In vitro and *in vivo* activities of linezolid alone and combined with vancomycin and imipenem against *Staphylococcus aureus* with reduced susceptibility to glycopeptides

Sandra Ribes[a,*], Maria Eugenia Pachón-Ibáñez[a,*], Maria Ángeles Domínguez[b], Raúl Fernández[b], Fe Tubau[b], Javier Ariza[a], Francesc Gudiol[a], and Carmen Cabellos[a].

***** Both authors equally contributed to this work.

[a] Laboratory of Experimental Infection, Infectious Diseases Department, IDIBELL-Hospital Universitari de Bellvitge, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain.

[b] Microbiology Department, IDIBELL-Hospital Universitari de Bellvitge, Feixa Llarga s/n, 08907 L'Hospitalet de Llobregat, Barcelona, Spain.

Corresponding author:

Dr. Sandra Ribes

Mailing address: Department of Neurology, University of Göttingen, Robert-Koch-Str. 40, D-37075 Göttingen, Germany.

Phone: +49 551 396689; fax: +49 551 399337

E-mail: sribes@med.uni-goettingen.de

ABSTRACT

Objective: To evaluate the *in vitro* and *in vivo* efficacies of linezolid (35 mg/kg/5 h), vancomycin (60 mg/kg/5 h), imipenem (30 mg/kg/5 h), linezolid+imipenem, linezolid+vancomycin, and vancomycin+imipenem against two clinical *S. aureus* isolates with reduced susceptibility to glycopeptides using time kill curves and the murine peritonitis model.

Material and Methods: Time kill curves were performed over 24 hours. Murine peritonitis model: Peritonitis was induced by intraperitoneal inoculation of 10^8 CFU/ml of each bacterial strain. Four hours later (0 h), mice were randomly assigned to a control group or to therapeutic groups receiving subcutaneous treatment for 25 h. Bacterial counts in peritoneal fluid, bacteraemia and mortality rates were determined.

Results: Time-kill curves: Addition of linezolid to imipenem yielded synergistic results after 24 h. Addition of linezolid decreased vancomycin activity. In the animal model, vancomycin and linezolid monotherapies produced comparable bacterial decreases in mice infected with each strain but linezolid achieved higher rates of blood sterilization. Linezolid tested either in monotherapy or in combination showed similar efficacy against both strains in terms of bacterial killing, number of negative blood cultures and survival. Linezolid and vancomycin were moderately bactericidal and similar in efficacy against glycopeptide intermediate or resistant *S. aureus*.

Conclusions: Linezolid combinations, as effective as linezolid tested alone, could be considered as alternative options for the treatment of GISA infections.

Keywords: GISA; heteroresistance; linezolid; peritonitis; experimental infections

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most important cause of antibiotic-resistant healthcare-associated infections [1]. In addition, clinical isolates of *S. aureus* with heterogeneous resistance to vancomycin (hGISA) and, more rarely, glycopeptide-intermediate resistant strains (GISA) have emerged worldwide over the past years [2-4].

Reduced vancomycin and teicoplanin activities against hGISA and GISA isolates have been reported in experimental studies [5-7], while in the clinical setting vancomycin has appeared to be sub-optimal in deep-seated and difficult-to-treat infections caused by these strains [7, 8]. Furthermore, the antagonistic effect or false synergy showed by *in vitro* studies with the combination of glycopeptides and *β*-lactams refuses its use as a potentially promising alternative to glycopeptide monotherapy [9,10]. The oxazolidinone linezolid, one of the new treatment options for multidrug-resistant Gram-positive bacteria [11-13], shows high *in vitro* activity against resistant staphylococcal strains [13, 14]. In patients with MRSA infections, linezolid has shown comparable efficacy to vancomycin [15, 16]. Moreover, it shows excellent oral bioavailability and does not require dose adjustment for renal insufficiency [14]. Its unique mechanism of action by inhibiting ribosomal protein synthesis at an early stage of bacterial replication leads to the absence of cross resistance with other antimicrobials [15]. Although linezolid-nonsusceptible strains are unusual [17], long courses of oxazolidinone therapy could select resistant mutants [18], hence the use of a combined strategy might be considered in clinical practice. To date, the efficacy of linezolid as part of a combination has been studied against MRSA strains but very few data has been reported against hGISA or GISA strains [19, 20].

Linezolid plus *β*-lactams exhibited bactericidal and synergistic activity against MRSA and hGISA strains in experimental models of endocarditis and meningitis [19]. Linezolid plus rifampicin was an effective prophylactic regimen for preventing staphylococcal prosthetic vascular graft infection, although the combination did not show higher efficacy than linezolid monotherapy [20].

We aimed to evaluate and compare the efficacies of linezolid alone and in combination with either vancomycin or imipenem against two *S. aureus* strains with reduced susceptibility to glycopeptides.

MATERIALS AND METHODS

Bacterial strains

Two clinical isolates of *S. aureus* with different degrees of resistance to glycopeptides were included: a hGISA strain isolated in our hospital and belonging to the Iberian clone, growing on 4 mg/l vancomycin Mueller–Hinton plates with a sub-population frequency of 3.6×10^{-6} CFU/ml (this strain was equivalent to the Mu3 heteroresistant strain) [8]; and a GISA strain (Mu50, ATCC 700699) reported as the first GISA strain [3]. MICs (mg/l), determined using the Etest and the macrodilution method [21], were for the hGISA strain: cloxacillin, 1024; cefotaxime, 1024; teicoplanin 8; vancomycin (VAN), 2; linezolid (LZD), 1; and imipenem (IMP), 32. MICs for the GISA strain were: cloxacillin, 1024; cefotaxime, 2048; teicoplanin, 16; VAN, 8; LZD, 2; and IMP, 64.

In vitro time-kill studies

The bactericidal activities of the drugs were tested in glass tubes containing Mueller–Hinton broth and a final inoculum of 1×10^5 to 1×10^6 CFU/ml [21].

Linezolid (Pfizer, Madrid, Spain), imipenem-cilastatin (Merck, Sharp & Dohme, Madrid, Spain) and vancomycin (Normon, Madrid, Spain) were provided by the manufacturers. Antibiotics were tested for a range of concentrations according to their MICs and their achievable levels in human serum: linezolid concentrations ranging from 1/4 x to 8 x MIC, vancomycin concentrations ranging from 1/4 x to 1 x MIC, imipenem levels from 1/8 x to 1 x MIC, as well as concentrations of 1/4 x, 1/2 x and 1x MIC of each drug in combination. In all experiments, growth control was assessed using an extra tube without antibiotic. At 0, 6 and 24 h of incubation, aliquots of 100 µl were taken from each tube to perform direct and 10-fold dilutions, and were cultured onto 5% sheep blood agar plates (SBA) at 37ºC for 24 h. Experiments were performed in duplicate**.** The following effects were studied in combinations after 24 h of incubation: **a** bactericidal effect was defined as a decrease in the initial inoculum of ≥ 3 $\log_{10}$CFU/ml. Synergy of a combination was defined as a > 2 $\log_{10}$CFU/ml reduction over the most active agent alone, with one of the drugs at subinhibitory concentration. An indifferent effect was defined as < 1 log (increase or decrease) in killing.

Pharmacokinetics

Pharmacokinetic studies were performed to select dose regimens that result in serum concentrations similar to those found in humans [6, 22]. Groups of 21 healthy mice were used for each pharmacokinetic study. A single weight-adjusted antibiotic dose was administered subcutaneously (sc) to each animal. At different time points, sets of three animals were anaesthetised intraperitoneally (ip), and blood samples (0.5 ml) were obtained by an intracardiac puncture. Blood was centrifuged and serum stored at -80°C.

Pharmacokinetic and pharmacodynamic parameters were obtained by a computer-assisted method (PK Functions for Microsoft Excel. Usansky, Desai and Tang-Liu, Pharmacokinetics and Drug Metabolism Dept, Allergan, Irvine, CA 92606) after determination of antibiotic concentrations over the time. Based on the obtained parameters the final selected doses were: vancomycin 60 mg/kg every 5 h (300 mg/kg/day), linezolid 35 mg/kg every 5 h (175 mg/kg/day) and imipenem 30 mg/kg every 5 h (150 mg/kg/day).

Mouse peritonitis model

This mouse peritonitis model has been previously characterized in our laboratory [6,23]. Inbred, female C57BL/6 mice (6 weeks; 14–16 g) were used (Harlan Int. Ibérica, S.A., Barcelona, Spain). Inoculation was performed via a 26-gauge syringe by ip injection of 0.5 ml of the inoculum consisting of a 5 x 10^8 CFU/ml staphylococcal suspension with 5% (w/v) mucin in sterile saline. A group of control mice (n ≥ 18) were killed 4 h after inoculation (hour 0) and antibiotic sc therapy was initiated. The rest of the mice were randomized to the control group receiving saline (n ≥ 25) or to one of the following therapeutic schedules (n ≥ 10 per therapy): Linezolid, vancomycin, imipenem, linezolid+vancomycin, linezolid+imipenem and vancomycin+imipenem receiving sc treatment over 25 h. At 25 **h** of therapy (5 **h** after the last antibiotic dose), mice were anaesthetised ip with ketamine/xylazine and peritoneal washes were performed by injecting 2 ml of sterile saline ip followed by a massage of the abdomen. Immediately, 0.1 ml of blood was withdrawn by cardiac puncture and animals were then sacrificed by cervical dislocation. Next, the abdomen was opened and 0.2 ml of peritoneal fluid (PF) was recovered from peritoneum. Undiluted and ten-fold diluted PF samples (0.1 ml) were plated on SBA plates to

perform bacterial determinations. Mortality was recorded after 25 h of therapy. Blood samples were grown in TSB at 37ºC for 24 h and then 0.1 ml of broth was cultured on SBA plates to assess *S. aureus* bacteraemia.

Antibiotic assays

Vancomycin serum concentrations were determined by fluorescent polarization immunoassay using a TDx analyzer (ABBOTT CIENTIFICA, S.A., Diagnostics Division, Costa Brava 13, 28034 Madrid, Spain) with a detection limit of 2.0 µg/ml. Serum concentrations of linezolid and imipenem were measured using the agar disc diffusion method and *Bacillus subtilis* ATCC 12432 and *Escherichia coli* ATCC 25922, respectively, as assay organisms**.** Standard curves were constructed using mouse plasma. Assay validation indicated linearity (r^2 value) of 0.9709 for imipenem and 0.9887 for linezolid. The detection limit was 0.5 µg/ml and 2 µg/ml for imipenem and lnezolid, respectively.

Statistics

Statistical analysis was performed with SPSS 12.0. ANOVA with Dunnett's post-hoc tests was used to analyse multiple bacterial count comparisons between therapeutic and control groups. Two-tailed Fisher's exact test was used for analyse survival and bacteraemia data. A *P* value of < 0.05 was considered statistically significant.

RESULTS

***In vitro* time–kill studies**

Linezolid achieved a bacterial decrease up to 2 log CFU/ml when tested at 4-16 mg/l against both strains. Vancomycin achieved a bacterial decrease of 2 log

CFU/ml when studied at 2 and 8 mg/l against the hGISA and the GISA strain, respectively. Imipenem failed to inhibit bacterial growth at any tested concentration (8-64 mg/l).

In killing curves with the hGISA strain, linezolid combined with vancomycin showed lower activity than vancomycin alone. Vancomycin activity was decreased between 1-1.5 $\log_{10}$CFU/ml at 24 h. The same combination improved the activities of antibiotics tested alone against the GISA strain (Figure 1).

The combination of linezolid at concentrations above the MIC and imipenem did not improve upon the activity of linezolid tested alone against either strain. The addition of sub-MIC concentrations of linezolid to imipenem produced a synergistic effect against both strains (Figure 2).

The combination of vancomycin with imipenem was bactericidal and synergistic against the hGISA strain. Vancomycin tested at 2 mg/l in combination with imipenem (8-64 mg/l) was also bactericidal and improved upon the activity of vancomycin alone against the GISA strain.

Pharmacokinetics.

Linezolid and vancomycin free maximum concentrations in serum were 18.16 and 37.73 mg/l, respectively (with a protein binding of 26 % for linezolid and 25 % for vancomycin [24, 25]) Imipenem free maximum concentration found in serum was 38.26 mg/l**.** Drug serum concentrations in humans are 12-15 mg/l for linezolid (dose 600 mg/12 h), 30-40 mg/l for vancomycin (dose 1 g/12 h) [26] and 32.1 mg/L (dose 500 mg/6 h) for imipenem [27]

Mortality and bacteraemia rates. In control mice, mortality rates were 90% and 69% after 25 h of infection with the hGISA and GISA strains, respectively. At the same time point, mortality in mice infected with the hGISA strain was 0%

in all therapeutic groups except for the imipenem (20%, 2/10) and the vancomycin plus imipenem (7%, 1/14) groups. In mice infected with the GISA strain, mortality was 0% in all treated animals except for those receiving imipenem monotherapy (45.5%, 5/11). Data from GISA-infected animals treated with imipenem monotherapy were not considered for any statistical analysis because of the low number of animals that survived after 25 h of therapy (n=6).

Bacteremia in control animals at 0 h, expressed as percentage of positive blood cultures, was 100% for each strain. Bacteraemia rates in control and therapeutic groups after 25 h of therapy are shown in Table 1. Imipenem alone and in combination with vancomcycin failed in blood bacterial clearance. Llinezolid alone and its combinations significantly reduced the bacteraemia rates achieved by the control group in hGISA-infected mice ($P < 0.04$). Mice treated with linezolid combinations also showed lower number of positive blood cultures than imipenem-treated group ($P < 0.04$). In GISA-infected mice, linezolid alone and in combination with imipenem significantly reduced the bacteraemia rates reached by the control ($P \leq 0.02$) and the vancomycin plus imipenem ($P < 0.05$) groups.

Murine peritonitis model. Therapeutic efficacy. Bacterial counts in PF (mean $\log_{10}$CFU/ml ± SD) of control animals at hour 0 were 8.17 ± 0.81 for the hGISA strain (n = 25) and 7.82 ± 0.57 for the GISA strain (n = 18). Bacterial counts in PF of control and treated mice after 25 h are shown in Table 2. Efficacy of an antibiotic therapy was defined as the decrease in the number of CFU (Δlog CFU/ml) in PF between 0 and 25 h. All regimens were statistically more effective than the control group for both strains ($P < 0.001$). Linezolid monotherapy produced similar bacterial decreases against both isolates.

Linezolid was as effective as vancomycin against the hGISA strain but slightly improved vancomycin activity against the GISA strain.

Linezolid combinations showed comparable efficacies to linezolid monotherapy against both strains. The association of linezolid with vancomycin was more active reducing bacterial counts than vancomycin alone in mice infected with the GISA strain. The addition of linezolid to imipenem showed enhanced activity upon imipenem alone against both strains. The association of linezolid with either vancomycin or imipenem showed higher activity than vancomycin plus imipenem against both strains (P = 0.048, linezolid plus vancomycin *vs* vancomycin plus imipenem against the GISA strain).

DISCUSSION

The increasing incidence of nosocomial infections due to *S. aureus* antibiotic-resistant strains and the report of therapeutic failures associated with standard glycopeptide therapy highlight the importance of identifying new synergistic drug combinations [1, 7, 8]. Linezolid has demonstrated good activity against most staphylococci, including methicillin-resistant strains [12, 13].

Linezolid was tested *in vitro* at achievable concentrations in human serum after oral administration of 500 and 600 mg regimens [22]. At 4-16 mg/l linezolid was effective against both hGISA and GISA strains. Its association with different drugs exerted distinct effects. Linezolid combined to imipenem was synergistic against both strains. The synergistic interaction between low concentrations of linezolid and imipenem has been previously reported against MRSA strains [28]. An indifferent effect was the most common result achieved with the interaction between linezolid and vancomycin accordingly to previous studies

involving MRSA and hGISA strains [29, 30]. Of particular interest was our finding of a synergistic killing with sub-MIC concentrations of both antibiotics in combination against the GISA strain. This enhanced effect has been reported on another GISA strain in an *in vitro* pharmacodynamic model [31].

In the murine peritonitis model caused by the hGISA strain no differences were found between linezolid and vancomycin monotherapies in terms of bacterial counts in peritoneal fluid and survival. In contrast, in GISA-infected mice linezolid showed a slightly higher activity than vancomycin, although this did not reach statistical significance. Moreover, linezolid achieved higher percentages of blood culture sterilization in comparison to vancomycin against both isolates. The use of combined regimens would be a good approach to improve the effectiveness of linezolid in the management of drug-resistant infections. In our experimental setting, the addition of linezolid to vancomycin showed similar efficacy but decreased the bacteraemia rates in comparison to vancomycin and linezolid monotherapies against the hGISA strain. The same combination enhanced vancomycin activity against the GISA strain but did not improve the rates of blood sterilisation achieved with monotherapy regimens.

Even though *β*-lactam antibiotics do not show any activity against MRSA and hGISA strains, their use in combination with linezolid has been shown to be highly effective against MRSA strains *in vitro* and in experimental endocarditis [28]. In our study, linezolid combined to imipenem was an effective therapy in mice infected with hGISA/GISA strains in terms of bacterial and bacteraemia reduction.

Our study found some discrepancies between *in vitro* and *in vivo* results. It should be emphasized that *in vitro* interaction may not translate into clinical

efficacy, mainly because of the diversity of mechanisms involved in *in vivo* antibiotic interactions which can not be analyze by the use of *in vitro* techniques [33]. In deed, experts recommend to use the *in vivo* efficacy more than the *in vitro* data when selecting an antistaphylococcal drug as a therapeutic option [34].

To date, few studies have addressed the role of linezolid combinations against glycopeptide-resistant *S. aureus.* The present study confirms the antistaphylococcal activity of linezolid in association with vancomycin or imipenem indicating that linezolid combinations preserves the activity of linezolid alone and might be considered as therapeutic options in the management of infections caused by *S. aureus* strains with reduced susceptibility to glycopeptides.

ACKNOWLEDGEMENTS

Part of this work was presented at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 17-20 September 2007. Funding: This work was partially supported by the Spanish Network for Infectious Diseases Research (REIPI RD06/0008/0022) and by a grant from Pfizer (Spain). M.P-I was supported by a grant from REIPI.

REFERENCES

1. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt, SM, Boxrud DJ, Etienne J et al (2003) Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA 290: 2976-2984.
2. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y (2003) Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. Clin Infect Dis 36: 53-59.
3. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FC (1997) Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 40: 135-136.
4. Maor Y, Rahav G, Belausov N, Ben-David D, Smollan G, Keller N (2007) Prevalence and characteristics of heteroresistant vancomycin-intermediate *Staphylococcus aureus* bacteremia in a tertiary care center. J Clin Microbiol 45: 1511-1514.
5. Climo MW, Patron RL, Archer GL (1999) Combinations of vancomycin and beta-lactams are synergistic against staphylococci with reduced susceptibilities to vancomycin. Antimicrob Agents Chemother 43: 1747-1753.
6. Domenech A, Ribes S, Cabellos C et al (2004) A mouse peritonitis model for the study of glycopeptide efficacy in GISA infections. Microb Drug Resist 10: 346-353.
7. Moore MR, Perdreau-Remington F, Chambers HF (2003) Vancomycin treatment failure associated with heterogeneous vancomycin-

intermediate *Staphylococcus aureus* in a patient with endocarditis and in the rabbit model of endocarditis. Antimicrob Agents Chemother 47: 1262-1266.

8. Ariza J, Pujol M, Cabo J, Pena C, Fernandez N, Linares J et al (1999) Vancomycin in surgical infections due to methicillin-resistant *Staphylococcus aureus* with heterogeneous resistance to vancomycin. Lancet 8: 1587-1588.
9. Aritaka N, Hanaki H, Cui L, Hiramatsu K (2001) Combination effect of vancomycin and beta-lactams against a *Staphylococcus aureus* strain, Mu3, with heterogeneous resistance to vancomycin. Antimicrob Agents Chemother 45: 1292-1294.
10. Goldstein FW, Atoui R, Ben Ali A, Nguyen JC, Ly A, Kitzis MD (2004) False synergy between vancomycin and beta-lactams against glycopeptide-intermediate *Staphylococcus aureus* (GISA) caused by inappropriate testing methods. Clin Microbiol Infect 10: 342-345.
11. Drew RH (2007) Emerging options for treatment of invasive, multidrug-resistant *Staphylococcus aureus* infections. Pharmacotherapy 27: 227-249.
12. Lentino JR, Narita M, Yu V (2008). New antimicrobial agents as therapy for resistant gram-positive cocci. Eur J Clin Microbiol Infect Dis 27: 3-15.
13. Rybak MJ, Hershberger E, Moldovan T, Grucz RG (2000) *In vitro* activities of daptomycin, vancomycin, linezolid, and quinupristin-dalfopristin against Staphylococci and Enterococci, including

vancomycin- intermediate and -resistant strains. Antimicrob Agents Chemother 44: 1062-1066.

14. Perry CM, Jarvis B (2001) Linezolid: a review of its use in the management of serious gram-positive infections. Drugs 61: 525-551.
15. Rubinstein E, Cammarata S, Oliphant T, Wunderink R (2001) Linezolid (PNU-100766) versus vancomycin in the treatment of hospitalized patients with nosocomial pneumonia: a randomized, double-blind, multicenter study. Clin Infect Dis 32: 402-412.
16. Stevens DL, Herr D, Lampiris H, Hunt JL, Batts DH, Hafkin B (2002) Linezolid versus vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* infections. Clin Infect Dis 34: 1481-1490.
17. Jones RN, Fritsche TR, Sader HS, Ross JE (2007) Zyvox Annual Appraisal of Potency and Spectrum Program Results for 2006: an activity and spectrum analysis of linezolid using clinical isolates from 16 countries. Diagn Microbiol Infect Dis 59: 199-209.
18. Wilson P, Andrews JA, Charlesworth R, Walesby R, Singer M, Farrell DJ et al (2003) Linezolid resistance in clinical isolates of *Staphylococcus aureus*. J Antimicrob Chemother 51: 186-188.
19. Jacqueline C, Caillon J, Le Mabecque V, Miegeville AF, Hamel A, Bugnon D et al (2007) *In vivo* efficacy of ceftaroline (PPI-0903), a new broad-spectrum cephalosporin, compared with linezolid and vancomycin against methicillin-resistant and vancomycin-intermediate *Staphylococcus aureus* in a rabbit endocarditis model. Antimicrob Agents Chemother 51: 3397-3400.

20. Sacar M, Sacar S, Kaleli I, Onem G, Turgut H, Goksin I et al (2007) Linezolid alone and in combination with rifampicin prevents experimental vascular graft infection due to methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis*. J Surg Res 139: 170-175.

21. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Approved standard M7-A6. 6th edn. Wayne, Pa., 2005.

22. MacGowan AP (2003) Pharmacokinetic and pharmacodynamic profile of linezolid in healthy volunteers and patients with Gram-positive infections. J Antimicrob Chemother 51 (suppl 2): ii17-25.

23. Domenech A. Ribes S, Cabellos C, Taberner F, Tubau F, Dominguez MA et al (2005) Experimental study on the efficacy of the combinations of glycopeptides and B-lactams against *Staphylococcus aureus* with reduced susceptibility to glycopeptides. J. Antimicrob. Chemother 56: 709-716.

24. Gentry-Nielsen MJ, Olsen KM, Preheim LC (2002) Pharmacodynamic activity and efficacy of linezolid in a rat model of pneumococcal pneumonia. Antimicrob Agents Chemother 46: 1345-1351.

25. Knudsen JD, Frunsted K, Esperen F, Frimodt-Moller N (1997) Activity of vancomycin and teicoplanin against penicillin resistant pneumococci *in vitro* and *in vivo* and correlation to pharmacokinetic parameters in the mouse peritonitis model. Antimicrob Agents Chemother 41: 1910-1915.

26. Murillo O, Doménech A, Garcia A, Tubau F, Cabellos C, Gudiol F et al (2006) Efficacy of high doses of levofloxacin in experimental foreign-

body infection by methicillin susceptible *Staphylococcus aureus*. Antimicrob Agents and Chemother 50: 4011-4017.

27. Paradis D, Vallee F, Allard S, Bisson C, Daviau N, Drapeau Ch et al (1992) Comparative study of pharmacokinetic and serum bactericidal activities of cefpirome, ceftazidime, ceftiaxone, imipenem and ciprofloxacin. Antimicrob Agents Chemother 36: 2085-2092.

28. Jacqueline C, Navas D, Batard E, Miegeville AF, Le Mabecque V, Kergueris MF et al (2005) *In vitro* and *in vivo* synergistic activities of linezolid combined with subinhibitory concentrations of imipenem against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 49: 45-51.

29. Jacqueline C, Caillon J, Le Mabecque V, Miegeville AF, Donnio PY, Bugnon D et al (2003) *In vitro* activity of linezolid alone and in combination with gentamicin, vancomycin or rifampicin against methicillin-resistant *Staphylococcus aureus* by time-kill curve methods. J Antimicrob Chemother 51: 857-864.

30. Sahuquillo Arce JM, Colombo Gainza E, Gil Brusola A, Ortiz Estevez R, Canton E, Gobernado M (2006) *In vitro* activity of linezolid in combination with doxycycline, fosfomycin, levofloxacin, rifampicin and vancomycin against methicillin-susceptible *Staphylococcus aureus*. Rev Esp Quimioter 19: 252-257.

31. Allen GP, Cha R, Rybak MJ (2002) *In vitro* activities of quinupristin-dalfopristin and cefepime, alone and in combination with various antimicrobials, against multidrug-resistant staphylococci and

enterococci in an *in vitro* pharmacodynamic model. Antimicrob Agents Chemother 46: 2606-2612.

32. Tsiodras S, Gold HS, Sakoulas G, Eliopoulos GM, Wennersten C, Venkataraman L et al (2001) Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. Lancet 358: 207-208.

33. Fantin B, Carbon C (1992) *In vivo* antibiotic synergism: contribution of animal models. Antimicrob Agents Chemother 36: 907–912.

34. Cunha BA (2005) Methicillin-resistant *Staphylococcus aureus*: clinical manifestations and antimicrobial therapy. Clin Microbiol Infect 11: 33-42.

Table 1. Bacteraemia rates of control and therapeutic groups of infected mice after 25 h of subcutaneous therapy. Data are expressed as percentages of positive blood cultures (n≥ 10 mice/group except for imipenem against the GISA strain where n = 6).

LZD, linezolid; VAN, vancomcyin; IMP, imipenem

Therapy (25h)	% Positive blood cultures	
	hGISA strain	GISA strain
Control	100	100
LZD 35 mg/kg/5 h	73[a]	67[a, c]
VAN 60 mg/kg/5 h	93	81
IMP 30 mg/kg/5 h	100	81*
LZD + VAN	64[a, b]	79
LZD + IMP	64[a, b]	67[a, c]
VAN + IMP	93	100

[a] $P < 0.04$ *vs.* Control group

[b] $P < 0.04$ *vs.* IMP group

[c] $P < 0.05$ *vs.* VAN + IMP group

* Small “n”; this group was excluded from statistical studies.

Table 2. Bacterial counts in peritoneal fluid (PF) for therapeutic and control groups after 25 h of subcutaneous therapy.

Therapy (25h)	PF bacterial counts ± SD (log CFU/ml) [n]	
	hGISA strain	GISA strain
Control	8.19 ± 0.57 [29]	8.29 ± 0.9 [29]
LZD 35 mg/kg/5 h	5.88 ± 0.61 [15][a]	5.60 ± 0.61 [18][a, b]
VAN 60 mg/kg/5 h	5.90 ± 0.31 [14][a]	6.02 ± 0.56 [16][a]
IMP 30 mg/kg/5 h	6.40 ± 0.80 [10][a]	7.38 ± 1.38 [6]*
LZD + VAN	5.94 ± 0.30 [14][a]	5.61 ± 0.56 [14][a, b]
LZD + IMP	5.81 ± 0.41 [14][a]	5.74 ± 0.52 [15][a]
VAN + IMP	6.15 ± 0.68 [14][a]	6.28 ± 0.49 [14][a]

[a] $P < 0.001$ *vs.* Control group

[b] $P < 0.05$ *vs.* VAN + IMP group

* Small “n”; this group was excluded from statistical analysis.

Fig.1

Figure 1. Time-kill curves of the combinations of linezolid plus vancomycin that improved the activities of both monotherapies against the GISA strain.

LZD, linezolid; VAN, vancomycin.

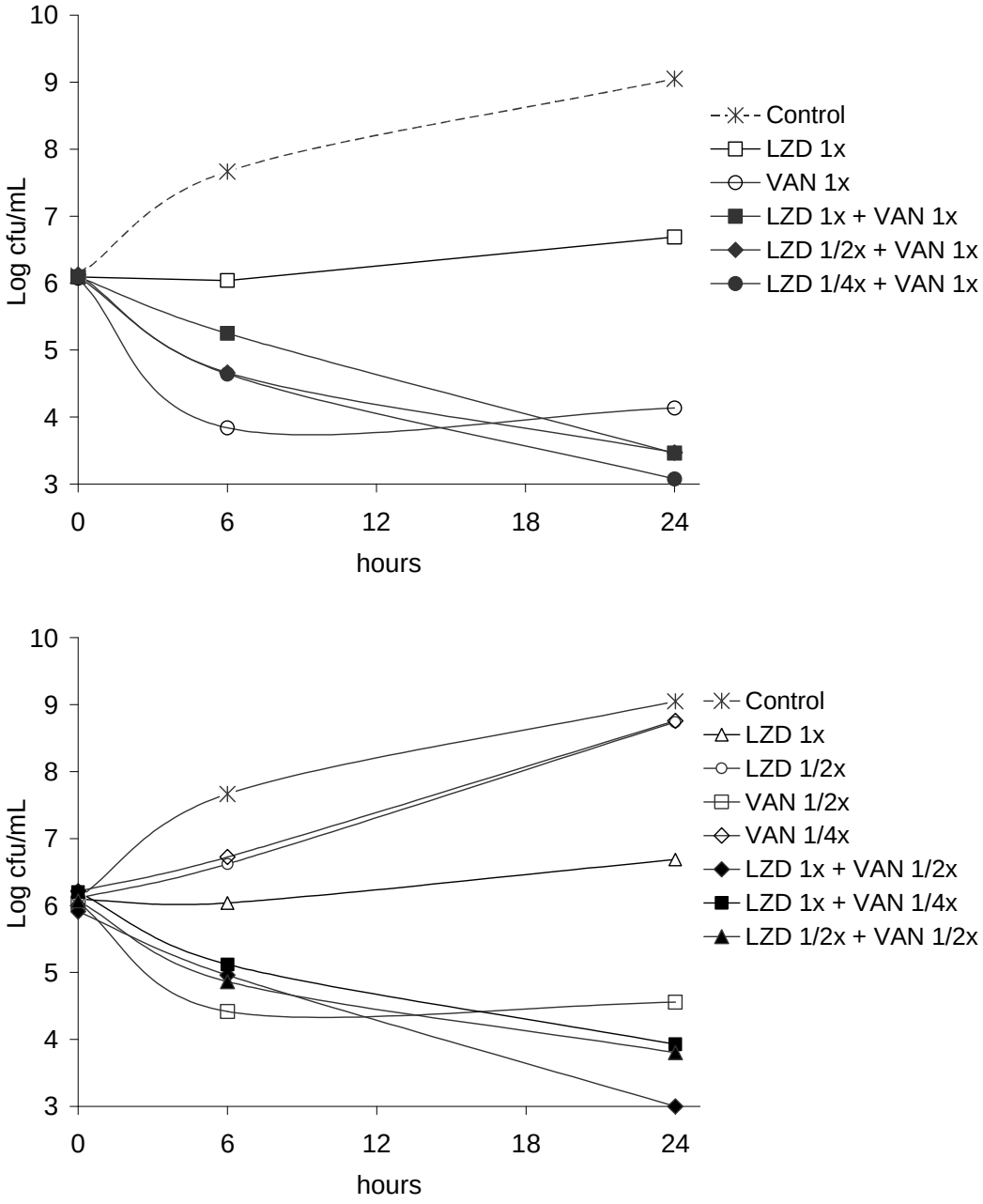

Fig.2

Figure 2. Time-kill curves with synergistic activity for linezolid in combination with imipenem against hGISA and GISA strains.

LZD, linezolid; IMP, imipenem