Title: Efficacy of linezolid in monotherapy q12h *vs.* q8h *vs*. combined with daptomycin in the treatment of experimental endocarditis caused by methicillin-resistant (MRSA) and glycopeptide-intermediate (GISA) *Staphylococcus aureus* strains.

Running Title: Linezolid monotherapy or combined with daptomycin in the treatment of MRSA/GISA experimental endocarditis.

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Synopsis

Background: Daptomycin (DAP) is highly effective against methicillin-resistant *Staphylococcus aureus* (MRSA), but limited by poor penetration into the central nervous system and disactivated by pulmonary surfactant. Linezolid (LIN), despite bacteriostatic activity, is effective in MRSA pneumonia and has excellent central nervous system penetration. Therefore, it is not uncommon in clinical practice to administer both antibiotics simultaneously in patients with MRSA bacteremia/endocarditis with pneumonia/meningitis.

Objectives: Determine the efficacy of LIN monotherapy administered every 12 h *versus* every 8 h *versus* the combination of LIN-q12h plus DAP against MRSA and glycopeptide-intermediate *S. aureus* (GISA) experimental endocarditis (EE).

Methods: *In vitro* time-kill studies were performed using standard inoculum (10^5 cfu) of one GISA and five MRSA strains. MRSA-277 and GISA-700788 were selected for the EE model. Animals were treated with LIN-q12h (600 mg/kg/12h), LIN-q8h (600 mg/kg/8h), DAP (6 mg/kg/day), or DAP plus LIN-q12h simulating the human-like serum levels.

Results: LIN-q8h and LIN-q12h showed similar activity in MRSA-277 EE ($p=0.150$). Against GISA-700788 endocarditis, LIN-q8h was more effective than LIN-q12h ($p<0.001$). DAP monotherapy was very effective against MRSA-277 and GISA-700788 EE, sterilizing 70% and 60% of valvular vegetations, respectively. DAP plus LIN-q12h was similarly effective at 60% and 56%, respectively. This combination showed neither *in vitro* nor *in vivo* antagonism, and DAP-non-susceptible (DNS) strains were not

recovered.

Conclusion: Increasing LIN to 600 mg/8h may be useful as initial treatment in clinical practice. DAP plus LIN-q12h showed no antagonism and can be used for the treatment of MRSA/GISA bacteremia/endocarditis with pulmonary and/or meningeal involvement.

Introduction

Staphylococcus aureus continues to be a major cause of hospital and community-acquired infections[1], with rates of bacteremia and infective endocarditis (IE) due to this bacterium increasing in recent decades[2,3]. The increasing prevalence of healthcare-associated *S. aureus* IE may partly reflect the growing use of medical devices and interventional procedures[4–6]. Unfortunately, *S. aureus* is characterized by the development of resistance to each new class of antistaphylococcal drugs, penicillins, sulfonamides, tetracyclines, and glycopeptides[7].

Methicillin-resistant *S. aureus* (MRSA) infections have had limited treatment options for decades. According to current United States and European treatment recommendations, vancomycin (VAN) and daptomycin (DAP) remain first-line therapies for MRSA bacteremia and IE[8,9]. However, these first-line therapeutic agents are not without limitations, and, despite their use, clinical outcomes of severe MRSA infections remain largely unsatisfactory. Regarding VAN, prior exposure to this drug (or other glycopeptide antibiotics) appears to be a strong risk factor for developing *S. aureus* isolates with reduced susceptibility to vancomycin (MIC > 1.0 μg/ml), which might be a significant risk factor for treatment failure[10,11]. Moreover, the increase in reports of *S. aureus* with intermediate resistance to glycopeptides (GISA) and heteroresistant MRSA to VAN (hVISA) have emerged worldwide, further complicating the management of severe MRSA infections.

Daptomycin is a lipopeptide antibiotic that disrupts bacterial cell membrane potential, leading to rapid, dose-dependent bactericidal effects[12]. The use of DAP has increased over the past decade, and many clinical reports have documented the rapid development of DAP resistance[13,14]. Furthermore, this drug exhibits a poor diffusion into the central nervous system (CNS)[15,16] and is inactivated by pulmonary

surfactant[17,18], limiting its effectiveness in treating MRSA bacteremia associated with meningitis or pneumonia. On the other hand, LIN is an oxazolidinone that exerts bacteriostatic activity by inhibiting the ribosomal synthesis of bacterial proteins[19,20]. LIN should be considered as an alternative treatment in cases of MRSA bacteremia or IE complicated by drug allergy, although its use is limited by a relative scarcity of data and side effects related to prolonged use[5,21]. In the clinical setting of *S. aureus* bacteremia originating from pneumonia, LIN is preferable if the strain is susceptible[8], moreover this drug offers an excellent diffusion into the lung and CNS[16,22] for patients with meningeal involvement and, finally, it can be considered a suitable option for sequential therapy from i.v. to oral route in selected IE cases[23].

Combination therapies offer advantage over monotherapies given their wide-spectrum, acquisition of synergistic effect and ability to prevent the emergence of drug resistance[24]. However, *in vitro* studies have shown antagonism when LIN is combined with VAN, suggesting this combination should be avoided[25–27]. In clinical practice, the combination of DAP and LIN is often used to treat MRSA bacteremia or endocarditis in patients with pneumonia or meningitis. There are several case reports and case series of successful treatment of MRSA with DAP/LIN, despite limited systematic evaluation of its efficacy. In addition, the DAP/LIN combination has not been sufficiently studied *in vitro* and *in vivo* models, and published data are still scarce. Some *in vitro* studies using checkerboard analysis have shown indifference or additive effects with DAP/LIN, while time-kill assays indicated antagonism[28,29]. However, in biofilm models and simulated endocardial vegetations, the DAP/LIN combination was more effective than either drug alone[30,31]. Further studies are therefore needed to elucidate the efficacy of combining DAP with LIN for treatment of MRSA infections.

This study has two objectives: first, to evaluate the *in vivo* efficacy of LIN every 12 hours or every 8 hours (q8h); second, to assess the efficacy of the combination of LIN-q12h and DAP against MRSA and GISA strains using a human-like pharmacokinetics model of experimental endocarditis in rabbits.

Materials and Methods

Bacterial strains

We studied six *S. aureus* strains. Five strains of methicillin-resistant *S. aureus* (MRSA) isolated from patients with endocarditis (MRSA-196, MRSA-277, MRSA-513, MRSA-726, and MRSA-835) at Hospital Clínic-Universitat de Barcelona (Barcelona, Spain) and one MRSA strain with reduced susceptibility to glycopeptides *S. aureus* (GISA) from the ATCC collection (ATCC-700788). Two strains were selected for *in vivo* studies (MRSA-277 and GISA-700788). These strains were kept frozen at -80° C in skim milk.

Antimicrobials agents

LIN powder (lot (D2) 1800-8180-JLH) was supplied by Pfizer (Kalamazoo, MI. USA). DAP powder was supplied by MSD, and VAN was purchased commercially from Sigma-Aldrich. The drugs were prepared according to the manufacturers' recommendations.

In vitro studies

LIN, DAP and VAN minimum inhibitory concentrations (MIC) were determined in duplicate by microdilution method in cation-adjusted Mueller-Hinton broth (MHB;

Oxoid Ltd., Hampshire, England) liquid medium. For testing DAP, MHB was supplemented with calcium chloride at 50 mg/L. The experiments were carried out according to the recommended procedures of the CLSI[32], using *S. aureus* ATCC-29213 as the reference control strain for assay validation.

Time killing methodology as described previously[33] to evaluate the activity of DAP plus LIN against all strains. A final inoculum of 5×10^5 cfu/mL was used. Antibiotic concentration of 1×MIC were selected for testing.

Animals

New Zealand white rabbits (2.5 kg) provided by San Bernardo farm (Pamplona, Spain) were used. The animals were housed in the animal facilities of the Scientific and Technological Centers of the University of Barcelona's School of Medicine (CCiTUB). The Committee of Animals Ethics of the University of Barcelona approved all animal experimentation in this study.

Pharmacokinetics studies

To reproduce human serum pharmacokinetics (PK) in rabbits, antibiotics were administered using a computer-controlled infusion system (KD Scientific Inc. Model 200. Boston. MA. USA) designed to reproduce human serum PK in rabbits. The *in vivo* experimental pharmacokinetics of DAP have been described elsewhere[34]. The following antibiotic regimens and doses were chosen for simulation in the rabbits: DAP at 6 mg/kg intravenous (i.v.) every 24 hours and LIN at 600 mg i.v. every 12 or 8 hours.

The LIN PK parameters in rabbits were estimated. An open one-compartment model was used to estimate the elimination rate constant (*Kel*) and the volume of distribution (*V*) after an i.v. injection of LIN in five healthy rabbits. To determine the

concentrations of LIN in serum samples, blood was drawn from a carotid catheter at 0.08, 0.25, 0.42, 0.5, 1, 2, 3, 4, 5, and 6 hours after administration of a single i.v. injection of 20 mg/kg. LIN concentrations in rabbit serum were determined by high-performance liquid chromatographic (HPLC) assay, developed by the Pharmacology Laboratory at the Centre de Diagnòstic Biomèdic, Hospital Clínic, Barcelona. Serum samples were prepared by mixing a solution of perchloric acid and 100 µL of serum. The HPLC analysis used a reversed-phase C18 analytical column and a mobile phase consisting of an isocratic mixture of 15-mM phosphate buffer (pH 5)-acetonitrile and UV monitoring. The method was linear over the therapeutic concentration range of 0.5–100 µg/mL. The quantification limit was 0.5 µg/mL in plasma for a sample size of 200 µL. The accuracy of the method ranged from 94.4–106.1%, and the precision values ranged from 0.88–6.0 % intra-day and from 3.7–5.6 % inter-day.

A mathematical model[35] was applied to derive the required infusion doses to simulate human-like PK of LIN in rabbits.

Different PK parameters were estimated based on an open, one-compartment model to compare the PK of LIN in rabbits[35–37], the human-adapted model, and humans. The area under the concentration-time curve from zero hours to infinity ($AUC_{0\rightarrow\infty}$) was calculated as C_0/K_{el}, and the time over MIC was calculated as (ln C_0-ln MIC) K_{el}.

Endocarditis model

Experimental aortic valve infective endocarditis was induced in New Zealand rabbits as described previously by our group[38,39]. The different groups of animals were infected via the marginal ear vein with 1 mL of saline solution containing 10^5 cfu of either the MRSA-277 or the GISA-700788 strain in the stationary phase of growth. The animals were treated for 48 hours using a computer-controlled pump. After completion of the

205 treatment, six additional half-lives of the antibiotics were left to elapse, allowing for the
206 growth of residual viable bacteria in the endocardial vegetations. After this, the animals
207 were anaesthetized and euthanized using an intravenous bolus of pentobarbital. Aortic
208 valve vegetations were obtained, weighed, homogenized in 2 mL of saline solution, and
209 quantitative and qualitative cultures were performed.
210
211 **Treatment groups**
212 The infected rabbits were separated into different treatment arms simulating human PK.
213 The groups included: LIN monotherapy at dosages of 600 mg every 12h and 600 mg
214 every 8h, DAP monotherapy at 6 mg/kg/day, and combined therapy with DAP
215 6 mg/kg/day plus LIN-q12h. Each group included at least 10 animals.
216
217 **Analysis of endocardial vegetations**
218 The cfu counts recovered from vegetations were expressed as the number of $\log^{10}$ cfu
219 per gram of vegetation ($\log^{10}$ cfu/g veg). The result was assigned a value of zero and the
220 vegetation was considered sterile if there was no growth from the initial quantitative and
221 qualitative cultures or from the homogenates cultured for a week.
222
223 ***Statistical Analysis.***
224 The results were expressed as the median and the IQR of the number of $\log^{10}$ cfu/g veg.
225 The Mann–Whitney non-parametric test was used to compare the $\log^{10}$ cfu tissue values
226 among the different treatment groups. The Fisher exact test was used to compare the
227 rate of sterilized vegetations and analyze whether there were differences between
228 treatment groups.
229

Results

In vitro studies

The MICs from *in vitro* susceptibility testing of LIN, DAP and VAN are shown in Table 1. All isolates were uniformly susceptible to LIN with a range of 2-4 mg/L, to DAP with a range of 0.25-0.5 mg/L and to VAN a range of 0.5- 2 mg/L, except for the GISA-700788 strain with an VAN MIC of 8 mg/L.

Synergy studies

Figure 1 shows the results of the time-killing curves for the combination of DAP plus LIN in the standard inoculum when tested at MIC concentrations, showed indifference to all MRSA strains, including the GISA strain. Furthermore, after 24 hours of incubation, all isolates recovered from the DAP monotherapy and combinations showed no change in MIC to DAP. No antagonism was detected in the time-killing curves.

Pharmacokinetics studies.

The values of the PK parameters for DAP have been previously described[34]. The results of the human-like approach to the data for LIN were determined in five healthy rabbits after receiving an i.v.-bolus dose of 20 mg/kg (values are means ± standard deviations [SDs]): K_{el}, 1.25 ± 0.08 h^{-1}; elimination $t_{½}$ ($t_{½\beta}$) 0.54± 0.08 h; and the area under the concentration-time curve from time zero to infinity ($AUC_{0\rightarrow\infty}$), 10.55 ± 2.31 µg.h/mL (Table 2). The PK profile in human serum after i.v. administration of 600 mg of LIN was also reproduced in rabbits (Figure 2). The PK data for the human-adapted model (K_{el}, 0.17 ± 0.03 h^{-1}; $t_{½\beta}$, 4.22± 0.8 h; $AUC_{0\rightarrow\infty}$, 70.81 ± 7.5 µg.h/mL) were similar to those of 600 mg of LIN administered i.v. in humans (Table 2).

Antibiotic concentrations in serum.

The DAP concentration detection range was from 0.8 to 72.9 µg/mL, and the C_{max}/C_{min} ratio was 86/15 mg/L, as previously described[40]. After the administration of LIN that simulated a 600 mg dose i.v. every 12 hours (q12h) in humans, the corresponding peak and trough levels on the first day were 10 ± 1.7 and 1.4 ± 0.8 µg/mL, and on the second day of treatment the peak and trough levels were 16 ± 3.8 and 2.8 ± 1.1 µg /mL. When the rabbits were treated with LIN with 600 mg i.v. every 8 hours (q8h) the peak and trough levels reached on the first day of treatment were 8.1 ± 2 and 6.2 ± 2 µg /mL, and on the second day 19 ± 2 and .8.3 ± 3 µg /mL. These results show that with 48 hours of treatment there was accumulation of LIN with time, and, when it was given q8h, the levels reached were higher (Table 3).

Pharmacodynamic studies

To determine whether rabbits were treated appropriately, different pharmacodynamic parameters were measured. The results showed that the concentration of LIN reached in the serum of rabbits treated with either q12h or q8h was always higher than the MIC for both the MRSA-277 and the GISA-700788 strains. The pharmacodynamic parameter that best predicts outcome is the 24-hour area under the concentration-time curve over the MIC for the infecting microorganism (AUC/MIC ratio). This ratio showed that, with both regimens of LIN during the two days of treatment, the rabbits were not under treated. Table 4 shows one summary of these results.

Experimental endocarditis studies

Linezolid q12h versus q8h

All control rabbits had infected aortic valve vegetations, with a median bacterial of 9.1–10 $\log^{10}$ cfu/g veg for both strains. The treatments of rabbits with IE for both the MRSA-277 and the GISA-700788 strains with LIN at q12h and at q8h showed no significant differences with the untreated control group, and sterile vegetations were not found in any case (**Figure 3**). However, for treating IE caused by MRSA-277, the treatment with LIN-q8h was more effective than LIN-q12h in reducing the number of colonies. Additionally, the regimen of 600 mg LIN-q12h was effective and reducing the colonies number in the vegetations from rabbits infected with the 700788-GISA strain ($p<0.001$) (**Figure 3**).

Linezolid plus daptomycin

Comparisons between treated groups revealed that after 48 hours of treatment, DAP monotherapy (6 mg/kg/24h) was able to significantly reduce the density of microorganisms in the vegetations of MRSA-277 (P=0.002) and GISA-700788 (P=0.011). Additionally, the sterilization of vegetations was achieved by 70% and 60%, respectively (Figure 4). Regarding combination therapy, DAP monotherapy exhibited comparable efficacy to DAP plus LIN, demonstrating a notable sterilization rate of 60% (6/10) against MRSA-277 and 56% (5/9) against GISA-700788. This resulted in a substantial decrease in bacterial density concentration and a marked increase in the percentage of vegetation sterilization for both strains (Figure 4). On the other hand, it is important to highlight that the combination of DAP with LIN did not show an antagonistic effect, and isolates with changes in the MIC to DAP were not recovered.

The *in vivo* activity of LIN in monotherapy or combined therapies of LIN/DAP is summarized in ***Supplemental material tables 1-2***.

Discussion

The present study evaluated the efficacy of linezolid by comparing the q12h and q8h dosing regimens and exploring its combination with daptomycin against MRSA and GISA strains. According to our findings, the activity of LIN within the first 48 hours can be enhanced by administering the antibiotic every eight hours. In the same manner, the combination of LIN with DAP was as effective as DAP monotherapy in reducing bacterial counts in valve vegetations caused by MRSA/GISA strains, while preventing the emergence of daptomycin non-susceptible (DNS) strains. Importantly, our experiments did not detect relevant *in vitro* or *in vivo* antagonistic phenomena. These findings offer valuable experimental insights with potentially significant clinical implications.

As previously commented, VAN remains the primary treatment for MRSA endocarditis[41,42], despite drawbacks like slow bacterial killing, nephrotoxicity, and rising resistance. DAP is a suitable alternative, often recommended in combination with beta-lactams or fosfomycin to prevent resistance[43]. Due to its bacteriostatic effect, LIN has relegated it to a marginal mention in the guidelines[41,42]. However, numerous clinical experiences have been published in recent years about its role as a salvage drug in IE[21]. The largest retrospective cohort was that published by the Spanish group GAMES, which included 295 episodes of IE treated (84 of them with IE due to MRSA strains)[44]. In their work, with an adequate analysis adjusted by propensity score, the researchers observed higher in-hospital mortality in the group that received LIN for more than seven days and/or as an initial antibiotic treatment, precluding its use as monotherapy in IE. In our study, an experimental endocarditis model in rabbits infected with MRSA or GISA strains was used. As a first step, we evaluated the efficacy of LIN in monotherapy, administered to simulate human-level pharmacokinetics. Animals were treated with LIN-q12h (600 mg/kg/12h) or LIN-q8h (600 mg/kg/18h). We found that

LIN-q8h proved significantly more effective than both the untreated and the LIN-q12h groups ($p<0.001$) in reducing colony numbers for MRSA-277 and GISA-700788. The serum levels of LIN achieved through intermittent infusion with both regimens indicated that the LIN concentration was consistently higher than the MIC for the strains analyzed. However, at these concentrations and as reported previously, LIN could not sterilize the vegetation of animals infected with different *S. aureus* strains. For Jacqueline C et al[45]., in a human-like pharmacokinetics model using the same dose of LIN, an intermittent dosing regimen failed to exhibit a bactericidal effect. However, by continuous infusion maintaining a T>MIC of 100% and achieving a steady-state serum concentration with an increased ratio to the MIC over five days of treatment, these researchers demonstrated drug accumulation and bactericidal activity *in vivo*[45]. Other studies not using human-like pharmacokinetics, found that oral LIN at 50 and 75 mg/kg orally q8h for five days resulted in a significant decrease in the bacterial valve vegetation counts[46–48]. In the same way, orally administering LIN at 75 mg/kg q8h could maintain plasma and valve vegetation drug levels above the MIC for the entire dosing interval over multiple days, with a progressive reduction in bacterial counts in infected valves[49]. Interestingly, continuous infusion provided concentrations five times the MIC throughout the treatment[45], and oral administration three times a day yielded serum and vegetation concentrations above 2 mg/L after the second day of treatment and a kinetic profile close to that of continuous infusion[45,49–51]. Nevertheless, the potential beneficial effect of high-dose as induction therapy should be weighed together with the possible toxicity, which was not assessed by our model[52,53].

Several clinical trials have explored the potential synergistic effects in MRSA bacteremia, including combinations of VAN with beta-lactams[54,55], DAP with fosfomycin[56], and DAP with ceftaroline[57], although with little specific information for

IE cases. Moreover, as previously mentioned, there are clinical circumstances in which the combination of DAP/LIN could be rational: we are referring specifically to endocarditis with concomitant CNS involvement and bacteremia pneumonia due to MRSA. However, there was concern that this combination could present *in vivo* antagonism eventually causing therapeutic failures. *In vitro* studies performed retrospectively showed indifference in chequerboard analysis and antagonism in time-kill assays for the combination of LIN/DAP[27,58]. On the contrary, another study, using an *in vitro* combined drug sensitivity test, showed synergy between DAP and LIN against MRSA, with no antagonistic effect[59]. In our *in vitro* studies, the combination of DAP/LIN had an indifferent effect, with no antagonistic interaction observed. In the second part of our *in vivo* experimental study, intravenous LIN at 600 mg/12h was administered alone or in combination with DAP at 6 mg/kg. Our findings showed that DAP monotherapy was significantly more effective than LIN in sterilizing endocardial vegetations caused by MRSA-277 and GISA-700788. Similar results showed >5 log cfu reduction and 5/8 sterilization rate using the same DAP dose[60]. Despite this, our data reflect the combination of DAP and LIN exhibiting activity similar to that of DAP monotherapy but effectively preventing the emergence of DNS strains. On the contrary, other *in vitro* and *in vivo* studies have reported greater efficacy of daptomycin combined with linezolid. Steed et al.[61] found that the combination of LIN/DAP showed a bactericidal effect in an *in vitro* pharmacokinetic/pharmacodynamic study of simulated endocardial vegetations infected by DNS MRSA strains. Additionally, Parra-Ruiz et al.,[62] working on an *in vitro* pharmacokinetic/pharmacodynamic biofilm model found DAP/LIN combination therapy more effective than monotherapy and showed bactericidal activity against biofilms of MRSA.

This study has some limitations. First, the efficacy of the combination of

DAP/LIN was evaluated *in vivo* using only two MRSA and GISA strains, but antagonism was not detected either *in vivo*, and the *in vitro* TKC was done in six strains with standard inoculum. Second, despite these efficacy studies being performed in animals and not humans, the experimental endocarditis model in rabbits is an ideal discriminatory model, with numerous examples demonstrating good translation into clinical studies (e.g., *Enterococcus faecalis* IE treated with ampicillin and ceftriaxone, or MRSA endocarditis treated with fosfomycin and imipenem[63,64]). Lastly, these *in vivo* studies did not assess the safety of LIN at 600 mg q8h or the DAP/LIN combinations. Therefore, the potential toxicity of high doses of LIN or the combination of DAP/LIN in humans should be closely monitored.

In summary, our experiments have yielded some relevant findings regarding the potential role of LIN in the treatment of endocarditis. The activity of LIN within the first 48 hours can be enhanced by administering the antibiotic every eight hours. In the same manner, the of combination of LIN with DAP is as effective as DAP monotherapy in reducing bacterial counts in valve vegetations against MRSA/GISA strains and prevents the emergence of DNS strains. Importantly, our experiments did not detect any relevant *in vitro* or *in vivo* antagonistic phenomena. From a clinical perspective, these two antibiotics in combination could be considered for treating patients with bacteremia or endocarditis, particularly those also suffering from MRSA meningitis or pneumonia.

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Table 1. MIC values of daptomycin and linezolid for MRSA and GISA strains.

Strains	Daptomycin MIC (mg/L)	Linezolid MIC (mg/L)	Vancomycin MIC (mg/L)
MRSA-196	0.5	1	0.5
MRSA-277	0.5	2	2
MRSA-513	0.5	2	1
MRSA-726	0.25	1	0.5
MRSA-835	0.25	4	1
GISA-700788	0.5	2	8

MIC (minimal inhibitory concentration). According with CLSI breakpoints for *Staphylococcus spp*. were defined in Susceptible (S) and Resistant (R) the following antimicrobials: Daptomycin S: ≤ 1 mg/L, Linezolid S: ≤ 4 mg/L; R: ≥ 8 mg/L and Vancomycin S: ≤ 2 mg/L; R: ≥ 8 mg/L.

653 **Table 2.** Pharmacokinetic parameters

Linezolid	
Previously reported human values (single dose)	
Dose	600 mg i.v.[a]
C_{max}/C_{min} (μg/mL)	--/--
K (h^{-1})	0.135 [a]
T½β (h)	5 [a]
AUC (μg-h/ml)	76.1 [a]
Protein binding	31%
Animal values (n = 5)[b]	
K (h^{-1} ± SD)	1.25 ± 0.08
T½β (h ± SD)	0.5 ± 0.08
AUC (μg-h/ml ± SD)	10.5 ± 2.31
Protein binding	
Human-like values (n = 5)[c]	
K (h^{-1} ± SD)	0.17 ± 0.03
T½β (h ± SD)	4.22 ± 0.8
AUC (μg-h/ml ± SD)	70.81 ± 7.5

[a]Data from et al. Eur J Clin Pharmacol 2002, 57: 793-797

[b]Animal parameters are for healthy rabbits treated with linezolid 20 mg/Kg.

[c]Data for human-like pharmacokinetics of linezolid 600 mg

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655 **Table 3** Linezolid serum levels during the treatment following the human like model

656 with the two regimens of administration.

657

Treatment	**Day 1**			**Day 2**	
	C_{max} (µg/mL)	C 8h (µg/mL)	C 24h (µg/mL)	C_{max} (µg/mL)	C 48h (µg/mL)
Linezolid-q12h*	10 ± 1.7	2.5 + 1.4	1.4 + 0.8	16.5 ± 3.8	2.8 ± 1.1
Linezolid-q8h**	8.1 ± 2	2 + 0.8	6.2 + 2	19 ± 2	8.3 ± 3

*Simulating 600 mg/12h i.v.; **Simulating 600 mg/8h i.v.

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659

660 **Table** 4. Pharmacodynamic parameters of linezolid used in the treatment of IE

661 against MRSA-277 and GISA-700788 strains.

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Treatment	**MRSA-277**			**GISA-700788**		
	MIC (mg/L)	Time>MIC	AUC/MIC	MIC (mg/L)	Time>MIC	AUC/MIC
Linezolid-q12h*	1	12 h (100%)	146.88	2	10h (83%)	73.44
Linezolid-q8h**	1	8 h (100%)	182.1	2	8 h (100%)	91.05

*Simulating 600 mg/12h iv; **Simulating 600 mg/8h iv.

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Figure 1. **Time-kill curves for MRSA-196, MRSA-277, MRSA-277, MRSA-513, MRSA-726, MRSA835 and GISA-700788**. The strains were incubated with daptomycin (DAP) plus linezolid (LIN) at concentrations of 1×MIC for both antibiotics. Values are means ± standard deviations from two independent experiments. The black line indicates bactericidal activity.

Figure 2. The pharmacokinetic profile in human serum after i.v. administration of 600 mg of LIN in rabbits. Concentration-time curves for single dose of linezolid delivered to rabbit at 20 mg/kg, before and after of application mathematics model to obtain the required infusion doses to simulate human kinetics of LIN.

Figure 3. **The treatment of rabbits with IE for (A.-) MRSA-277 and (B.-) GISA-700788 strains with LIN q12h (simulating 600 mg/12h i.v.) or q8h (simulating 600 mg/8h i.v.).** Densities of MRSA/GISA in aortic vegetations in the IE model due to 10^5 cfu/mL challenges of study strains. Each dot represents one animal. Horizontal black bars indicate mean and interquartile MRSA/GISA densities.

Figure 4. **The treatment of rabbits with IE for (A.-) MRSA-277 and (B.-) GISA-700788 strains with DAP combined with LIN**. Densities of MRSA/GISA in aortic vegetations in the IE model due to 10^5 cfu/mL challenges of study strains. The number of rabbits with sterile vegetations/total number of rabbits (%) is shown for each treatment group under the abscissae. Each dot represents one animal. Horizontal black bars indicate mean and interquartile MRSA/GISA densities.

690 Figure 1

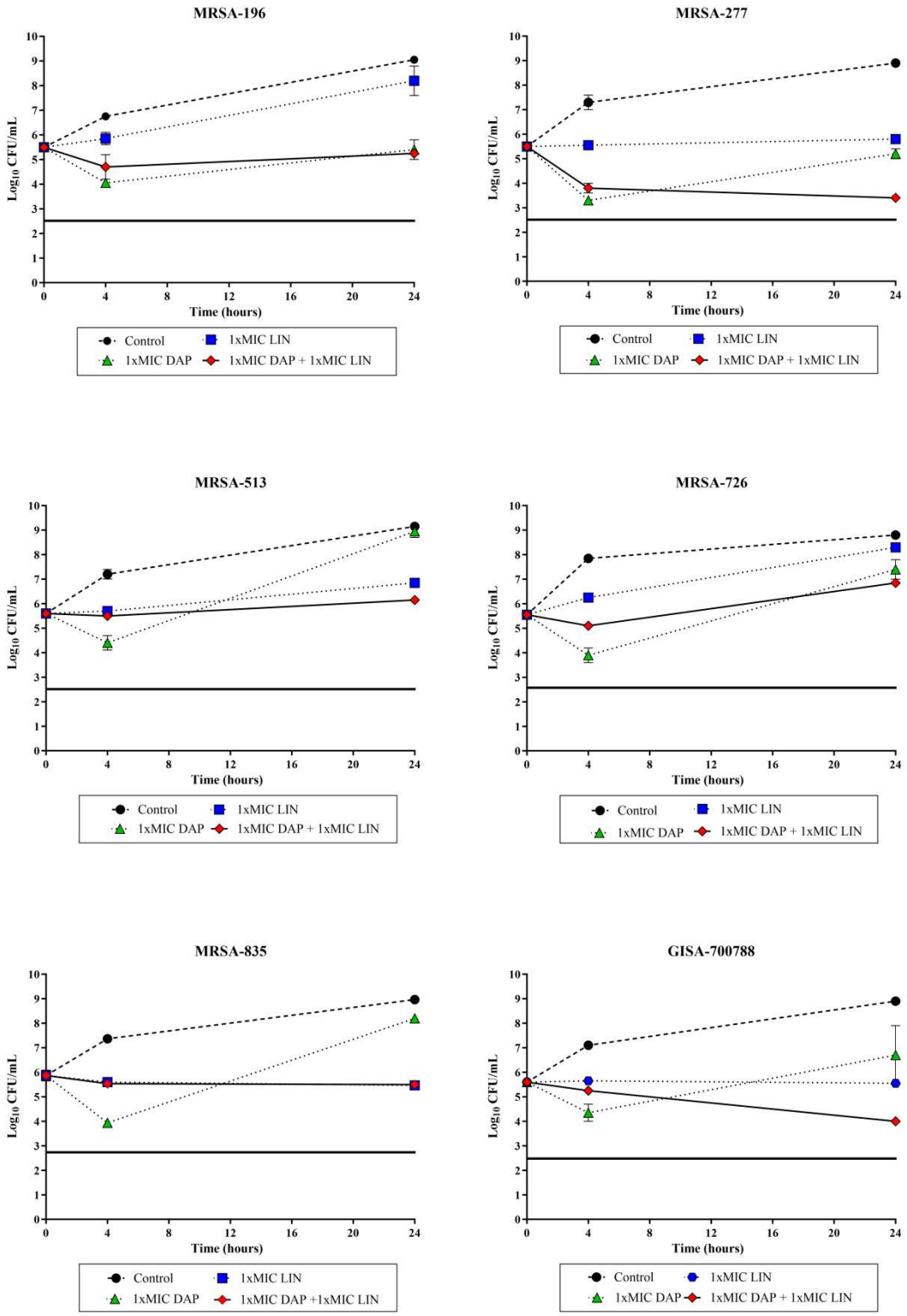

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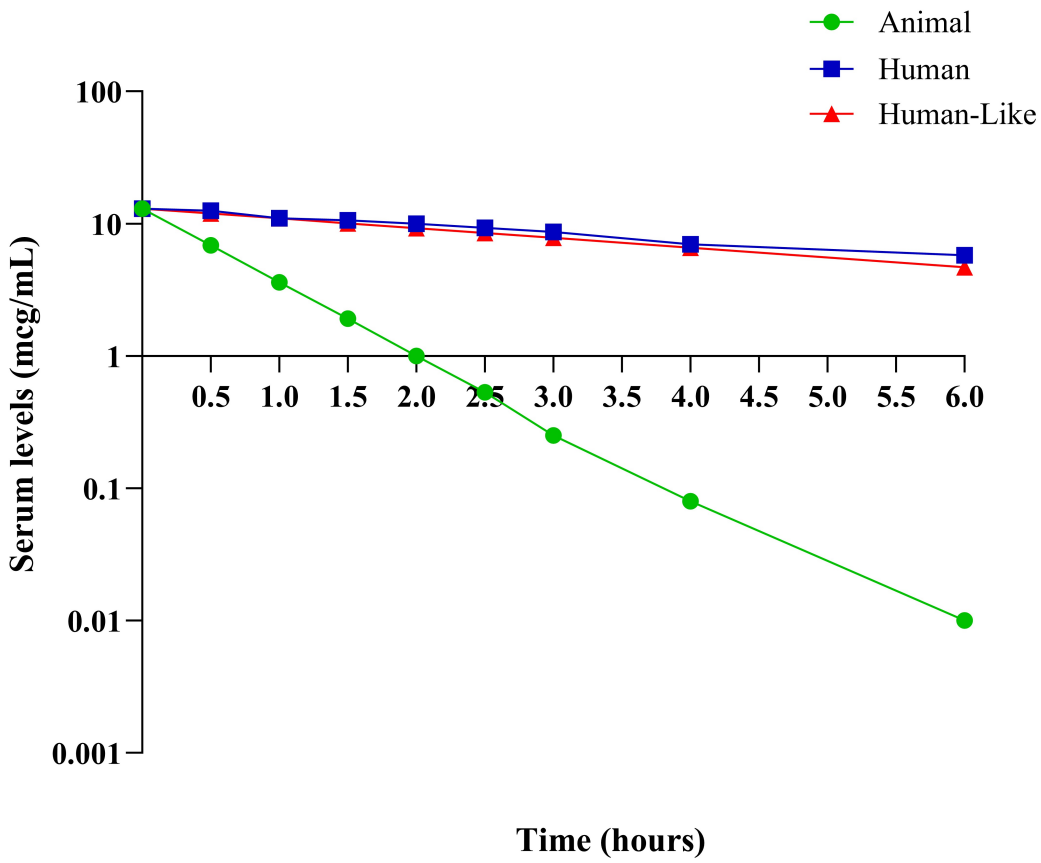
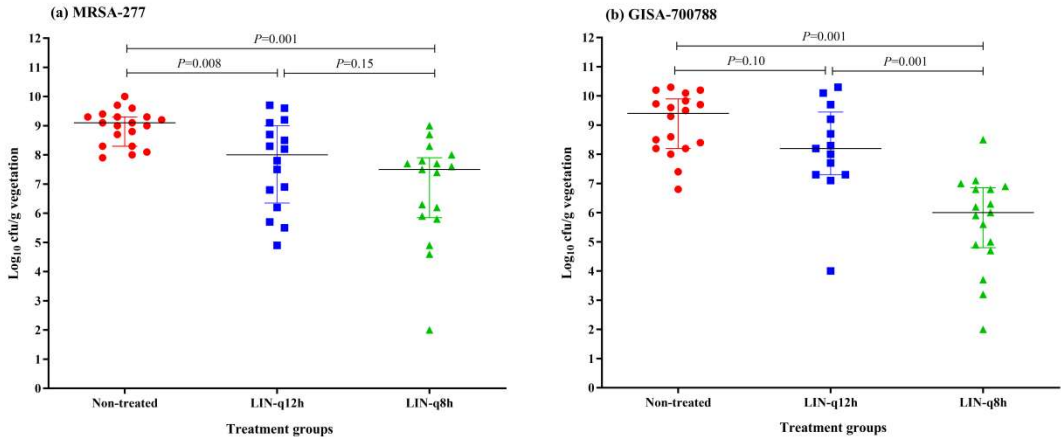
Figure 2


Animal
Human
Human-Like
100
10
1
0.1
0.01
0.001
Serum levels (mcg/mL)
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Time (hours)

704 Figure 3



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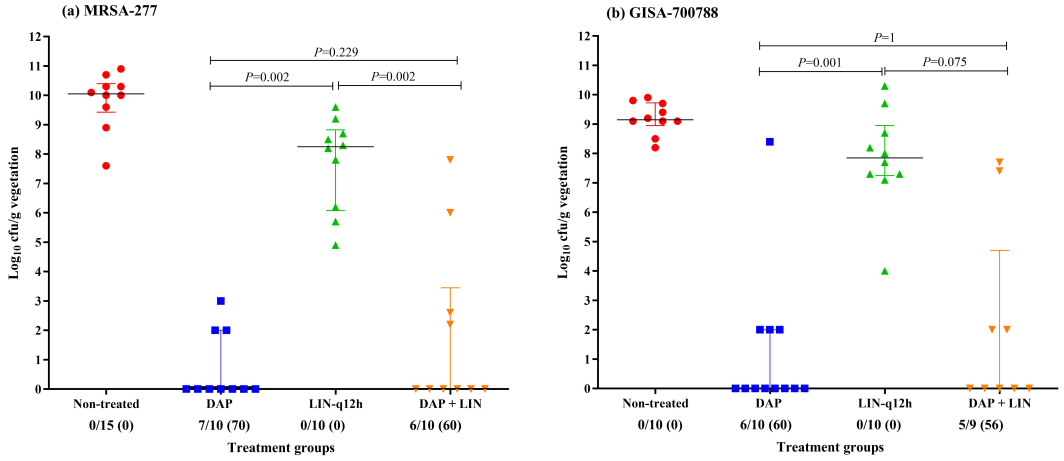
706 Figure 4



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