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Pharmacodynamics of early, high-dose linezolid against vancomycin-resistant enterococci with elevated MICs and pre-existing genetic mutations

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Objectives: Vancomycin-resistant enterococci (VRE) have emerged as an important nosocomial pathogen in medical centres worldwide. This study evaluated the impact of front-loading of linezolid on bacterial killing and suppression of resistance against VRE strains with defined genetic mutations.

Methods: Time–killing experiments over 48 h assessed the concentration effect relationship of linezolid against eight strains of vancomycin-resistant *Enterococcus faecalis*. A hollow fibre infection model (HFIM) simulated traditional and front-loaded human therapeutic linezolid regimens against VRE strains at 10^6 cfu/mL over 240 h. Translational modelling was performed using S-ADAPT and NONMEM.

Results: Over 48 h in time–kill experiments, linezolid displayed bacteriostatic activity with >2 $\log_{10}$ cfu/mL killing for all strains with an MIC of 4 and minimal activity against VRE with MICs of 16 and 64 mg/L. Against one strain with no resistant alleles (MIC 4 mg/L), 600 mg of linezolid every 12 h achieved maximal reductions of 0.96 $\log_{10}$ cfu/mL over 240 h in the HFIM, whereas front-loaded 1200 mg of linezolid every 12 h $\times$10 doses or 2400 mg of linezolid every 12 h $\times$10 doses followed by 600 mg of linezolid every 12 h provided significantly improved killing with maximal reductions of 3.02 and 3.46 $\log_{10}$ cfu/mL. Front-loaded regimens suppressed amplification of resistant subpopulations against VRE strains with no resistant alleles (MIC 4 mg/L) and postponed regrowth of resistant subpopulations against a VRE with 3.2 resistant alleles (MIC 4 mg/L). Modelling yielded excellent population fits ($r=0.934$) and identified the number of sensitive alleles as a critical covariate.

Conclusions: Early, high-dose regimens of linezolid provided promising killing against selected susceptible strains and may be clinically beneficial if early bactericidal activity is necessary.

Keywords: oxazolidinones, pharmacokinetics, resistance, pharmacogenomics

Introduction

Vancomycin-resistant enterococci (VRE) have become a prevalent nosocomial pathogen in medical centres throughout the USA.[1] Over the last decade, the incidence of VRE infections has been increasing, and nearly 50% of enterococci isolated from patients in intensive care units are vancomycin resistant.[1,2] In addition, these organisms are persistent nosocomial pathogens capable of prolonged survival on environmental surfaces and frequently colonize the intestinal and genital tract in humans.[3,4] Additionally, VRE bloodstream infections typically occur in critically ill patients, which highlights the need to focus on optimal treatment for these aggressive and persistent pathogens.[2]

Linezolid represents the first marketed drug belonging to the oxazolidinone class of antimicrobials. Linezolid displays activity against aerobic and facultative Gram-positive microorganisms, including VRE. Although resistance to linezolid occurs *in vitro* at

a low frequency of 1×10^{-9} to 1×10^{-11}, it has been attributed[5] to a single nucleotide change in 23S rDNA at bp 2576. There have been few studies directed toward defining the pharmacokinetic-pharmacodynamic relationship of linezolid against VRE as it relates to amplification of resistance during therapy.[6–8] Additionally, there has been limited information regarding whether higher exposure regimens of linezolid display activity against intermediate (MIC 4.0 mg/L) or resistant strains (MIC ≥8 mg/L) of VRE with pre-defined, pre-existing genetic mutations.

We hypothesized that increasing the dose and exposure of linezolid during the first 5 days of therapy may have potential utility to rapidly reduce bacterial burden and suppress or postpone resistance against these difficult-to-treat strains. Indeed, this proposed 'front-loading' strategy significantly differs from the loading dose approach. The loading dose approach seeks to achieve a steady state more rapidly by giving a larger dose on day 1, whereas the front-loading approach explored in this study uses significantly higher doses for multiple days to achieve more bacterial killing and prevent the emergence of resistance.

The hollow fibre infection model (HFIM) presents a sophisticated *in vitro* system that can simulate the time course of linezolid concentrations in humans for normal and front-loaded regimens over 10 days. We are not aware of a published translational, mechanism-based model that simultaneously described time–kill and HFIM data. To our knowledge, there are also no published reports that quantitatively implemented the effect of sensitive and resistant alleles on bacterial killing by oxazolidinones.

Therefore we utilized VRE strains with varying susceptibility and a number of sensitive and resistant alleles to evaluate the benefit of front-loaded regimens with different dose intensity during the first 5 days to gain insight into the utility of linezolid front-loading against increasingly resistant VRE strains and to aid in the design of future oxazolidinones. A translational mechanism-based model was developed to support the translation from time–kill to hollow fibre data and to estimate the impact of sensitive and resistant alleles.

Materials and methods

Bacterial isolates

Eight clinical vancomycin-resistant *Enterococcus faecalis* isolates with varying susceptibility and genetically defined resistant alleles were utilized and obtained from Focus Technologies' clinical surveillance programme Phase IV studies. The number of resistant alleles was quantified by quantitative real-time PCR, by amplification of 72 bp of 23S rDNA by 5′ nuclease real-time PCR, as previously described.[9] *E. faecalis* strains utilized in time–killing experiments included: (i) 4408 (linezolid MIC of 4.0 mg/L, 4.3 sensitive alleles, 0 resistant alleles); (ii) 4407 (linezolid MIC of 4.0 mg/L, 3.5 sensitive alleles, 0 resistant alleles); (iii) 4422 (linezolid MIC of 4 mg/L, 2.4 sensitive alleles, 3.2 resistant alleles); (iv) 4412 (linezolid MIC of 16 mg/L, 1.2 sensitive alleles, 3.2 resistant alleles); (v) 4424 (linezolid MIC of 16 mg/L, 1.0 sensitive alleles, 3.4 resistant alleles); (vi) 4397 (linezolid MIC of 32 mg/L, 1.3 sensitive alleles, 3.5 resistant alleles); (vii) 4405 (linezolid MIC of 32 mg/L, 0 sensitive alleles, 3.5 resistant alleles); and (viii) 4393 (linezolid MIC of 64 mg/L, 0 sensitive alleles, 4.0 resistant alleles).

Based on the time–kill experiments, bacterial strains that demonstrated adequate response to linezolid (maximal reductions of at least 2 $\log_{10}$ cfu/mL) at 48 h were selected for further evaluation of front-loaded regimens in the HFIM. These strains were selected since they were responsive to higher concentrations of linezolid and were expected to benefit from a front-loading strategy. Therefore *E. faecalis* 4407, 4408 and 4422 were subsequently evaluated in the HFIM. This allowed a comparison of killing and prevention of resistance for strains with the same linezolid MIC (4 mg/L), but with different numbers of resistant alleles. Strains that exhibited a high level of resistance (such as 4393, with a linezolid MIC of 64 mg/L) were not evaluated in the HFIM, as linezolid monotherapy would not be considered as a treatment option against these strains in clinical practice. However, we considered it important to gain insight into the concentration response relationship against these highly resistant strains using time–kill studies. ATCC 29212 *E. faecalis* (linezolid MIC of 1.0 mg/L) was utilized as a vancomycin-susceptible control.

Antibiotic, susceptibility testing and medium

Linezolid analytical grade powder was obtained from Pfizer Global Research and Development, Groton, CT, USA. MIC values were determined by broth microdilution according to the CLSI. Brain heart infusion (BHI) broth (Difco, Detroit, MI, USA) and tryptic soy agar (TSA) with 5% sheep blood were used for all time–kill experiments and hollow fibre experiments.

Time–kill experiments

To first evaluate the full concentration–effect relationship, time–kill experiments were performed as previously described against a starting inoculum of 10^6 cfu/mL over 48 h.[10] In brief, for bacterial inocula preparation, fresh bacterial colonies from overnight growth were added to standard BHI broth to provide a bacterial suspension, which was diluted to achieve a starting inoculum of approximately 10^6 cfu/mL. Quantitative cultures were determined on TSA plates with 5% sheep blood. The limit of detection was 10^2 cfu/mL. Time–kill experiments were conducted at concentrations of 0–512 mg/L to characterize linezolid pharmacodynamics against each strain. Linezolid concentrations included clinically achievable and higher concentrations to evaluate the full concentration response profile of linezolid and to determine whether higher concentrations would display a pharmacodynamic benefit.

HFIM

An HFIM, adapted from Louie *et al.*,[11] was used to evaluate the effect of selected linezolid dosing regimens on the change in bacterial burden and suppression of resistance of *Enterococcus faecalis* over 240 h as previously described. In brief, a cellulosic cartridge (C3008, FiberCell Systems Inc., Frederick, MD, USA) was utilized for all experiments. The determination of bacterial counts for each experiment was performed by obtaining samples at 0, 24, 48, 72, 96, 144, 192 and 240 h. Samples quantified the total population, and aliquots of the diluted sample were plated in quintuplicate on BHI plates containing linezolid at 2, 4 and 8 times the MIC in order to quantify the resistant subpopulations.

Experimental design and simulated linezolid regimens

The experimental design consisted of a no-treatment control arm and three simulated regimens including one traditional regimen and two front-loaded regimens. The following linezolid regimens were administered using an apparent half-life of 4.8 h and a protein binding of

31%,[12] resulting in a free (i.e. non-protein bound) maximal concentration (fC_{max}) and a free AUC at steady state over 24 h ($fAUC_{0\text{-}24}$):

Traditional regimen: 600 mg every 12 h (fC_{max} 10.4 mg/L, $fAUC_{0-24}$ 124).
Front-loaded regimen: 1200 mg every 12 h ×10 doses on days 0–5 (fC_{max} 20.8, $fAUC_{0-24}$ 248) followed by 600 mg every 12 h ×10 doses on days 5–10 (fC_{max} 10.4, $fAUC_{0-24}$ 124)
Front-loaded regimen: 2400 mg ×10 doses on days 0–5 (fC_{max} 41.6, $fAUC_{0-24}$ 495) followed by 600 mg every 12 h ×10 doses on days 5–10 (fC_{max} 10.4, $fAUC_{0-24}$ 124)

Pharmacokinetic and pharmacokinetic-pharmacodynamic analysis

Samples from the central reservoir from the HFIM were stored at −80°C until they were assayed for concentrations of linezolid determined using a validated HPLC assay.[13] In brief, samples were measured using a system consisting of a ThermoFinnegan P4000 HPLC pump (San Jose, CA, USA) with model AS1000 fixed-volume autosampler, a model UV2000 ultraviolet detector, a Gateway Series e computer (Poway, CA, USA) and the Chromquest HPLC data management system. The plasma standard curve for linezolid ranged from 0.5 to 30 mg/L. The within-sample precision [percentage coefficient of variation (CV%)] of validation in a single standard concentration was 0.69%, and the overall validation precision across all standards was 1.04%–4.39%. The measured drug concentrations were within 10% of the targeted values.

Mechanism-based population pharmacodynamic modelling

Population pharmacodynamic modelling was performed to: (i) estimate the exposure response relationship for the time–kill studies; (ii) propose front-loaded regimens to be evaluated in the HFIM; and (iii) develop the first translational model that can bridge between time–kill and hollow fibre studies.

Structural model

We adapted a previously described life-cycle growth model with one bacterial population and included the effect of linezolid on inhibition of protein synthesis.[14] Linezolid was assumed to inhibit protein synthesis:

$$\frac{dP}{dt} = k_{Prot} \cdot \left[\left(1 - \frac{C_{Drug}}{IC_{50} + C_{Drug}}\right) - P\right] \qquad \text{Initial condition (IC)} : 100\% \quad (1)$$

where P is the protein pool, IC_{50} is the linezolid concentration (C_{Drug}) associated with half-maximal inhibition of protein synthesis and k_{Prot} is the turnover rate constant of the protein pool. The parameterization of equation (1) yields a steady-state of 1 for the protein pool (in the absence of linezolid), representing 100% of its hypothetical baseline. The lack of proteins (Lack) was calculated as (1 − P) and the probability of death during replication ($Prob_{death}$) was described via a maximal probability of death ($Imax_{Rep}$) at a pronounced lack of the protein pool:

$$Prob_{death} = Imax_{Rep} \cdot Lack \quad (2)$$

The model contained one bacterial population that was split into a growing (S1) and a replicating state (S2), as described previously.[14] Bacteria in states S1 and S2 have the same susceptibility, but differ in their growth phase. Bacteria in state S2 are immediately before replication and bacteria in state S1 are preparing for replication. The differential equations for S1 and S2 were

$$\frac{dS1}{dt} = 2 \cdot PLAT \cdot (1 - Prob_{Death}) \cdot k_{21} \cdot S2 - k_{12} \cdot S1 \qquad IC : 10^{\log cfu_o} \quad (3)$$

$$\frac{dS2}{dt} = -k_{21} \cdot S2 + k_{12} \cdot S1 \qquad IC : 0 \quad (4)$$

k_{12} and k_{21} are the first-order transfer rate constants between both states and log cfu_o represents the $\log_{10}$ of the initial inoculum. The total population (cfu_{tot}) is the sum of S1 and S2. The plateau factor (PLAT) is defined as $1 - [cfu_{tot}/(cfu_{tot} + cfu_{max})]$, with cfu_{max} representing the maximum population size. The factor 2 represents the doubling of cells during the replication process.

Covariate effect model

The number of sensitive alleles (Nsen) was used as a covariate for the IC_{50} of linezolid for the inhibition of protein synthesis via a Hill function:

$$IC_{50} = IC_{50Sen0} \cdot \left(1 - \frac{Imax_{Sen} \cdot Nsen^{HSen}}{N50_{Sen}^{HSen} + Nsen^{HSen}}\right) \cdot f_{HFIM} \quad (5)$$

Thus IC_{50} is affected by Nsen as defined by equation (5). IC_{50Sen0} represents the typical IC_{50} for a strain with no sensitive alleles, $Imax_{Sen}$ is the maximum fractional decline of IC_{50} and H_{Sen} is the Hill coefficient. f_{HFIM} represents the estimated ratio of IC_{50} in the HFIM compared with the static time–kill model. The number of resistant alleles (Nres) was used as a covariate for the mean generation time (MGT_{12}) for the transfer of bacteria from state S1 to S2 (k_{12} was calculated as $1/MGT_{12}$; see Table 1 for further parameter explanations). An effect of linezolid to further prolong the MGT_{12} potentially due to a lack of proteins was explored.

$$MGT_{12} = MGT_0 \cdot \left(1 + \frac{Smax_{Res} \cdot Nres^{HRes}}{N50_{Res}^{HRes} + Nres^{HRes}}\right) \quad (6)$$

Parameter variability model

The inter-strain variability of parameters estimated on a log scale was described by a normal distribution. The $Imax_{Rep}$ and $Imax_{Sen}$ were constrained between 0 and 1 using a logistic transformation as described previously.[15] All other parameters were described by a log-normal distribution.

Residual error model and computation

Candidate models were fit simultaneously to: (i) all viable count profiles from eight strains studied in time–kill experiments; or (ii) all viable count profiles of the total population from the time–kill and hollow fibre studies using an additive residual error model on a $\log_{10}$ scale. Estimation in NONMEM® VI (level 6.2) used the first-order conditional estimation method with the interaction option and estimation in parallelized S-ADAPT (version 1.57) used the importance sampling Monte Carlo parametric expectation maximization algorithm.[16] The SADAPT-TRAN facilitator tool was used to support model building and evaluated in S-ADAPT.[15,17]

Model selection was based on the maximum likelihood objective function, plausibility of parameter estimates, standard diagnostic plots, visual predictive checks and the reduction in the unexplained (random) inter-strain variability due to inclusion of a covariate effect.[18]

Results

Killing profile of linezolid against VRE with defined genetic mutations

Linezolid time–kill experiments (Figure 1) were first conducted to characterize the pharmacodynamic profile of linezolid against eight VRE strains to evaluate the full concentration response and select potential strains and regimens to be evaluated in the HFIM. Linezolid displayed bacteriostatic activity, with bacterial killing for all strains $<1.5\ \log_{10}$ cfu/mL over 48 h. A concentration-dependent response was evident, with increasing concentrations resulting in additional killing. Selected time–kill

Table 1. Estimates of the population pharmacodynamic model based on data from eight strains in the time–kill experiments and four strains in the HFIM

Parameter	Symbol	Unit	Mean (% relative standard error)	Inter-strain variability[b] (SE%)
Initial inoculum in time–kill	$\log_{10}$ [cfu_o (TK)]	—	6.07 (0.4%)	0.19 (20%)
Initial inoculum in HFIM	$\log_{10}$ [cfu_o (HFIM)]	—	6.47 (1.3%)	0.25 (44%)
Maximum inoculum in time–kill	$\log_{10}$ [cfu_{max} (TK)]	—	8.44 (0.6%)	0.29 (27%)
Maximum inoculum in HFIM	$\log_{10}$ [cfu_{max} (HFIM)]	—	10.8 (1.5%)	0.18 (215%)
Mean generation time in time–kill	MGT_{12} (TK)	min	79.8 (5.9%)	0.29 (66%)
Mean generation time in HFIM	MGT_{12} (HFIM)	min	144 (15%)	0.25 (71%)
Mean turnover time of protein pool	$T_{Prot}=1/k_{Prot}$	min	6.50 (9.5%)	
Maximal extent of inhibition of successful replication	$Imax_{Rep}$	—	0.561 (12%)	range 0.552–0.571
Linezolid concentration causing 50% of $Imax_{Rep}$ for a strain with no sensitive alleles	IC_{50Sen0}	mg/L	10.8 (16%)	49 (26%)
Covariate effects				
Maximal fractional decrease in IC_{50} in the presence of ∼3.6 or more sensitive alleles[a]	$Imax_{Sen}$	—	0.904[a] (2.8%)	
Number of sensitive alleles associated with 50% of $Imax_{Sen}$	$N50_{Sen}$	—	1.48 (4.5%)	
Hill coefficient for $N50_{Sen}$	H_{Sen}	—	5.17 (2.3%)	
IC_{50} in the hollow fibre divided by IC_{50} in time–kill for strain 4422	f_{HFIM} (4422)	—	0.895 (4.7%)	
IC_{50} in the follow fibre divided by IC_{50} in time–kill for strains 4407 and 4408	f_{HFIM} (4407 and 4408)	—	0.205 (6.6%)	
Maximal fractional increase in MGT_{12} due to resistant alleles	$Smax_{Res}$	—	0.228 (6.5%)	
Number of resistant alleles causing 50% of $Smax_{Res}$	$N50_{Res}$	—	2.19 (4.7%)	
Hill coefficient for $N50_{Res}$	H_{Res}	—	2.75 (3.4%)	
SD of additive residual error on $\log_{10}$ scale	SDCF	—	0.251 (2.6%)	

[a]This estimate means that the typical IC_{50} for a strain with ∼3.6 or more sensitive alleles was 1.04 mg/L.
[b]The variability estimates include both the variability between different strains and the variability between different viable count profiles within the same strain.

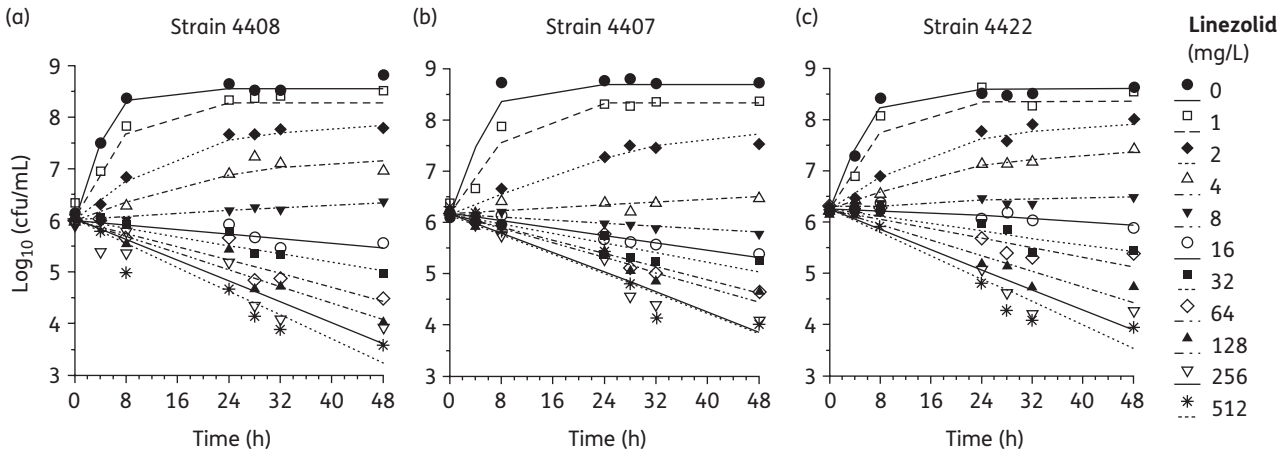

Figure 1. Observed and individual fitted viable counts (in NONMEM®) from the time–kill experiments for linezolid versus three VRE strains with an MIC of 4 mg/L.

experiments are shown in Figure 1(a–c). Against increasingly resistant strains with an MIC ≥16, linezolid generally displayed minimal killing with <1 $\log_{10}$ cfu/mL maximal activity over 48 h (data not shown).

Modelling bacterial growth and killing as a function of the numbers of sensitive and resistant alleles as covariates

Figure 2 shows the structure of the mechanism-based model with linezolid inhibiting protein synthesis. The subsequent depletion of the protein pool stimulated the probability of death during replication. The presence of ∼3.6 or more sensitive alleles was estimated to decrease the IC_{50} of linezolid from 10.8 mg/L for strains with no sensitive allele to 1.04 mg/L for strains with at least 3.6 sensitive alleles (Table 1).

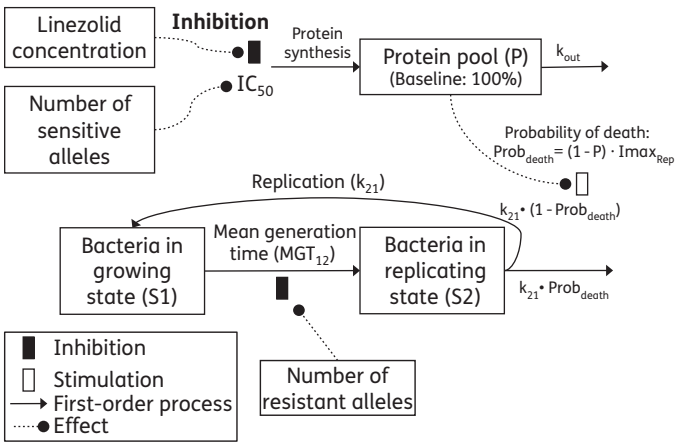

Figure 2. Life cycle growth model with one bacterial population residing in a growing state (S1; after replication) or in a replicating state (S2; immediately before replication). Linezolid inhibits protein synthesis. The number of sensitive alleles affects the linezolid concentration yielding half-maximal inhibition (IC_{50}). The lack of a protein pool stimulates the probability of death ($Prob_{death}$) during replication. The number of resistant alleles inhibited the mean generation time ($MGT_{12} = 1/k_{12}$).

The number of sensitive alleles (Nsen) was assumed to affect the binding affinity of linezolid to its target, which is represented by the IC_{50} as a drug-related parameter. Inclusion of the covariate effect of Nsen on the IC_{50} of linezolid explained approximately 80% of the variance in IC_{50}. To consider a potentially decreased biofitness of strains with resistant alleles (Nres) compared with strains without resistant alleles, an effect of Nres on the mean generation time (MGT_{12}; a drug-independent system parameter) was additionally considered. Strains with resistant alleles were estimated to have an up to 23% longer mean generation time compared with strains without resistant alleles. These covariate effects underlined the benefits of using genetic information in a quantitative model to predict the bacterial susceptibility (IC_{50}) and the rate of bacterial growth and killing.

The maximum extent of inhibition of successful replication (equal to the maximum probability of death) was 0.561 (Table 1), in agreement with the slow killing by linezolid. A probability of death of 50% would result in net stasis (if two parent cells try to replicate, one cell successfully doubles and the other dies; i.e. two parent cells generate two daughter cells). A probability of death of 100% would result in all replicating cells dying and cause the maximum rate of killing to be equal to $-k_{12}$, as described previously.[14]

Figure 1 shows the individual curve fits for the time–kill data for the intermediary model that was estimated in NONMEM based on the time–kill data. The characterization of the pharmacokinetic-pharmacodynamic relationship between linezolid concentrations and killing allowed for the selection of clinical regimens of linezolid to be simulated in the HFIM.

Impact of linezolid front-loaded regimens on the total and resistant bacterial population

Traditional and front-loaded regimens for linezolid in humans were subsequently evaluated in an HFIM against four strains of VRE as shown in Figure 3(a–d). Against VRE strains with a linezolid MIC of 4.0 mg/L, the greatest benefit of front-loading was evident in strain 4408 (Figure 3a). Against this strain the traditional regimen of linezolid 600 mg every 12 h demonstrated a gradual reduction in bacterial counts over the study duration: maximal reductions were 0.96 $\log_{10}$ cfu/mL. There was a

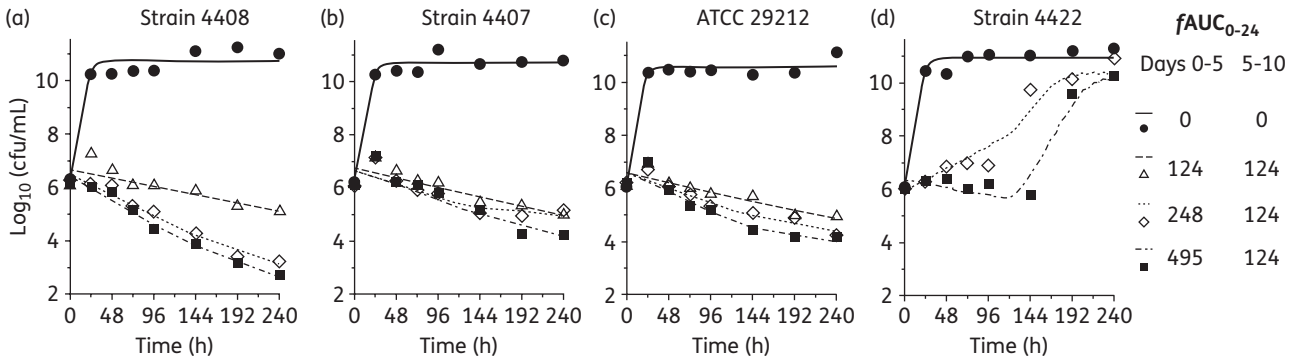

Figure 3. Observations and individual fitted viable counts (from S-ADAPT) for the HFIM plotted over time. Strains 4408, 4407 and 4422 had a linezolid MIC of 4 mg/L and strain ATCC 29212 had an MIC of 1 mg/L. Strains 4408 and 4407 carried no resistant allele, whereas strain 4422 carried 3.2 resistant alleles. The number of sensitive alleles was 4.3 for strain 4408, 3.5 for strain 4407 and 2.4 for strain 4422. The $fAUC_{0-24}$ is provided for the time of front-loading (0–120 h) and for the maintenance therapy (120–240 h).

significant improvement in bacterial killing for front-loaded regimens over a short duration, which demonstrated bactericidal activity at the 240 h study endpoint: 1200 mg every 12 h ×10 doses or 2400 mg every 12 h ×10 doses followed by 600 mg every 12 h with reductions of 3.02 and 3.46 $\log_{10}$ cfu/mL. All regimens suppressed the amplification of resistant subpopulations against VRE strains with an MIC of 4.0 mg/L. Interestingly, against strain 4422, which displayed an MIC of 4.0 mg/L and carried 3.2 resistant alleles, both front-loaded regimens displayed limited activity, resulting in eventual regrowth, and amplified the development of resistance, as shown in Figure 4(a–c).

Simultaneous modelling of the time–kill and hollow fibre data

The model developed in NONMEM based on the time–kill data over 48 h yielded unbiased and precise individual and population fits for the time–kill study (Figure 5a). This model was used to provide *in silico* predictions of the hollow fibre study without using any of the hollow fibre data. The *in silico* predictions showed that the model based on the time–kill data could excellently predict the first 48 h of therapy in the hollow fibre (Figure 5b, closed symbols), yielded reasonable predictions for days 3 and 4, but could only well predict counts in the hollow fibre for one of four strains on days 6–10. This was expected, since this model was only based on time–kill data over 48 h.

Upon re-estimation of the parameter values in S-ADAPT based on all data, the final model yielded excellent individual and population fits for both the time–kill and hollow fibre datasets (Figures 3 and 5c). The observed versus individual fitted log (cfu/mL) yielded a slope of 1.000 and an *r* of 0.986 and the observed versus population fitted log (cfu/mL) yielded a slope of 0.999 and an *r* of 0.934. Interestingly, to optimally translate between the time–kill and the hollow fibre experiments, the IC_{50} estimate in the hollow fibre model was similar (0.895 times) to the estimate in the time–kill for strain 4422 carrying resistant alleles. However, the IC_{50} for strains 4407 and 4408 carrying no resistant alleles was only 0.205 times the IC_{50} estimate for the time–kill experiments for the respective strain (Table 1). This suggested some strain-to-strain variability for the translation from time–kill to hollow fibre experiments.

Discussion

VRE continues to be a persistent, difficult-to-treat pathogen posing significant challenges for clinicians as it relates to optimal treatment.[1,2] Linezolid displays a unique mechanism of action by inhibiting bacterial protein synthesis through binding of 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex. Linezolid resistance in enterococci has been reported, attributed to a single conserved site in the 23S rDNA (G2576T).[5] This was demonstrated in early clinical trials where resistance to linezolid developed in patients infected with VRE who received a dose of 600 mg every 12 h.[5]

Therefore, to combat the increasing resistance, we evaluated the potential benefit of 'front-loading', a strategy to optimize the pharmacodynamic profile of an antibiotic through the administration of high doses early in therapy for a short duration. The

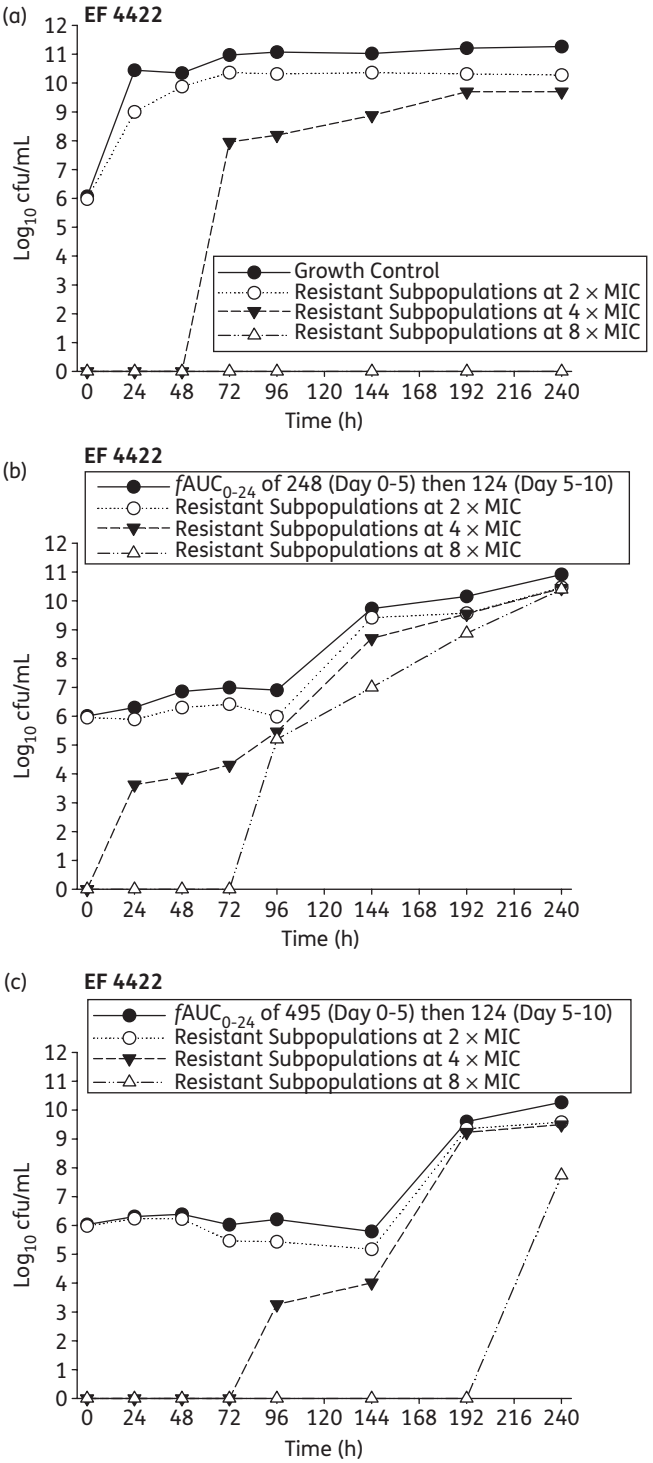

Figure 4. HFIM experiments simulating linezolid front-loaded regimens against strain 4422 (linezolid MIC of 4 mg/L, 2.4 sensitive alleles, 3.2 resistant alleles). Front-loaded regimens resulted in amplification of resistant subpopulations that grew on 2×, 4× and 8× MIC plates. The total and resistant subpopulations for the following regimens were as follows: (a) growth control; (b) front-loaded regimen: $fAUC_{0-24}$ of 248 on days 0–5 then $fAUC_{0-24}$ of 124 on days 5–10; (c) front-loaded regimen: $fAUC_{0-24}$ of 495 on days 0–5 then $fAUC_{0-24}$ of 124 on days 5–10.

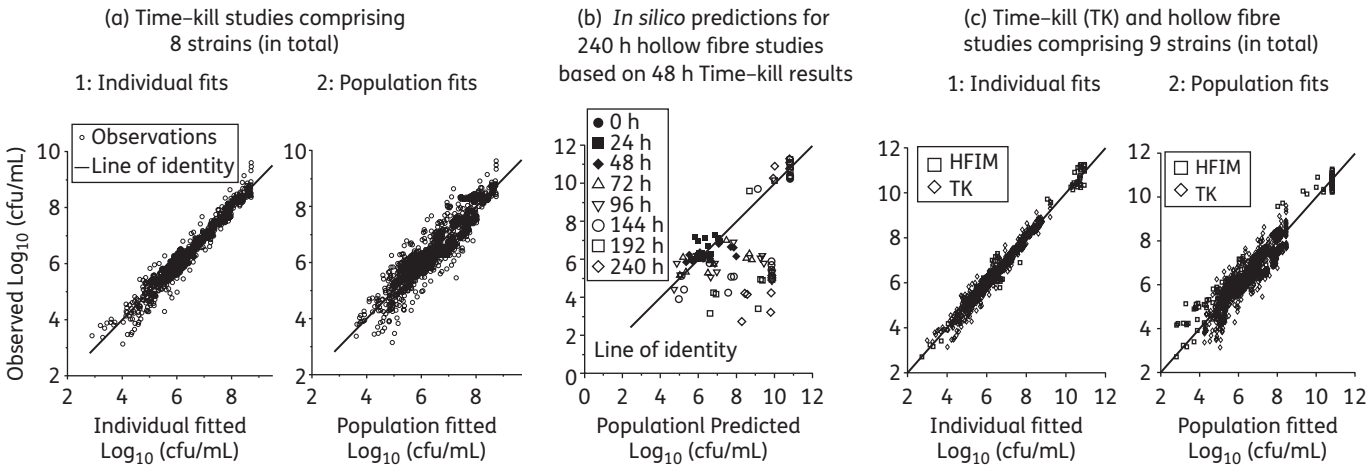

Figure 5. Observed versus individual fitted (a1 and c1), population fitted (a2 and c2) and *in silico* predicted (b) viable counts for the total bacterial population. (a1 and a2) NONMEM results for estimation of eight strains in the 48 h time–kill studies. (b) *In silico* predictions of the hollow fibre study based on the time–kill results with filled symbols representing the first 48 h and open symbols referring to observations at 72–240 h. (c1 and c2) Final model estimated using S-ADAPT (see Table 1) that simultaneously described all data from nine strains in total.

effect of administering linezolid in this fashion had not been examined. We first determined in time–kill experiments that although linezolid has long been considered a bacteriostatic, concentration-independent antimicrobial agent, there was a concentration-dependent response, with increasing concentrations resulting in more killing. Concentration-dependent pharmacodynamics of linezolid in enterococci and staphylococci have been previously reported.[6–8] All tested standard and front-loaded regimens with *f*AUC/MICs between 31 and 495 on days 1–5 and an *f*AUC/MIC of 31 or 124 on days 6–10 prevented emergence of resistance over 10 days for strains 4408, 4407 and ATCC 29212. Strains 4408, 4407 and presumably also ATCC 29212 carried no resistant allele. The *f*AUC/MICs of 31–495 that prevented resistance were in part lower than the previously identified optimal $fAUC_{0-24}$/MIC target of 230 assessed by Zinner *et al*.[8] Further studies are required to elucidate these differences.

Our results for *E. faecalis* strain 4422 (MIC 4 mg/L) showed that very high linezolid doses of 2400 mg every 12 h achieving an $fAUC_{0-24}$/MIC of 495 during the first 5 days could not prevent emergence of resistance. The latter results were in agreement with the $fAUC_{0-24}$/MIC target of 230 identified by Zinner *et al*.[8] Overall, these results suggest that there was notable strain-to-strain variability, with an *f*AUC/MIC of 31 preventing resistance for some strains whereas other strains required an $fAUC_{0-24}$/MIC of 230 (or >124) to prevent resistance.

Front-loading yielded the most pronounced benefit in the rate and extent of killing for strain 4408 (MIC 4 mg/L) carrying 4.3 sensitive alleles and no resistant allele. However, this benefit in killing was less pronounced for strains 4407 and ATCC 29212. For strain 4422 carrying 3.2 resistant alleles at baseline, emergence of resistance was observed for all tested regimens resulting in regrowth over 240 h. Strains 4408, 4407 and 4422 all had the same MIC of 4 mg/L to linezolid. The presence of resistant alleles was associated with the emergence of resistance for strain 4422, and a lack of resistant alleles successfully predicted no emergence of resistance for strains 4407 and 4408. While all studied dosage regimens failed with resistance against strain 4422, the time to emergence of resistance was notably delayed by high-intensity front-loading (Figure 4).

Such front-loading may leave the immune system or a combination antibiotic more time to kill the bacteria less susceptible to linezolid. Although resistance to linezolid has been uncommon in clinical trials, a low spontaneous mutation rate has been reported in VRE.[5] The current study provides further evidence that suboptimal dosing leads to selection of linezolid-resistant VRE. Our study suggested that front-loading may provide additional killing for some, but not all, strains that lack resistant alleles and that high-intensity front-loading may delay, but not prevent, emergence of resistance for strains with an MIC of 4 mg/L carrying resistant alleles. To predict the impact of high-intensity front-loading on platelet-related toxicity of linezolid, the present model should be combined with a mechanism-based toxicodynamic model such as the model proposed by Sasaki *et al*.[19] The toxicodynamic model should ideally account for the time course of linezolid concentrations (as opposed to steady-state AUCs) and for the time course of platelet counts. Such a simulation analysis has the capability to optimize both the intensity and duration of front-loading for future studies.

The population pharmacodynamic model estimated in the present study yielded excellent fits for the time–kill data and for a simultaneous analysis of all data (Figures 3 and 5c). Schmidt *et al*.[20] modelled the effect of two oxazolidinones against methicillin-resistant *Staphylococcus aureus* in a static time–kill and dynamic one-compartment model over 24 h. We chose to assess a longer duration of therapy and developed a translational model that can translate between 48 h time–kill and 10 day HFIMs. The present model additionally proposed an approach to use the number of sensitive alleles as an important covariate to predict the IC_{50} of linezolid. This covariate explained approximately 80% of the variance in IC_{50}, which may be quite beneficial to predict the treatment response to linezolid if the number of alleles is available. This combined genomic and mathematical modelling approach offers the possibility for dose individualization and warrants further investigation.

These findings on front-loading may have significant implications for the optimal treatment of VRE infections. First, they provide evidence that the pharmacodynamic profile of linezolid is concentration dependent at higher exposures and that increased dosage regimens of linezolid may be useful to achieve additional killing in difficult-to-treat strains.[21] Therefore we propose that front-loading may have clinical utility in selected infections that are deep seated, comprise high bacterial inocula, which may be subject to penetration barriers, or require longer durations of treatment (>10 days).[22] In these specific clinical situations, such as the case in bi-lobar pneumonia, where early aggressive therapy is necessary and therapeutic options are limited, exploring novel strategies such as front-loading is warranted. On the other hand, for less severe infections such as uncomplicated skin and soft tissue infections, which have been proven to have high response rates to traditional doses (600 mg every 12 h), there may be limited utility in such an approach. Second, although front-loaded regimens demonstrated increased killing activity, this is balanced with the potential for toxicity, as increasing the cumulative exposure (AUC), primarily driven by duration, has been associated with haematologic toxicities including myelosuppression, anaemia and neutropenia.[23] Our data suggest that front-loading linezolid regimens against VRE to achieve greater efficacy may be considered as a means to decrease the total duration of therapy. Additional lower exposure regimens that follow initial high-intensity regimens or shortening the course of therapy due to front-loading requires additional study and may be promising. We acknowledge a number of potential limitations in the current study. First, these results may not be translatable to other strains that display lower MICs, as only VRE with elevated MICs were selected for analysis in the current study. Second, there have been no human studies evaluating linezolid at such high exposure levels. Therefore additional animal and human studies are necessary before these results are considered in clinical practice. Overall, high-dose regimens of linezolid administered in a front-loading fashion are promising from a pharmacodynamic standpoint for early bactericidal activity and to postpone and potentially suppress resistance.

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