Effect of SNPs in human ABCB1 on daptomycin pharmacokinetics in Caucasian patients.

Effect of Single Nucleotide Polymorphisms of human *ABCB1* on Daptomycin Pharmacokinetics in Caucasian Patients

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Sir,

Daptomycin is a cyclic lipopeptide antibiotic, active against Gram-positive bacteria. It is excreted unchanged primarily by the kidneys (78%) and dosage adjustment is required in patients with renal impairment [1].

Daptomycin was shown to be substrate of P-glycoprotein (P-gp, MDR1) *in vitro* [2]. P-gp is an efflux transporter constitutively expressed in many human tissues with high levels in kidney and adrenal glands where it has a major influence on drug disposition [3]. P-gp is encoded in humans by the highly polymorphic *ABCB1* gene [4]. For example, an effect of single nucleotide polymorphism (SNP) in *ABCB1* on the pharmacokinetic (PK) profile of digoxin, fexofenadine, nelfinavir, tacrolimus, azytromycin, cloxacillin, rifampicin was reported [5, 6]. We aimed at evaluating the influence of SNPs in *ABCB1* on PK of daptomycin.

Adult patients, presenting to Amedeo di Savoia Hospital (Turin, Italy), in therapy with intravenous daptomycin according to the official indications were enrolled. Main exclusion criteria were: age <18 years, estimated creatinine clearance <30 mL/min, septic shock, concomitant therapy with drugs that may inhibit (indinavir, nelfinavir, ritonavir, saquinavir, erythromycin, clarithromycin, itraconazole) or induce (rifampicin, midazolam) P-gp function. The study was approved by ethics committee (ASL TO-2, number 44824/13). Sampling was performed after written informed consent was obtained. Blood samples for PK analysis were collected at steady state (after three days of treatment) before dose and after 0.5, 1.5, 5, 9 and 24 h drug administration.

Genomic DNA was extracted from blood using the QIAamp DNA Mini Kit (Qiagen, Venlo, Netherlands). SNPs (3435 C>T, rs1045642; 1236 C>T, rs1128503; 2677 G>T, rs2032582) were analyzed using the TaqMan assays (Applied Biosystems, Foster City, CA) by Real-Time PCR (BIORAD, Hercules, CA) reaction.

Daptomycin plasma levels were assayed using two validated HPLC-MS and UPLC-PDA (Photo Diode Array) methods [7, 8]. Lower limit of quantification was 1.56 mg/L and 0.781 mg/L, for the

HPLC-MS and UPLC-PDA method, respectively. Intra and inter-day accuracy (CV%) and precision (relative standard deviation, R.S.D.%) were lower than 15% for both methods.

Area under the curve from 0 to 24h (AUC_{0-24h}), volume of distribution at steady state (V_{ss}), clearance at steady state (CL_{ss}), and half life ($t_{1/2}$) were estimated using Kinetica 5.0 software (Thermo Scientific, Waltham, MA).

A total of 23 Caucasians patients were included in the study: 16 patients (69.6%) were male.

Median (interquartile range, IQR) age, weight, body mass index (BMI) were 61 years (46-71), 74 kg (60-86), and 26 (21-29), respectively. Median daptomycin daily dosage was 6 mg/kg (5-7).

Median (IQR) C_{max}, C_{min}, AUC_{0-24h}, V_{ss}, CL_{ss}, and $t_{1/2}$ were: 97.1 mg/L (78.1-110.4), 15.0 mg/L (10.3-21.8), 875.5 mg*h/L (634.4-1109.2), 8.7 L (6.8-9.9), 0.539 L/h (0.404-0.638), 11.0 h (8.4-12.1), respectively. At linear regression analysis using Spearman's rank correlation a trend between daptomycin dosage and $AUC_{0-24h,}$ which is affected by CL_{ss}, was observed (ρ, 0.404; P= 0.056). In order to evaluate the influence of genotype on daptomycin PK, we normalized C_{max}, C_{min}, and AUC_{0-24h} for daptomycin dosage (i.e. AUC_{0-24h}/dosage). The allele frequencies for 3435T, 1236T, and 2677T were 26.1%, 21.7%, and 17.4%, respectively. Mann-Whitney test indicated that median dose- normalized AUC_{0-24h} were higher in patients with 3435 TT genotype, compared with CC or CT genotype (2.61 [1.96-3.14] versus 1.77 [1.51-1.98]; P=0.021). Similarly, CL_{ss} was lower in patients with TT genotype compared to CC or CT genotype patients (0.38 [0.32-0.51] versus 0.57 [0.51-0.66]; P=0.021) (Figure 1). Multiple regression analysis showed that 3435 TT was the only individual factor predictive of dose-normalized AUC_{0-24h} (P= 0.01) and clearance (P=0.012).

3435 C>T SNP is the only silent polymorphism that might influence P-gp expression in humans, probably by altering protein folding and function and changing the substrate specificity [10]. In this study we found for the first time an influence of *ABCB1* 3435 C>T polymorphism on daptomycin dose-normalized AUC_{0-24h} and CLss. The following limitations should be noted: low number of patients, patients in treatment with different daptomycin dosage. These preliminary findings may

explain the high inter-subjects PK variability in daptomycin disposition and could represent the starting point in individualization of therapy especially in patients with renal impairment.

Part of these data were showed as oral presentation (A-1769) at the 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco, CA) .

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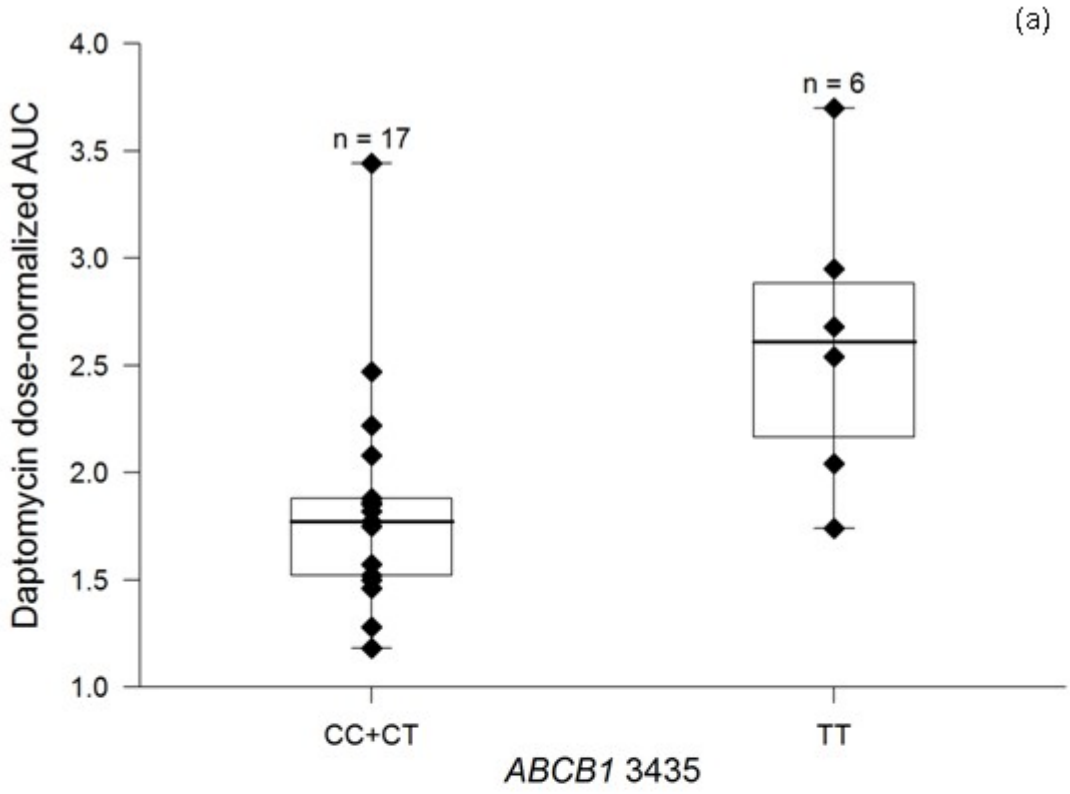
Transparency declarations

None to declare.


(a)
Daptomycin dose-normalized AUC
4.0
3.5
3.0
2.5
2.0
1.5
1.0
n = 17
n = 6
CC+CT
TT
ABCB1 3435

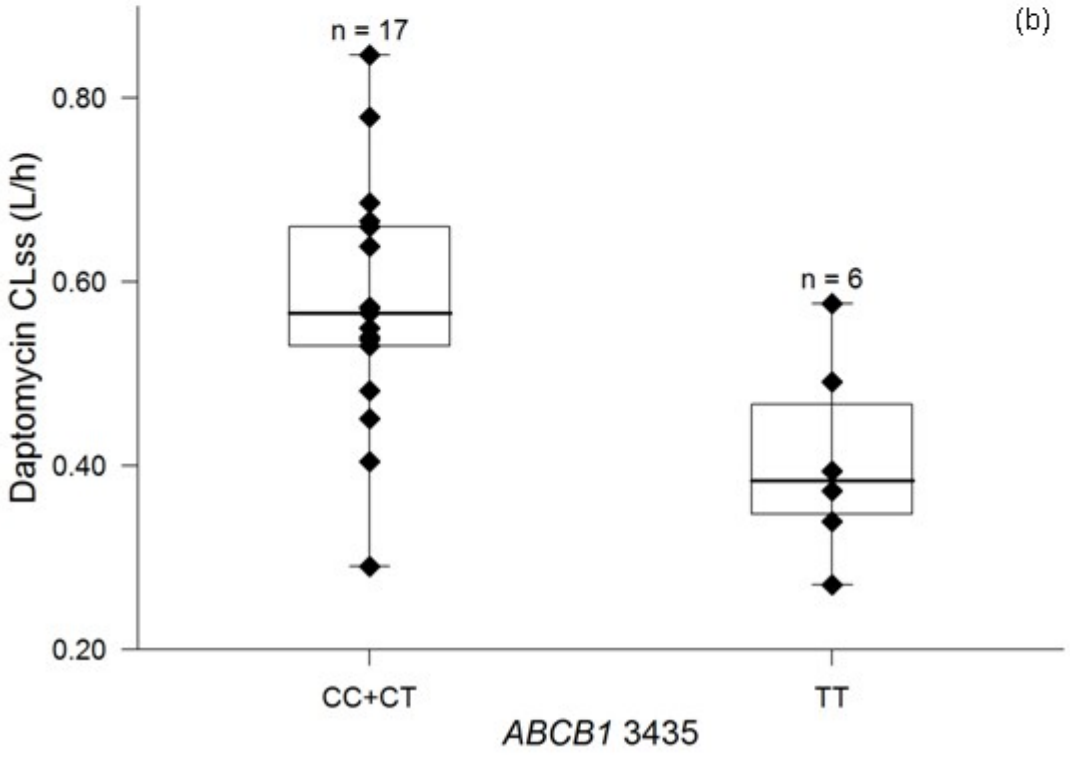

(b)
Daptomycin CLss (L/h)
0.80
0.60
0.40
0.20
n = 17
n = 6
CC+CT
TT
ABCB1 3435

Figure 1. Influence of *ABCB1* 3435 C>T genotype on daptomycin dose normalized $AUC_{0\text{-}24h}$ (a) and daptomycin clearance (b). AUC, area under the concentration-time curve; CL_{ss}, clearance at steady state.

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