time curve over 24 h (AUC_{0-24}) and CSF penetration as the comparison of areas under the curve ($AUC_{CSF\ 24}/AUC_{serum\ 24}$). Dosification of linezolid, 20 mg/kg/4 h was decided based on these data.

2.6. Antibiotic assays

Antibiotic assays were performed in triplicate. The blood and serum concentrations of vancomycin levels were determined using fluorescent polarization immunoassay (FPIA) using a TDX analyzer (ABBOTT CIENTÍFICA, S.A., Diagnostics Division, Costa Brava 13, 28,034 Madrid, Spain) with a detection limit of 2.0 μg/ml. Blood and serum linezolid concentrations were measured by the agar disc diffusion method, using *Bacillus subtilis* ATCC 12432 as assay organism.

2.7. Statistical analysis

The variables analysed were: Changes in bacterial CSF concentration (log cfu/mL), WBC count in CSF (cells/μL), and lactate and