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Genetic polymorphisms of *ABCB1* (P-glycoprotein) as a covariate influencing daptomycin pharmacokinetics: a population analysis in patients with bone and joint infection

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Background: Daptomycin has been recognized as a therapeutic option for the treatment of bone and joint infection (BJI). Gene polymorphism of *ABCB1*, the gene encoding P-glycoprotein (P-gp), may influence daptomycin pharmacokinetics (PK).

Objectives: We aimed to examine population PK of daptomycin and its determinants, including genetic factors, in patients with BJI.

Patients and methods: We analysed data from patients who received daptomycin for BJI between 2012 and 2016 in our regional reference centre and who had measured daptomycin concentrations and P-gp genotyping. A population approach was used to analyse PK data. In covariate analysis, we examined the influence of three single nucleotide variations (SNVs) of *ABCB1* (*3435C>T*, *2677G>T/A* and *1236C>T*) and that of the corresponding haplotype on daptomycin PK parameters. Simulations performed with the final model examined the influence of covariates on the probability to achieve pharmacodynamic (PD) targets.

Results: Data from 81 patients were analysed. Daptomycin body CL (CL_{DAP}) correlated with CL_{CR} and was 23% greater in males than in females. Daptomycin central *V* (*V*1) was allometrically scaled to body weight and was 25% lower in patients with homozygous CGC *ABCB1* haplotype than in patients with any other genotype. Simulations performed with the model showed that sex and P-gp haplotype may influence the PTA for high MIC values and that a dosage of 10 mg/kg/24 h would optimize efficacy.

Conclusions: Daptomycin dosages higher than currently recommended should be evaluated in patients with BJI. Gender and P-gp gene polymorphism should be further examined as determinants of dosage requirements.

Introduction

Daptomycin is a lipopeptide antibacterial agent that is active against Gram-positive bacteria. Currently daptomycin is approved for the treatment of complicated skin and soft tissue infections, and bacteraemia and endocarditis caused by *Staphylococcus aureus*. Although still off-label usage, daptomycin is recognized as a therapeutic option for staphylococcal bone and joint infection (BJI) by the IDSA, with a recommended dosage of 6 mg/kg.[1]

Experimental studies have demonstrated that the antimicrobial effect of daptomycin is concentration dependent, with both C_{max}/MIC and AUC/MIC being correlated with the killing effect.[2–4] In addition, it has been shown that serum creatine phosphokinase

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(CK) elevation, a marker of muscular toxicity of daptomycin, was linked with the trough concentration of daptomycin (C_{min}), with a greater risk for $C_{min} \geq 24.3$ mg/L.[5] These pharmacokinetic (PK)/pharmacodynamic (PD) relationships are arguments supporting therapeutic drug monitoring (TDM) of daptomycin, although this practice is not officially recommended.[6]

It has been suggested that higher doses of daptomycin (>6 mg/kg and even >9 mg/kg) may be more effective,[7–10] but concerns have been raised about a potentially greater risk of adverse drug reactions.[8,11,12]

Previous population PK studies on daptomycin have shown that renal function, body weight and acute infection were the most significant covariates influencing daptomycin PK.[13–15] It is uncertain how these results can be extrapolated to patients with BJI because these patients usually receive prolonged treatment. To our knowledge, our group has performed the only daptomycin population PK study in patients with BJI. This study showed substantial inter-individual variability in daptomycin PK, but also significant intra-individual variability of daptomycin body CL (CL_{DAP}), the latter being uncorrelated with changes in renal function.[16]

P-glycoprotein (P-gp) is a transmembrane efflux transport protein encoded by the ATP-binding cassette B1 (ABCB1) gene, *ABCB1* [also known as the MDR 1 gene (*MDR1*)]. P-gp is expressed in many cells and tissues (e.g. enterocytes, hepatocytes and renal tubular cells) and has been shown to influence the disposition of many drugs.[17] Gene polymorphisms of *ABCB1* have been associated with changes in drug PK and effects.[18]

It has been shown that P-gp influences the intracellular concentration and activity of daptomycin in macrophages and kidney cells.[19] A recent study in 23 patients has shown an association between gene polymorphisms of *ABCB1* and CL_{DAP}.[20]

Our aims were to study the population PK of daptomycin and its determinants, including *ABCB1* genotype, in patients with BJI, and to assess the potential influence of those determinants on daptomycin efficacy and toxicity.

Patients and methods

Patient population and data collection

This was a single-centre retrospective study in our regional reference centre for the management of complex BJI (CRIOAc Lyon; http://www.crioac-lyon.fr). We analysed data from all patients who received daptomycin for BJI between 2012 and 2016 as salvage therapy in usual care and practice and had measured daptomycin concentrations as well as determination of *ABCB1* genotype.

Patients' written informed consent was obtained before P-gp genotyping, in accordance with French regulation. Patients were informed orally and by letter that data were being collected in an officially declared national database. This study was subject to a declaration to the local Commission for Data Protection and Liberties (number 17-057) and is registered on ClinicalTrial.gov (number NCT03134521).

Daptomycin TDM has been performed in our centre since 2012 to ensure patient safety, as doses of daptomycin greater than those recommended for approved indications are often used for BJI and the muscular toxicity of daptomycin has been related to drug concentration. TDM can also permit avoidance of underexposure that may compromise the efficacy of this concentration-dependent agent. Daptomycin concentrations were measured using an HPLC assay with a photodiode array detector. The method was adapted from an earlier publication.[21] The lower limit of quantification was 2 mg/L. The interday precision was less than 11% with a bias lower than 8%. TDM of daptomycin typically included three timepoints: pre-dose (C_{min}); 30 min after the end of the 30 min infusion (C_{max}); and a last sample obtained 5 to 6 h post-infusion. TDM could be performed on several occasions (on average every month) in each patient. Data collected on all occasions in all patients were analysed simultaneously.

The three most common single-nucleotide variations (SNVs) of *ABCB1* (*3435C>T* rs1045642, p.I1145I; *2677G>T/A* rs2032582, p.A893S/T; and *1236C>T* rs1128503, p.G412G) were detected using a Taqman™ real-time PCR assay. These variants are characterized by strong linkage disequilibrium and the haplotypes CGC/CGC and TTT/TTT have been described in most ethnic groups.[18] In the present study, Hardy–Weinberg and linkage disequilibrium analyses were performed by using the Genepop and SNPStats web tools.[22,23]

Other variables were collected from the computerized patient files, including age, sex, weight, serum creatinine, estimated glomerular filtration rate (eGFR) estimated by the four-variable modification of diet in renal disease (MDRD) equation, CL_{CR} estimated by the Cockcroft–Gault equation, serum CK, dosing history of daptomycin and concomitant use of rifampicin, which is a known inducer of P-gp that may be used in combination with daptomycin in patients with BJI.

Population PK analysis

A population approach was used to analyse PK data. Non-linear mixed-effects modelling was performed using the Stochastic Approximation Expectation Maximization (SAEM) algorithm implemented in the Monolix software (version 4.2.2, Lixoft, Antony, France). Various structural, residual error, covariance and covariate models were assessed using a stepwise approach.

We assumed log-normal distributions of PK parameters, which means an exponential model of inter-individual variability. As most patients had daptomycin TDM repeated on several occasions, we also incorporated intra-individual variability into the model. This was implemented by using the inter-occasion variability (IOV) routine of Monolix. The inter-individual and intra-individual variabilities were coded in Monolix as follows:

$$P_{ik} = C_{ik}\mu \times \exp(\eta_i) \times \exp(\kappa_{ik}) \quad (1)$$

where P_{ik} is the parameter value of individual i on occasion k, C_{ik} is the matrix of covariates of individual i on occasion k, μ is the mean population parameter value (fixed effect) and η_i and κ_{ik} are the inter-individual and intra-individual variability parameters, respectively. The random effects η_i and κ_{ik} were assumed to follow normal distributions: $\eta_i \sim N(0, \Omega)$ and $\kappa_{ik} \sim N(0, \Gamma)$. Intra-individual variability was set on CL_{DAP} and central *V* only.

In covariate modelling, the following variables were examined: sex, age, body weight, serum creatinine, CL_{CR} estimated with the Cockcroft–Gault equation, eGFR estimated with the MDRD and with the Chronic Kidney Disease Epidemiology study (CKD-EPI) equations, and the co-administration of rifampicin. Regarding the genetic variables, we first considered each individual *ABCB1* SNV as a binary variable for the status WT or double mutation. For example, for the *3435C>T* SNV, we coded patients as carriers of the WT (CC, yes or no) and double-mutated (TT, yes or no) variant. We also examined the influence of the haplotype based on the three SNVs (*3435C>T*, *2677G>T/A* and *1236C>T*) and used the status CGC/CGC and TTT/TTT as binary variables.

The change in the objective function value (−2 log-likelihood) was used to assess the influence of covariates, assuming a χ^2 distribution with appropriate degrees of freedom. Statistical significance was set at a *P* value of 0.05 for forward selection and 0.01 for backward deletion of a covariate in the model.

The best final model was based on the classical criteria including objective function, parameter values along with their standard errors, plots of observed versus predicted concentrations, plots of residuals and visual

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predictive checks (simulation-based diagnostic based on 1000 replicates of each patient dataset).[24,25]

PK/PD simulations

PK/PD simulations were performed with the final model to investigate the influence of daptomycin dose and that of categorical covariates included in the final model (i.e. sex and *ABCB1* haplotype; see below) on the probability to achieve predefined target exposure. All simulations were performed with the Pmetrics R package.[26] Final estimates of the mean and variance of PK parameters obtained with Monolix were imported into Pmetrics.

We simulated patients with variable CL_{CR}. A truncated (minimum 31 mL/min; maximum 200 mL/min) normal distribution was used, with a mean of 108 mL/min and SD of 47 mL/min, in accordance with data from the study population. Because we simulated weight-based dosages, a fixed weight of 70 kg was used for all simulated patients. Three dosages were examined: 420 mg/24 h (6 mg/kg), 560 mg/24 h (8 mg/kg) and 700 mg/24 h (10 mg/kg). Results were analysed after the fifth dose to reflect the steady-state. We considered four groups of sex and *ABCB1* haplotypes: females without CGC/CGC haplotype, males without CGC/CGC haplotype, females with CGC/CGC haplotype and males with CGC/CGC haplotype. We simulated 1000 daptomycin PK profiles for each dosage and patient group.

Table 1. Characteristics of the study population

Characteristic	Value
Number of patients (female/male)	81 (34/47)
Age (years)	60 ± 18
Weight (kg)	79 ± 20
eGFR (mL/min/1.73 m^2)	100 ± 41
Measured daptomycin concentrations (*n*)	577
TDM occasions per patient (*n*)	2.5 ± 7.9
Daptomycin initial dose (mg/kg per 24 h)	8.0 ± 1.9

Data are given as mean ± SD, unless otherwise stated.

We then evaluated the probability of achieving predefined targets of efficacy for daptomycin into bone. First, we assumed that only the free fraction of daptomycin (i.e. unbound to plasma protein) can penetrate into bone tissue. This has been suggested by a PK study from Traunmuller *et al*.,[27] who reported a mean $fAUC_{24}$ ratio (tissue/plasma, over 24 h) of 1.17 into metatarsal bone. We then derived AUC_{24}/MIC and C_{max}/MIC targets for free plasma concentrations based on the PD study performed by Safdar *et al*.[2] in mice. This group reported that an AUC_{24}/MIC ratio ≥666 (total concentration) and a C_{max}/MIC ratio ≥129 (also in total concentrations) were associated with bactericidal effect (1 $\log_{10}$ killing) against *S. aureus*. As the rate of protein binding reported by Traunmuller *et al*.[27] in humans (91%) was similar to that reported in mice (90%),[2] we can assume that the exposure targets identified in the mouse model are valid in humans. After correction for 91% protein binding, this led to the following free plasma concentration targets: $fAUC_{24}$/MIC ≥66 and fC_{max}/MIC ≥12.

For each simulation, we evaluated the probability of achieving these efficacy targets for the following MIC values: 0.064, 0.125, 0.25, 0.5, 1, 2 and 4 mg/L. These are representative of MICs reported by EUCAST for *S. aureus*, for which the daptomycin epidemiological cut-off (ECOFF) is 1 mg/L (https://mic.eucast.org/Eucast2/). We considered a PTA ≥90% as optimal. Cumulative fraction of response was also calculated, by using the MIC distribution reported by EUCAST for *S. aureus*.

For each simulation, we also calculated the probability of achieving a safety target of C_{min} ≥24.3 mg/L, which was the threshold associated with increased risk of CK elevation identified by Bhavnani *et al*.[5]

Results

Population data

Data from 81 patients collected between March 2012 and December 2016 were analysed. A total of 577 daptomycin concentrations were collected, with 2.5 ± 7.9 TDM occasions per patient. Patient characteristics are shown in Table 1.

Results of Hardy–Weinberg and linkage disequilibrium analyses are shown in Table S1 (available as Supplementary data at *JAC* Online). Allele frequencies for the three loci were in accordance with Hardy–Weinberg equilibrium. Results also showed linkage

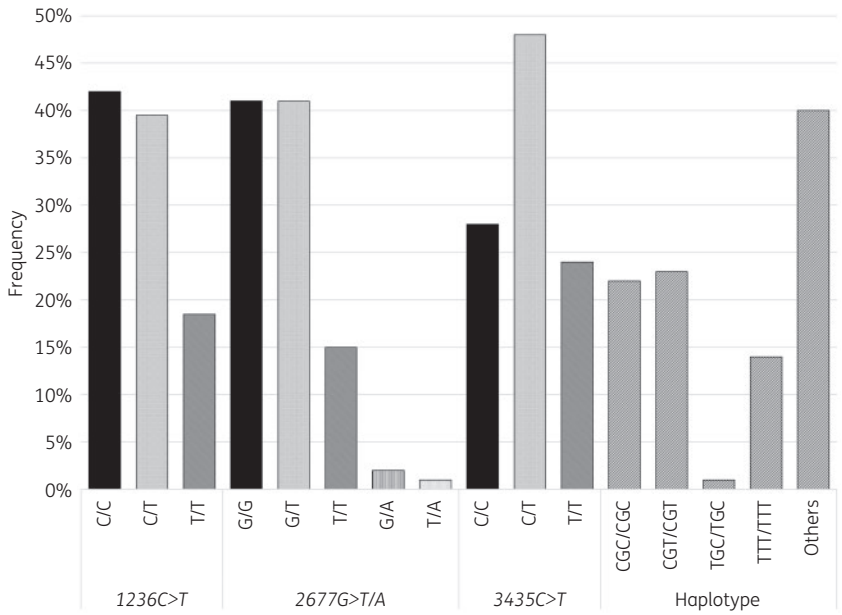

Figure 1. Distribution of *ABCB1* SNVs and haplotypes.

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disequilibrium, which confirmed haplotype association of the three SNVs. The distributions of *ABCB1* genotypes are shown in Figure 1. The haplotype CGC/CGC was found in 18 patients (22%).

Regarding measured concentrations, mean C_{min} (measured pre-dose concentration), C_{max} (measured concentration 30 min after the end of the infusion), AUC and $t_{1/2}$ on the first TDM occasion were 18.7 ± 14.0 mg/L, 72.0 ± 19.4 mg/L, 949 ± 337 mg·h/L and 18.5 ± 13.3 h, respectively.

We observed 45 C_{min} values (22.5%) >24.3 mg/L, from 30 patients. However, only three patients (10%) showed elevated CK. The elevation was mild (211, 359 and 429 U/L) and asymptomatic in all cases. Of note, three other patients had increased CK levels associated with C_{min} <24.3 mg/L. Overall, 7.4% of patients had elevated CK levels.

Table 2. Daptomycin population PK parameters from the final model

Parameter	Estimate (RSE, %)
Population CL_{DAP} (θ_{CL}, in L/h)	0.585 (4)
Influence of CL_{CR} on CL_{DAP} (β_{CLCR}, in L/h per unit of CL_{CR})	0.46 (10)[a]
Influence of sex on CL_{DAP} (β_{SEX})	0.208 (25)[a]
Population *V*1 (θ_{V1}, in L)	10.1 (8)
Influence of body weight on *V*1 (β_{BW})	0.543 (29)[a]
Influence of *ABCB1* haplotype CGC/CGC on *V*1 (β_{CGC})	−0.287 (29)[a]
Population *V*2 (θ_{V2}, in L)	3.39 (19)
Population Q (θ_{Q}, in L/h)	1.97 (41)
CL_{DAP} inter-individual variability (ω_{CL})	0.135 (20)
*V*1 inter-individual variability (ω_{V1})	0.231 (25)
CL_{DAP} intra-individual variability (γ_{CL})	0.215 (8)
*V*1 intra-individual variability (γ_{V1})	0.0965 (39)
Residual error term (b, proportional model)	0.194 (2)

RSE, relative standard error of estimate.
[a]Coefficient significantly different from zero ($P<0.001$, Wald test).
The relationship between covariates and individual values of CL_{DAP} and *V*1 were modelled as follows.
For CL_{DAP}: $CL_i = \theta_{CL} \times \left(\frac{CL_{CRi}}{102.5}\right)^{\beta_{CLCR}} \times \exp(sex \times \beta_{sex}) \times \exp(\eta CL_i) \times \exp(\kappa CL_i)$
where CL_{CRi} is the individual value of CL_{CR}, 102.5 is the median value of CL_{CR} in the population (in mL/min), sex is a binary variable (equal to 0 for female and 1 for male), ηCL_i is the inter-individual random effect, with $\eta CL_i \sim (0, \omega_{CL}^2)$ in the population, and κCL_i is the intra-individual random effect, with $\kappa CL_i \sim (0, \gamma_{CL}^2)$ in the population.
For *V*1: $V1_i = \theta_{V1} \times \left(\frac{BW_i}{83}\right)^{\beta_{BW}} \times \exp(CGC \times \beta_{CGC}) \times \exp(\eta V1_i) \times \exp(\kappa V1_i)$
where BW_i is the individual body weight, 83 is the median value of body weight in the population (in kg), CGC is a binary variable relative to *ABCB1* haplotype (equal to 1 for haplotype CGC/CGC and 0 for any other haplotype), $\eta V1_i$ is the inter-individual random effect, with $\eta V1_i \sim (0, \omega_{V1}^2)$ in the population, and $\kappa V1_i$ is the intra-individual random effect, with $\kappa V1_i \sim (0, \gamma_{V1}^2)$ in the population.
Note: the standard deviation of the random effects, ω_{CL} and ω_{V1}, are approximate values of the coefficients of variation of CL_{DAP} and *V*.

Population PK analysis

The final model was a two-compartment model, with four parameters: CL_{DAP}, daptomycin central *V* (*V*1) and peripheral *V* (*V*2) and intercompartment clearance (Q). Inter-individual variability could not be well estimated for *V*2 and Q, so only a fixed effect was estimated for these parameters in the final model. The introduction of IOV on CL_{DAP} and *V*1 much improved the fit, with a 125 point decrease in the objective function ($P<0.001$).

The population PK parameter values and parameter–covariate relationships are shown in Table 2. Parameters were all estimated with acceptable standard errors. It is noteworthy that intra-individual variability in CL_{DAP} was greater than inter-individual variability. Four covariates were included in the final model. CL_{CR} and sex were found to influence CL_{DAP}. Median CL_{DAP} was 23% greater in male than in female subjects (0.72 versus 0.585 L/h). Central *V* was allometrically scaled to body weight and was also influenced by *ABCB1* haplotype. Carriers of the CGC/CGC haplotype showed a 25% lower median *V*1 compared with subjects with any other haplotype (7.56 versus 10.1 L). This was the only significant relationship found between polymorphisms of P-gp and daptomycin PK parameters.

The final model adequately described the data, as shown in the predictions versus observations plot (Figure 2). The scatterplots of Normalized Prediction Distribution Error (NPDE) showed no tendency and the distribution of NPDE appeared to be Gaussian, as expected (Figure 3). Simulation-based visual predictive check showed overall good agreement between model predictions and observations (Figure 4).

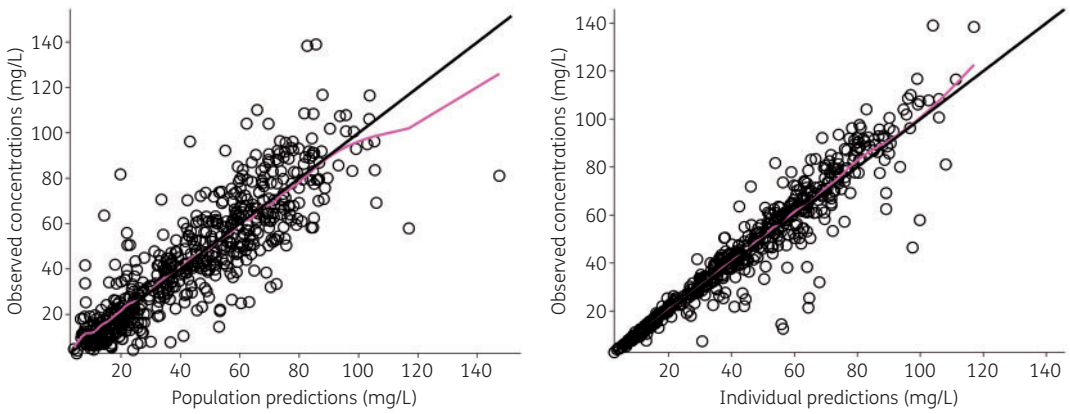

Figure 2. Observed daptomycin concentrations versus model predictions. The black line is the line of identity ($y = x$) and the magenta line is a spline regression. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

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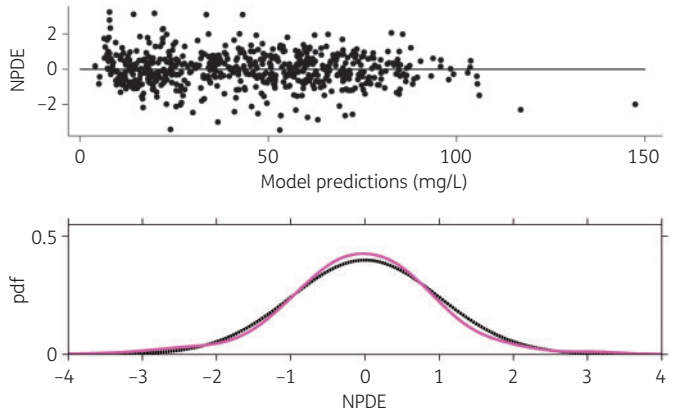

Figure 3. Scatterplot and distribution of NPDE. Upper panel, NPDE versus model predictions; lower panel, probability density function (pdf) of empirical (magenta line) and theoretical (black dashed line) NPDE. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

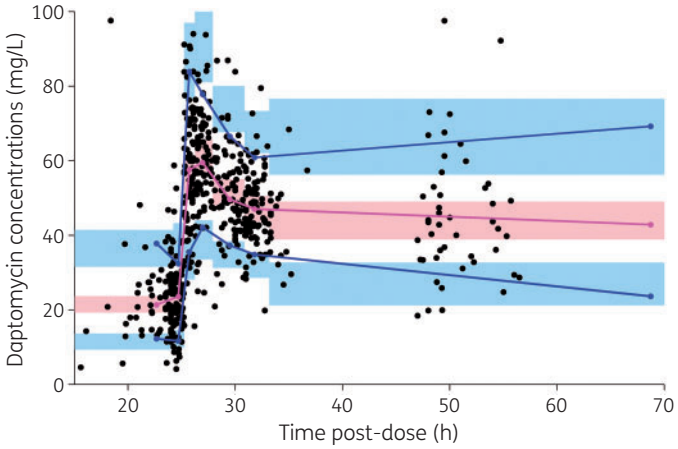

Figure 4. Prediction-corrected visual predictive check from the final model. The black dots are the observations. The blue solid lines are the 10th and 90th percentiles of prediction-corrected daptomycin concentrations and the magenta solid line is the median of observations (i.e. 50th percentile). The blue areas are the 90% CIs of the 10th and 90th simulated percentiles and the pink area is the 90% CI of the simulated median. Simulated percentiles were obtained from 1000 replicates of each individual dataset and the final model parameter estimates. Observations greater than 100 mg/L (n=9) are not shown for ease of graphical display. Of note, the x-axis represents the time after the previous dose, i.e. the dose administered the day before TDM was performed. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

PK simulation

Simulations were performed to calculate PK quantities (C_{max}, C_{min} and AUC) and derived probabilities to achieve defined PK/PD targets of efficacy (fAUC$_{24}$/MIC ≥66 and fC_{max}/MIC ≥12) and safety (C_{min} ≥24.3 mg/L). The results were calculated for three daptomycin dosages (6, 8 and 10 mg/kg/24 h) and the four groups of sex/*ABCB1* haplotype influencing daptomycin PK. The results are summarized in Table 3. Figure 5 shows the PTA for the two efficacy targets as a function of daptomycin MIC. First, the results shown in Table 3 illustrate the influence of sex and *ABCB1* haplotype on drug exposure. On average, daptomycin AUC, C_{max} and C_{min} were greater in females than in males, as a result of reduced CL_{DAP}. Subjects with CGC/CGC haplotype had higher C_{max} but comparable AUC compared with subjects with any mutated haplotype. The largest difference in drug exposure was observed between females with CGC/CGC haplotype and males without CGC/CGC haplotype, the former showing a 27% greater mean AUC and a 23% higher mean C_{max} (for the 6 mg/kg dose). Those differences in daptomycin exposure showed implications in terms of target attainment. For a given dosage and MIC, females appeared to have a higher probability of achieving the fAUC$_{24}$/MIC target, but also a higher probability of having C_{min} associated with a risk of CK elevation. P-gp genetic polymorphism mainly influenced the probability of achieving the fC_{max}/MIC target, with a greater probability in subjects with CGC/CGC haplotype.

The results also provided insights on the influence of daptomycin dosage and bacterial MIC on PTA. For the 6 mg/kg/24 h dosage, PTA was ≥90% only when the MIC was ≤0.5 mg/L for the fAUC$_{24}$/MIC target and when the MIC was ≤0.25 mg/L for the fC_{max}/MIC target. An increase in the dosage to 10 mg/kg/24 h was associated with greater PTA for MICs of 0.5–1 mg/L. PTA was still optimal for MIC values up to 1 mg/L for the fAUC$_{24}$/MIC target and MIC values up to 0.5 mg/L for the fC_{max}/MIC target. However, such an increase in the dosage was associated with a much greater probability of exceeding the C_{min} threshold for CK elevation. The overall probabilities were 5.5% and 37.8% for the 6 mg/kg and 10 mg/kg dosages, respectively.

Discussion

To our knowledge, the present work is the largest population PK study of daptomycin in patients with BJI. In addition, this is the largest study examining the influence of P-gp polymorphism on daptomycin PK. This study has provided several interesting pharmacological and clinical insights.

First, we have found that polymorphisms of *ABCB1*, the gene encoding P-gp, influenced daptomycin central *V*. Only the haplotype defined by the three most common *ABCB1* SNVs (*3435C>T*, *2677G>T/A* and *1236C>T*) showed a significant influence. Previous studies with digoxin (a sensitive substrate of P-gp) have shown that *ABCB1* haplotype analysis may be more relevant than single SNP analysis to study genotype–phenotype relationships.[28] Patients having the CGC/CGC haplotype showed a 25% lower median *V*1 compared with subjects with any other haplotype. This suggests that P-gp could play a role in daptomycin tissue distribution. We assume that patients with CGC/CGC haplotype would have a more effective efflux of daptomycin from tissue and so a more restricted *V*. By contrast, Baietto *et al.*[20] reported that the mutated genotype 3435T/T was associated with lower CL_{DAP} compared with CC or CT genotypes (median of 0.38 L/h and 0.57 L/h, respectively) in 23 patients. This discrepancy may be due to the approach used to estimate PK parameters, as Baietto *et al.*[20] used non-compartmental analysis to derive individual PK parameters. Covariance between parameters and between covariates was probably not taken into account, so P-gp genotype may not be an independent determinant of CL_{DAP} in this study. Physiologically, a possible influence of P-gp activity on CL_{DAP} is intriguing, considering

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Table 3. Simulated exposure and probability to achieve efficacy and toxicity targets stratified by dosage and categorical covariates

Daptomycin dosage and sex/*ABCB1* haplotype	C_{max} (mg/L)	C_{min} (mg/L)	AUC (mg·h/L)	PTA[a] *f*AUC/MIC ≥66	CFR[b] *f*AUC/MIC ≥66	PTA[a] fC_{max}/MIC ≥12	CFR[b] fC_{max}/MIC ≥12	PTA C_{min} ≥24.3 mg/L
6 mg/kg								
F/other	55.1 ± 8.5	16.9 ± 5.9	735 ± 159	0.626	0.993	0	0.807	0.11
M/other	49.9 ± 7.7	12.1 ± 4.9	601 ± 133	0.278	0.987	0	0.779	0.017
F/CGC	63.0 ± 9.5	14.9 ± 5.9	740 ± 164	0.63	0.994	0	0.900	0.079
M/CGC	57.7 ± 8.7	10.3 ± 4.7	603 ± 135	0.281	0.987	0	0.859	0.012
8 mg/kg								
F/other	73.4 ± 11.4	22.5 ± 7.9	980 ± 212	0.967	0.999	0.002	0.947	0.365
M/other	66.6 ± 10.3	16.1 ± 6.5	801 ± 178	0.761	0.996	0.001	0.915	0.112
F/CGC	83.9 ± 12.6	19.9 ± 7.9	987 ± 219	0.968	0.999	0.016	0.977	0.256
M/CGC	76.9 ± 11.6	13.8 ± 6.2	804 ± 181	0.763	0.996	0.006	0.969	0.063
10 mg/kg								
F/other	91.8 ± 14.2	28.1 ± 9.9	1225 ± 265	0.998	0.999	0.03	0.982	0.611
M/other	83.2 ± 12.9	20.1 ± 8.1	1001 ± 222	0.972	0.999	0.01	0.974	0.264
F/CGC	104.9 ± 15.8	24.9 ± 9.9	1233 ± 273	0.998	0.999	0.239	0.987	0.465
M/CGC	96.2 ± 14.5	17.2 ± 7.8	1005 ± 226	0.972	0.999	0.142	0.985	0.17

F, female; M, male.
Data are given as mean ± SD unless otherwise stated. All PK quantities were calculated after five doses of daptomycin. 'CGC' means homozygous CGC/CGC haplotype and 'other' means any other genotype.
[a]PTAs are given for a daptomycin MIC of 1 mg/L (ECOFF for *S. aureus* from EUCAST).
[b]CFR, cumulative fraction of response based on the distribution of daptomycin MIC reported by EUCAST for *S. aureus*.

that this agent is mainly eliminated by the kidneys. In addition, the renal CL of unbound daptomycin was found to be lower than the glomerular filtration rate in healthy subjects, which does not support major tubular secretion by transporters.[29] Further research is required to clarify the role of P-gp in daptomycin distribution and elimination.

This observation also raises questions about the role of P-gp in daptomycin distribution and antibacterial effect in bone tissue. In previous reports, daptomycin alone did not show significant activity within osteoblasts.[30,31] Although P-gp has not so far been described as being present on the osteoblast cell membrane, its presence on the membrane of osteosarcoma cells suggests that it may be found in bone cells.[32] Efflux by P-gp could explain the lack of intra-osteoblast activity of daptomycin. Also, as reported by Lemaire *et al.*,[19] inhibitors of P-gp could increase intracellular daptomycin concentration and its activity. It would be interesting to study intra-osteoblastic activity of daptomycin in combination with a P-gp inhibitor. In addition, the implication of *ABCB1* genotype on daptomycin tissue concentration and response should be considered in further studies.

The other covariates identified were in accordance with previous studies.[13,14] We confirmed the independent influence of sex on CL_{DAP}, which was first reported in the large population PK study conducted by the manufacturer[13] and also observed in our first PK study in patients with BJI.[16] The magnitude of this sex difference was remarkably similar in our study and that of Dvorchik *et al.*,[13] with about 20% lower CL_{DAP} in females, on average. The rationale for this role of gender on daptomycin PK remains unclear. The confounding role of renal function can be ruled out, as CL_{CR} was also included as a covariate in the CL_{DAP} model. Preliminary runs showed an influence of body weight on CL_{DAP}, but this covariate showed no additional influence once CL_{CR}, which incorporates body weight, was included in the model. One may hypothesize that gender could be a better categorical descriptor of the influence of body size on CL_{DAP}, but this requires further investigation.

PK/PD simulations performed with our final model showed that sex could influence the achievement of efficacy (*f*AUC/MIC ratio) and safety (C_{min}) targets. For the efficacy target, this was especially true for MICs ranging from 0.5 to 2 mg/L, with men showing lower PTA. From the safety perspective, women presented a greater risk than men of achieving C_{min} levels associated with CK elevation for a given dosage and P-gp genotype. P-gp haplotype mainly influenced the PTA for the fC_{max}/MIC target: patients without CGC/CGC haplotype showed lower PTA than patients with CGC/CGC haplotype. P-gp genotype marginally influenced C_{min} and the corresponding safety target. Overall, women with CGC/CGC haplotype were identified as a subgroup with the highest daptomycin exposure and a potentially greater risk of toxicity, while men without CGC/CGC haplotype showed the lowest exposure and may be at risk of suboptimal efficacy for relatively high *S. aureus* MIC values (0.5–1 mg/L).

Simulations also showed that 6 mg/kg/24 h did not achieve sufficient exposure in bone for MIC values up 1 mg/L in patients with normal or moderately impaired renal function (CL_{CR} >30 mL/min). A dose of 10 mg/kg/24 h appears necessary to achieve the *f*AUC/MIC target for MIC values up to the *S. aureus* ECOFF.

While a dose of 10 mg/kg/24 h appears to be better than 6 or 8 mg/kg/24 h for efficacy, it is associated with a significant increase in the probability to attain potentially toxic C_{min}. So, one has to balance the need for efficacy against the risk of muscular toxicity when using such a dosage of daptomycin. While the risk of muscular toxicity cannot be neglected, one should recall that the relationship between daptomycin C_{min} and CK elevation reported in the reference study from Bhavnani *et al.*[5] was probabilistic, with

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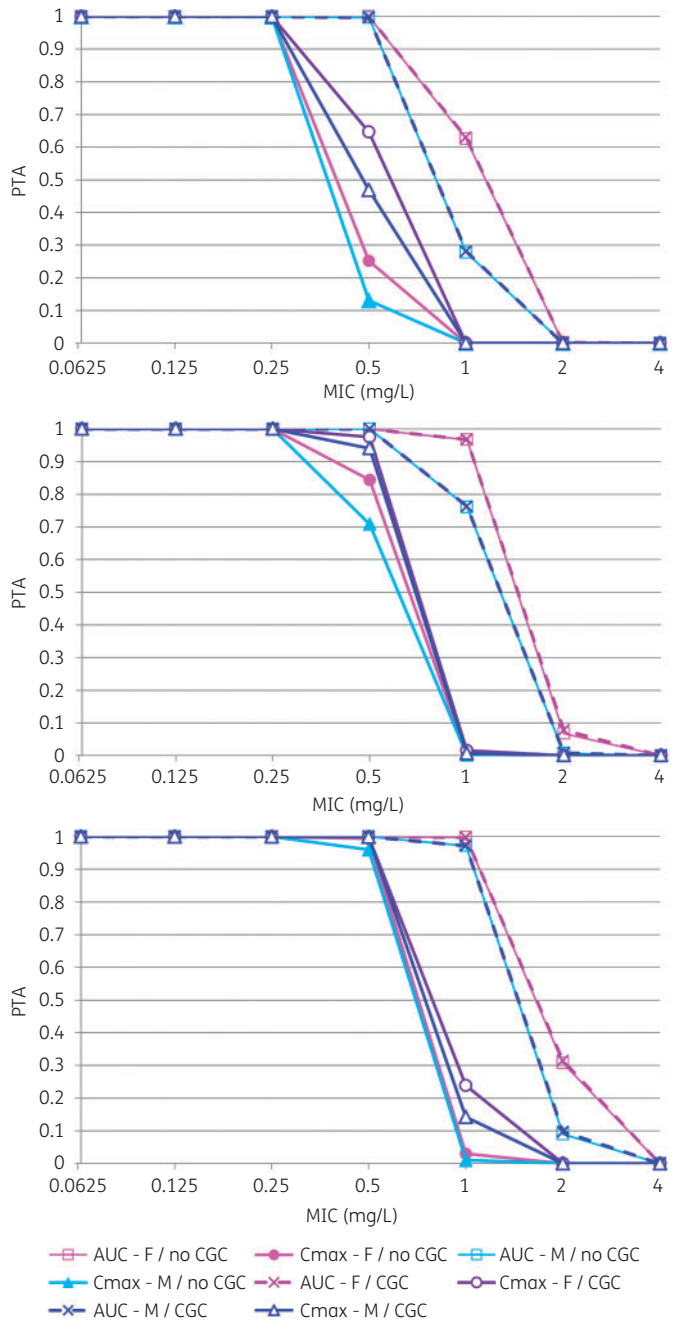

Figure 5. Probability of achieving target values of $fAUC_{24}$/MIC and fC_{max}/MIC ratio for various daptomycin dosages and covariate status. Top, 6 mg/kg/24 h; middle, 8 mg/kg/24 h; bottom, 10 mg/kg/24 h. In the figure legend, 'AUC' means $fAUC_{24}$/MIC target, while 'C_{max}' means fC_{max}/MIC target. 'CGC' means homozygous CGC/CGC haplotype, while 'no CGC' means any other genotype. This figure appears in colour in the online version of *JAC* and in black and white in the printed version of *JAC*.

relatively large imprecision in probability estimates above the threshold of 24.3 mg/L. In their subsequent PK/PD analysis, Bhavnani *et al*.[5] estimated that a dosage of 10 mg/kg/24 h would be associated with a 5.1% risk of muscular adverse events in patients with *S. aureus* bacteraemia.[33] In the present study, only 10% of patients with C_{min}>24.3 mg/L had CK elevation and none showed clinical signs. This lower rate of CK elevation could be explained by the study population, as patients with BJI often have limited mobility, and by the cessation of co-medications with muscular adverse effects such as statins[34] during daptomycin therapy, which was applied in the cohort.

An original feature of the present study was the quantification of intra-individual variability of CL_{DAP} and *V*. Interestingly, intra-individual variability in CL_{DAP} (21.5%) was greater than inter-individual variability unexplained by covariates (13.5%). It is likely that such intra-individual variability may be greater in other patient populations, such as critically ill patients who often present rapid changes in renal function and health status that influence daptomycin exposure.[35,36] Repeated TDM may be especially useful in these patients to control drug exposure.

This study has several limitations that are inherent to its design. Data were collected during routine patient care and some errors might have occurred. Except for rifampicin, we did not record all co-medications that may influence P-gp activity. The presence of fever or sepsis, which have been found to influence daptomycin PK,[13,14] was not recorded. Regarding P-gp genotyping, only the three most common *ABCB1* SNVs were investigated, although others have been described. We did not investigate the influence of possible inducers or inhibitors of P-gp other than rifampicin. Finally, microbiological data (species and MIC values) were not collected and clinical outcome was not analysed, which precludes a thorough PK/PD evaluation based on observed data.

Conclusions

This is the largest population PK study of daptomycin in patients with BJI. In addition to renal function and body weight, this study identified sex and P-gp genotype as covariates influencing daptomycin PK. PK/PD simulations performed with the final model showed that these covariates may influence the risk/benefit profile of daptomycin for high MIC values. This work also suggests that a dosage of 10 mg/kg/24 h would optimize the antibacterial effect of daptomycin in patients with BJI. Prospective clinical studies are necessary to confirm the efficacy and safety of such dosages in this clinical setting.

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Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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