**Pharmacodynamics of Teicoplanin Against Methicillin-Resistant Staphylococcus aureus**

**Running title: Pharmacodynamics of teicoplanin against MRSA**

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ABSTRACT

Objectives: The overall study aim was to identify the relevant pre-clinical teicoplanin PK-PD indices to predict efficacy and suppression of resistance in MRSA infection.

Methods: A hollow fibre infection model and a neutropenic murine thigh infection model were developed. The PK-PD data generated was modelled using a non-parametric population modelling approach with Pmetrics. The posterior Bayesian estimates derived were used to study the exposure-effect relationships. Monte Carlo Simulations from previously developed population PK models in adults and children were conducted to explore the PTA for teicoplanin dosage regimens against current EUCAST wild-typesusceptibility range.

Results: There was a concentration-dependent activity of teicoplanin in both, the in *vitro* and *in vivo* models. A total *in vivo* AUC/MIC of 610.4 (total exposure of 305.2 mg\*h/L) for an MRSA strain with an MIC of 0.5 mg/L was needed for efficacy (2 log10 cell kill) against a total bacterial population. A total AUC/MIC ratio of ~ 1500 (total exposure of ~ 750 mg\*h/L) was needed to suppress the emergence of resistance. The PTA analyses showed that adult and paediatric patients receiving a standard regimen were only successfully treated for the *in vivo* bactericidal target if the MIC of the strain was ≤ 0.125 mg/L in adults and ≤ 0.064 in children.

Conclusions: This study improves our understanding on teicoplanin pharmacodynamics against MRSA and defines an *in vivo* AUC/MIC target for efficacy and suppression of resistance. Additional studies are needed to further corroborate the PK-PD index in a variety of infection models and in patients.

INTRODUCTION

Teicoplanin is a glycopeptide with a similar spectrum of antibacterial activity to vancomycin.1,2 Teicoplanin is licensed in the EU and other countries for the treatment of moderate-to-severe methicillin-resistant Gram-positive infections in children and adults.1 Teicoplanin was developed before contemporary PK-PD techniques and there is little pharmacodynamic information that provides a rationale for the optimal use of this agent.

Drug exposure targets that are associated with a high probability of a successful clinical outcome are relatively poorly defined. Initial regimens were designed to achieve a Cmin of 10 mg/L.3 More recently, this drug exposure target has been increased to > 15 mg/L for the majority of clinical indications (e.g. bacteraemia, pneumonia, complicated skin and soft-tissue infections). Higher targets of 20 and 30-40 mg/L are now advocated for the treatment of bone/joint infections, and infective endocarditis, respectively.1 These updated targets are based on small retrospective studies in adults.4,5

Herein, we describe the pharmacodynamics of teicoplanin against MRSA. We used a well-characterised hollow fibre infection model (HFIM) and a murine thigh infection model of MRSA to establish these relationships. We considered both the antibacterial effect of teicoplanin and the emergence of resistance to teicoplanin as primary study endpoints. We evaluated the relevant PK-PD indices that best described the killing of susceptible sub-populations and prevent emergence of resistance. We finally bridged the experimental results to human patients to reflect on the adequacy of current EUCAST *in vitro* susceptibility breakpoints.

MATERIAL AND METHODS

*Organism, Susceptibility Studies and Mutational Frequency*

MRSA ATCC® 43300™(ATCC®, Middlesex, UK) was used for all experiments. The isolate was stored on beads at -80ºC. The MIC for the strain was determined using the EUCAST broth microdilution methodology on three separate occasions.6 The mutational frequency of a subpopulation with MIC >=8 mg/L was determined using teicoplanin drug containing MH agar (Sigma-Aldrich, Dorset, UK), as previously reported.7

*Bioanalytical Methodology*

Concentrations of teicoplanin were measured using a commercially available fluorescence polarization immunoassay (FPIA; Thermo Fisher Scientific, Germany). This is a homogeneous particle-enhanced turbidimetric immunoassay that utilizes the Quantitative Microsphere System (QMS) technology and was implemented on an automated analyzer Abbott Architect ci4100. The assay is based on competition between drug in the sample and drug coated onto a microparticle for antibody biding sites of the teicoplanin antibody reagent. A concentration-dependent agglutination inhibition curve was obtained with minimum and maximum rate of agglutination at the highest and lowest teicoplanin concentrations, respectively. The limit of quantification (LOQ) was < 3.0 mg/L. The dynamic range was 3-100 mg/L and overall (intra and inter-day) precision was < 6%.

*In vitro Model of MRSA Infection in a Hollow Fibre System*

Teicoplanin for intravenous infusion (Targocid 400 mg, Sanofi Aventis, Surrey, UK) was used for all experiments. A paediatric pharmacokinetic profile was simulated in the HFIM. An elimination half-life of 6.4 hours for teicoplanin was targeted in all the experiments, which was based on a median clearance estimate from a previous population pharmacokinetic model in children.8

For each experiment, fresh bacterial isolates were grown on blood agar (Oxoid Ltd., Hants, UK) and incubated at 37º C for 24 hours. Bacteria were then inoculated into the extra-capillary space of each hollow-fibre (HF) cartridge. The desired starting inoculum (~6 log10 CFU/mL) was confirmed by quantitative culture on MH agar plates (Thermo Fisher Scientific, Runcorn, UK). The HFIM was incubated at 37ºC in ambient air.

*In Vitro Pharmacokinetic and Pharmacodynamic Studies*

Initial dose-finding studies were conducted using concentration-time profiles of teicoplanin corresponding to human dosages of 10-100 mg/kg/day. Dosages that encompassed the exposure-response relationships for bacterial killing and the emergence of resistance were defined in preliminary experiments. The current standard dosing for teicoplanin in adults is 3 loading doses of 400 mg (~6mg/kg) 12 hourly, followed by a maintenance dose of 400 mg/day. In contrast, children receive 3 loading doses of 10 mg/kg 12 hourly, followed by a maintenance dose of 10 mg/kg daily.1

 A total daily dose of 10 and 30 mg/kg/ day of teicoplanin was administered in the following ways: (a) a bolus administered every 24 hours (q24h); (b) two half dosages administered q12h; and (c) the total daily dose infused over a 24 h period (CI). Treatment was initiated 24 hours post-inoculation and thexperiments were conducted for 7 days to simulate the typical duration of clinical therapy and provide the opportunity to observe the emergence of antimicrobial resistance. Fiveexperiments were conducted, each experiment contained 6 study arms (1 control drug-free arm and 5 teicoplanin-treated arms).

*In vitro Pharmacokinetic Studies*

PK samples (1 mL) were withdrawn from the central compartment of the HFIM before each dose and at 1, 3, 6, 12 and 24 hours. Sampling occurred during the first dose interval and at day 6 of therapy. The area under the teicoplanin concentration time curve (AUC0-24), maximum concentration (Cmax or peak) and minimum (Cmin) teicoplanin concentrations at steady state (144-168 h), were calculated from the Bayesian posterior estimates for the PK parameter values from each fibre (see below).

*In vitro Pharmacodynamic studies*

Bacterial samples (1 mL) were withdrawn from the extra-capillary space of each HF cartridges and ten-fold serial dilutions (100μL) were plated to both free and drug-containing agar plates. Sampling occurred at 0, 2, 6 and 24 hours post-infection and every 24 hours thereafter (immediately prior to dosing). Total and resistant subpopulations were quantified. The lower limit of quantification (LOQ) was 1 CFU/mL. To investigate whether the mutants that grew on drug-containing plates had an elevated MIC, approximately 10 colonies were selected from each plate and the MIC was re-estimated as previously described.

*Pharmacokinetic and Pharmacodynamic Studies in a murine thigh infection model*

A well-characterised neutropenic murine thigh infection model was used to provide a complementary perspective on the pharmacodynamics of teicoplanin to that provided by the HFIM. All experiments were conducted under UK Home office project license PPL 40/3630 and approved by the University of Liverpool’s Animal Welfare and Ethics Review Board. The mice were housed in vented HEPA-filtered cages, and food and water were provided ad libitum.

Male CD1 mice (16-20 g) were rendered neutropenic on day -4 and -1 with the intra-peritoneal administration of cyclophosphamide (Baxter, Liverpool, UK) (150 and 100 mg/kg, respectively).9 Each experiment (n=3) comprised a control and 3 cohort treated arms, nine mice were studied per cohort group. On day 1, mice were inoculated with 2 x 106 CFU/mL MRSA (43300) in each posterior thigh muscle in a 50 μL volume. Teicoplanin was commenced 2 h post-infection and was administered i.v. every 12 hours.

*In vivo Pharmacokinetic studies*

Groups of mice (n=9 per dosage cohort) received dosages of 2.5, 15 and 100 mg/kg/day (as multiple 12-hourly i.v. doses) that were chosen to investigate the total bactericidal effect and the suppression of resistance based on preliminary dose-finding studies. PK sampling was performed at: 0, 0.5, 1, 2, 4 and 12 hours post-dose. Three mice were used per dose-time-point. Samples were immediately spun and plasma stored at -80ºC until analysis.

*In vivo Pharmacodynamic studies*

At the time of sacrifice (at 2, 12 and 26 h post-infection) both thighs were aseptically removed and placed in separate culture tubes with 2 mL PBS. Samples were individually homogenised using a polytron disperser VDI 12 (VWR, Lutterworth, UK). One hundred μL aliquots from 10-fold serial dilutions were plated to drug free and MH agar containing teicoplanin 8 mg/L. The mean value of the bacterial burden from the left and right thigh from each mouse was determined. The mean and SD of a group of three mice was then calculated.

*PK-PD Mathematical Modelling*

A mathematical model was fitted to the experimental data from the HFIM and mice. The population PK program Pmetrics was used for all fitting (v.1.2.9, University of Southern California, Los Angeles, CA)10 for R (version 3.1.0, Institute for Statistics and Mathematics, Vienna, Austria). 11 The structural model took the form:

 (1) $\frac{dX1}{dt}= R\left(1\right)-\left(\frac{CL}{Vc}\right)\*X1$

(2) $\frac{dx2}{dt}=KgmaxS\*X2\*\left(1-\frac{\left(X2+X3\right)}{POPmax}\right)-\left(\frac{KkmaxS\*x2\*\left(\frac{x1}{Vc}\right)^{Hs}}{(C50S^{Hs}+\left(\frac{x1}{Vc}\right)^{Hs}}\right)$

(3) $\frac{dx3}{dt}=KgmaxR\*X3\*\left(1-\frac{\left(X2+X3\right)}{POPmax}\right)-\left(\frac{KkmaxR\*x3\*\left(\frac{x1}{Vc}\right)^{HR}}{(C50R^{HR}+\left(\frac{x1}{Vc}\right)^{HR}}\right)$

Equation 1 describes the rate of change of the amount of teicoplanin (mg) in the central compartment (X1). Equation 2 and 3 describe the rate of change of burden of a susceptible bacteria population (S) and a resistant/mutant (R) bacteria population in the HFIM/murine model. The rate of growth is a balance between bacterial growth and death. POPmax (CFU/mL) is the theoretical maximum bacterial density; KgmaxS/R (Log10CFU/h) is the maximum rate of bacterial growth in both subpopulations; KkmaxS/R (Log10 CFU/h) is the rate of bacterial killing induced by teicoplanin; C50S/R (mg/L) represent the teicoplanin drug concentrations that produce half-maximal killing in both sub-populations; HS/HR are the respective slope functions.

There were three output equations for the model: (1) Y(1)=X(1)/V, which described the time course of teicoplanin concentrations; (2) Y(2)=DLOG10(X(2)+X(3)), which described the time course of the total population; and Y(3)=DLOG10(X(3)), which described the time course of the resistant subpopulation.

The goodness of fit of each model to the data was assessed using a combination of the following: (1) the log-likelihood value, (2) the Akaike information criterion (AIC), (3) the coefficient of determination (*r2*) from the linear regression of the observed-predicted plots before and after the Bayesian step, and (4) a minimization of bias and imprecision values of the observed-predicted plots.

*Exposure-effect relationships and optimal targets*

To determine the PK-PD index that best described bacterial killing and the suppression of drug resistance, scatter plots from the *in vitro* dose-fractionation studies were constructed that related the *f*AUC:MIC, *f*Cmax:MIC and *f*Cmin:MIC with both the observed antibacterial effect and the emergence of a drug resistant subpopulation from the HFIM. The relevant PK-PD index was then further explored with the *in vivo* mice data. A non-linear regression model was fitted to the data using Prism software (GraphPad Software Inc., La Jolla, CA). The coefficient of determination and visual examination of the fit was used to discriminate the various pharmacodynamic indices.

*Monte Carlo Simulations and PTA analysis*

 Monte Carlo simulations were performed with Pmetrics. A population of 1,000 simulated patients was generated for each teicoplanin regimen. These simulated patients were used to examine the outcomes of candidate regimens of teicoplanin. For the adult simulations we used the population PK estimates and covariance matrix from a previously developed population PK model in adults.8 For the paediatric simulations, we used the population PK estimates and covariance matrix from a previously developed PK model in children (submitted for publication). For children, the weight-based dose of teicoplanin (mg per kg) was administered to each simulated patient as a 3-minute i.v. infusion. The weight-based dosage was converted to an absolute dosage for i.v. administration (as would happen at the bedside) by multiplying by the simulated weight (for example, a 20 kg child receiving 10 mg/kg would receive 200 mg of teicoplanin). The following covariate limits were used for the simulations: weight: 9-62.2 kg; age: 1-16 years old and estimated GFR (Schwartz): 49-178.1 mL/min/1.73 m2). A variety of candidate regimens were investigated: the standard teicoplanin dosing and regimens 2 and 3 with the same daily maintenance dose but different frequency of dosing (q24h vs q12h). We considered this relevant to evaluate the impact of the frequency in AUC and Cmin attained drug expsoures Estimation of various drug exposures in terms of AUC0-24 and Cmin at steady state (between days 3 and 4 of treatment) were performed for each dosage regimen for both adults and children. The selection of an AUC and Cmin time frame (72-96 h) is based on currently steady state SPC recommendations for TDM.

For the PTA analysis, a target AUC/MIC of stasis and 2 log10 cell kill in the murine model were used. The distribution of the MRSA MICs determined using EUCAST methodology from 0.032 to 16 mg/L were plotted.12 Fractional target attainment was calculated for the various regimens to identify the achievement of the AUC/MIC targets by comparing the PTA against the teicoplanin MIC distribution for MRSA.

RESULTS

*In vitro susceptibility and mutational frequency*

The modal teicoplanin MIC for the MRSA ATCC 43300 was 0.5 mg/L. The strain was oxacillin resistant by E-test (Oxoid). The mutational frequency (at 16 x MIC) was 1.02 x 10-7. MICs from resistant mutants at the end of *in vitro* experiments (168 h) were up to five two-fold dilutions higher (2 to ≥ 16 mg/L).

*Pharmacodynamics of Teicoplanin Against MRSA: Dose Finding Studies and Dose Fractionation Studies in the HFIM*

There was a dose-dependent decline in the total bacterial density following exposure to teicoplanin. Total bactericidal effect was achieved with dosages of ≥ 10 mg/kg/day q24h and suppression of resistance with dosages ≥ 30 mg/kg/day q24h.

 The results from the dose fractionation studies are shown in Figure 1. Non-linear regression analysis showed a strong correlation between *f*Cmax: MIC, *f*AUC:MIC and *f*Cmin:MICand bacterial killing (*r2* = 0.92, 0.9 and 0.86 respectively). There was also a strong association between *f*Cmax:MIC and *f*AUC:MIC and suppression of teicoplanin resistance (*r2* =0.79, 0.95 respectively). In contrast, a weaker relationship was apparent between *f*Cmin:MIC and suppression of teicoplanin resistance (*r2*=0.45).

*Murine MRSA thigh infection model*

 There was a dose-dependent decline in total bacterial density in the thighs of mice treated with teicoplanin. Teicoplanin was bactericidal (≥ 2 log10 cell kill) for all regimens. There was emergence of a resistant sub-population with all regimens investigated with the appearance of an inverted U (Figure 2).

*PK-PD mathematical modelling*

There was a good fit of the PK-PD data. For the *in vitro* model, the linear regression of observed versus predicted values before and after the Bayesian step had a coefficient of determination of a) *r2*= 0.83 and 0.9, respectively, for the PK data; b) *r2*= 0.55 and 0.7 for the PD data related to the total population and c) *r2*= 0.6 and 0.7 for the PD data related to the resistant bacterial population. For the *in vivo* model, the linear regression of the observed-predicted values for the population and for the Bayesian posterior had a coefficient of determination of a) r2=0.87 and 0.99, respectively; b) 0.94 and 0.95 and c) 0.47 and 0.63, all with minimal bias and imprecision.

The population PK-PD parameter estimates from the HFIM and the murine thigh model are shown in table 1. The estimated t1/2 of teicoplanin in the HFIM was 8.7 hours.

*Exposure-effect relationships and optimal targets*

In the HFIM, a teicoplanin *f*AUC/MIC ratio at steady state ≥ 576 mg\*h/L was associated with a 2 log10 CFU/mL decline in bacterial density and ≥ 1325 was required to suppress the emergence of resistant clones (Figure 1a). For the resistance studies, a characteristic “inverted U” was observed with maximal amplification of resistance with a *f*AUC:MIC of approximately 400-600 mg\*h/L (Figure 1a).

Based on the HFIM studies (above), the AUC:MIC was used as the dynamically linked index for both effect and the emergence of resistance in the mouse (Figure 2). A teicoplanin total drug AUC0-24/MIC ratio of ≈ 88.8 and 610.4 mg\*h/L was associated with stasis and near maximal bacterial killing (2 log10 cell kill), respectively. A total drug AUC0-24:MIC ratio ≥ 1400-1500 mg\*h/L was required for near complete suppression of emergence of resistance. Maximal amplification of resistance was seen with total drug AUC0-24/MIC ratios of 500-700 mg\*h/L (Figure 2b).

*Monte Carlo Simulations and PTA analysis*

The PTA analyses showed that adult and paediatric patients receiving a standard dosage regimen for a target of a total AUC/MIC ratio of 610.4 were only successfully treated (≥ 90% probability of success) if the MIC of the strain was ≤ 0.125 mg/L in adults and ≤ 0.064 in children. The achievement of AUC/MIC targets required for *in vivo* stasis (AUC/MIC 88.8) allowed a successful treatment of strains with MICs ≤ 1 in adults and ≤ 0.5 in children. If an increased teicoplanin dosage was used along with a pharmacodynamic target of 88.8, then a satisfactorily high probability of target attainment was achieved for MIC values ≤ 2mg/L in both adults and children. The PTA plots and fractional target attainment are shown in figure 3.

Calculations of drug exposures simulated at steady state (days 3-4) in terms of AUC0-24 and Cmin are shown in table 2 for each population (adults and children) and dosage regimen simulated.

DISCUSSION

In this study the AUC:MIC ratio was the pharmacodynamic index that best links the administration of teicoplanin with the antibacterial effect and the suppression of resistance. A total drug AUC/MIC ratio of 88.8 mg\*h/L and 610.4 results in stasis and a 2-log cell kill, respectively. A previous study in mice also suggests teicoplanin displays concentration-dependent antibacterial activity with both Cmax/MIC and AUC/MIC identified as the relevant pharmacodynamic indices.13 We demonstrate that all pharmacodynamic targets result in the amplification of resistant mutants (Figure 2). Thus, the antibacterial effect resulting from clinically relevant drug exposures is always tempered by the emergence of drug resistance, and the two are inextricably related. Higher teicoplanin exposure thresholds (e.g. AUC:MIC ≈ 1500) that are predicted to prevent the emergence of resistance are not obtainable using current regimens.

The magnitude of drug exposure that produces stasis in immunocompromised mice is relevant for patients with *S. aureus* bacteraemia (oritavancin and linezolid), and complicated skin and skin structure infections (linezolid and tigecycline).14–19 A teicoplanin AUC:MIC of 88.8 is likely relevant for patients with skin and skin structure infections. As it can be seen from Figure 3, such a target is readily achievable for simulated patients receiving currently licensed regimens with fractional target attainment rates of 84% and 94% in children and adults, respectively. For more serious staphylococcal infections, such as pneumonia, a higher pharmacodynamic target is generally required.18,20 An AUC:MIC of 610.4 produces logarithmic bacterial killing in mice. This estimate is consistent with several retrospective clinical studies in which teicoplanin AUCs ≥ 750-800 mg\*h/L and AUC:MIC≥ 900 are associated with favourable clinical outcomes in adults with serious MRSA infections.21–23 A higher AUC:MIC ratio is also consistent with the recent upward revision in target Cmin concentrations recommended in the SPC for the treatment of patients with deep-seated staphylococcal infections.1 The difficulty of achieving these higher exposure targets with currently recommended regimens is highlighted by the fact that fractional attainment rates are only 10% and 29% of simulated adults and children, respectively.

The administration of a fixed teicoplanin regimen is reasonable for skin and skin structure infections. Standard teicoplanin therapy results in target attainment in a majority of patients and the wild-type population can be largely covered. In contrast, more serious infections that require higher drug exposures require routine TDM. Too few patients receiving a fixed regimen achieve an adequate AUC:MIC of 610.4 and are at risk of concentration-dependent therapeutic failure. For these patients, drug measurement and dosage adjustment is required. An alternative strategy is the use of combination chemotherapy. There is a large amount of preclinical data that has explored the potential benefits of combining glycopeptides with rifampicin, fosfomycin and β-lactams.24–28 These ideas are being studied in several prospective clinical trials. ARREST (ISRCTN 37666216) is evaluating the potential benefit of adjunctive rifampicin in *S. aureus* bacteraemia,29 and CAMERA-2 (NCT02365493) is investigating the combination of glycopeptides with β-lactams to treat hospitalised patients with MRSA bacteraemia.30

Monte Carlo simulations and the PTA analysis suggest that patients infected with strains at the upper end of the wild type distribution may be difficult to treat. This appears to be the case even if a stasis endpoint is used. The ECOFF using EUCAST methodology is 2 mg/L.12 At this MIC, only 60% and 70% of children and adults, respectively, receiving a standard regimen are predicted to achieve a stasis target. This is strong evidence to consider a reduction in the breakpoint by at least a dilution to 1 mg/L.31 For more serious infections that require higher pharmacodynamic targets the situation is worse and the breakpoint divides the wild-type population.

We must acknowledge that only a single strain was studied in the murine model and HFIM. Nevertheless, two experimental models were used that yielded complementary information and allowed us to exploit the respective strengths of each. The murine thigh model is well characterised, has the advantage of simulating protein binding and is a mimic of complicated skin and skin structure infections that are common clinical problems. Nevertheless, the model implies some assumptions that need to be considered when interpreting the findings. Protein-binding in mice has been reported to be similar than in humans (~90%) after a single dose,3,32however, many factors can influence the degree of protein binding *in vivo,* particularly in the patient (i.e disease status, drug concentration, nature and concentration of proteins, interaction with other drugs and endogenous substances, etc). Its effect on the drug´s activity and tissue penetration in the murine model with respect to its clinical relevance, is still poorly understood and warrants further study 33.In contrast, the hollow fibre infection model enabled us to explore the pharmacodynamic relationships important for the suppression of resistance by means of using a higher inoculum size (10^9 vs 10^6 CFU/mL or g, respectively) and a longer duration of therapy (7 days *versus* 24 h). Notably, an inoculum effect needs to be taken into account as it has been shown to be particularly important in staphylococci, where 3-10 fold increase in drug exposures, were required for *in vivo* stasis as the inoculum increased from 5 to 7 log10.34The clinical relevance of these higher inocula used to evaluate the emergence of resistance remains to be cross-validated.

Together, the findings from these two model systems and pharmacodynamic analyses suggest that teicoplanin should be used with some caution for the treatment of strains with MICs at the upper edge of the wild type population. Therapeutic drug monitoring is a requirement for the treatment of serious infections to minimise the probability of concentration dependent therapeutic failure.

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TABLES and FIGURES

|  |  |
| --- | --- |
| Population PK parameter in vitro model(free drug concentrations) | Population PK parameter in vivo model(total drug concentrations) |
|  | Mean (SD) | Median | Mean (SD) | Median |
| Cl (L/h) | 0.04 (0.02) | 0.04 | 0.08 (0.05) | 0.06 |
| Vc (L) | 0.5 (0.2) | 0.5 | 0.6 (0.4) | 0.34 |
| Population PD parameter in vitro model | Population PD parameter in vivo model |
| KgmaxS (mg/L\*h-1) | 0.62 (0.19) | 0.68 | 0.42 (0.03) | 0.4 |
| KgmaxR (mg/L\*h-1) | 0.15 (0.09) | 0.2 | 0.09 (0.06) | 0.06 |
| Popmax (mg/L) | 1.6^109 (1.2^109) | 1.3^109 | 1.3^108(8^107) | 2^108 |
| H | 8.6 (2.6) | 9.9 | 13.7 (3.7) | 15.1 |
| HR | 13.9 (2.6) | 16 | 14.1 (5.2) | 17.1 |
| KkmaxS (mg/L\*h-1) | 0.7 (0.2) | 0.7 | 0.52 (0.06) | 0.51 |
| KkmaxR (mg/L\*h-1) | 0.7 (0.4) | 0.4 | 0.75 (0.15) | 0.79 |
| EC50S (mg/L) | 11.6 (3.6) | 11.8 | 1.2 (0.3) | 1.2 |
| EC50R (mg/L) | 40.2 (9.9) | 38.03 | 64.1 (9.8) | 70 |
| IC1 (mg/L) | 9.8^104 (9.4^104) | 10^103 | 4^104 (8^103) | 3.6^104 |
| IC2 (mg/L) | 20.2 (15.2) | 13.04 | 159 (162) | 206 |

Cl= Clearance ; Vc=Volume of distribution in the central compartment; Kgmax= maximum rate of bacterial growth; Popmax= theoretical maximum bacterial density; H=Hill slope; C50= Teicoplanin concentration producing half-maximal bacterial kill; IC= initial condition in bacterial density (1 for the susceptible population and 2 for the resistant population). S and R correspond to the total susceptible population and the resistant bacterial population, respectively.

Table 1. Population PK and PD parameter estimates from the HFIM and the neutropenic mice thigh model.

|  |  |  |
| --- | --- | --- |
| Dosage regimen simulated | AUC72-96 median (IQR) (mg\*h/L) | Cmin 96 h median (IQR) (mg/L) |
| Children |
| 1) Standard: 10 mg/kg x 3LD q12 + 10 mg/kg q24h  | 237.4 (114-475.5) | 5.4 (1.7-13.4) |
| 2) 30 mg/kg x 3LD q12h+30 mg/kg q24h  | 711.4 (342.1-1426.2) | 16.2 (5.1-40.1) |
| 3) 30 mg/kg x 3LD q12h+15 mg/kg q12h  | 560.05 (258.7-1298.6) | 19.7 (7.9-47.7) |
| Adults |
| 1) Standard: 400 mg x 3LD q12 +400 mg q24h | 205 (170.9-254.4) | 8.72 (7.23-10.8) |
| 2) 800 mg x 3LD q12h + 800 mg q24h | 410.7 (342.1-509.6) | 17.4 (14.5-21.6) |
| 3) 800 mg x 3LD q12h + 400 mg q12h | 499.03 (413.4-600.4) | 20.96 (17.2-25.5) |

Table 2. Simulated drug exposures (AUC72-96 and Cmin 96) in children and adults between days 3 and 4 of teicoplanin therapy for standard and dosage increased regimens.



Figure 1. Teicoplanin pharmacodynamics against MRSA in the HFIM. An Emax sigmoid model was fitted to the data (Bayesian individual posterior estimates from the population PK-PD model). a) *f*AUC (144-168 h) vs total and resistant bacterial density at the end of therapy (168 h); b) *f*Cmax (145 h) vs total and resistant bacterial density at the end of therapy and c) *f*Cmin (144 h) vs total and resistant bacterial density at the end of therapy. On the right colum, the effect against the resistant bacterial populations is shown. A dashed line shows the limit of quantification (LOQ) for bacterial load.



Figure 2. Teicoplanin total AUC/MIC ratio against MRSA total and resistant bacterial density from the 26 h mouse thigh infection model.

Effect=8.23-((5.24x (AUC:MIC)0.94 )/((AUC:MIC)0.94 +98.50.94). Each symbol (filled circle) represents the mean of data for three mice. Figure 2(a) shows the concentration-dependent decline in the total bacterial density reaching ≥ 2 log10 cell kill CFU/g after AUC/MIC ratios of 600 mg\*h/L. Thin dashed lines represent the mean bacterial load at the start of therapy (stasis) and 1 and 2 log10 drop cell kill. Thick dash line represents the LOQ. Figure 2(b) shows the characteristic “Inverted U” phenomenon in the resistant subpopulation with amplification of resistance at AUC/MIC ratios between ~500-700 mg\*h/L and near maximal effect at ≥1500 mg\*h/L.



Figure 3. Monte Carlo Simulations and PTA analysis: a) PTA in adult patients receiving standard and increased dosage regimens for a target AUC/MIC of 88.8 and 610.4 mg\*h/L by day 3-4 of therapy; b) PTA in paediatric patients (1-16 years old) with standard and increased dosage regimens for a target AUC/MIC of 88.8 and 610.4 mg\*h/L. The underlying continuous grey line on each plot represents the wild-type EUCAST reported distribution for teicoplanin against MRSA. The tables show the fractional target attainment for each of the simulated dosage regimens against the MRSA teicoplanin MIC distribution.