Predictive Biomarkers and Personalized Medicine

Impact of ABCB1 Allelic Variants on QTc Interval Prolongation


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Abstract

Purpose: Although the ABCB1 (P-glycoprotein) drug transporter is a constituent of several blood–tissue barriers (i.e., blood–brain and blood–nerve), its participation in a putative blood–heart barrier has been poorly explored. ABCB1 could decrease the intracardiac concentrations of drugs that cause QT prolongation and cardiotoxicity.

Experimental Design: ABCB1-related romidepsin transport kinetics were explored in LLC-PK1 cells transfected with different ABCB1 genetic variants. ABCB1 plasma and intracardiac concentrations were determined in Abcb1a/1b (−/−) mice and wild-type FVB controls. These same mice were used to evaluate romidepsin-induced heart rate-corrected QT interval (QTc) prolongation over time. Finally, a cohort of 83 individuals with available QTcB and ABCB1 genotyping data were used to compare allelic variation in ABCB1 versus QTc-prolongation phenotype.

Results: Here, we show that mice lacking the ABCB1-type P-glycoprotein have higher intracardiac concentrations of a model ABCB1 substrate, romidepsin, that correspond to changes in QT prolongation from baseline (∆QTc) over time. Consistent with this observation, we also show that patients carrying genetic variants that could raise ABCB1 expression in the cardiac endothelium have lower ∆QTc following a single dose of romidepsin.

Conclusions: To our knowledge, this is the first evidence that Abcb1-type P-glycoprotein can limit intracardiac exposure to a drug that mediates QT prolongation and suggests that certain commonly inherited polymorphisms in ABCB1 may serve as markers for QT prolongation following the administration of ABCB1-substrate drugs. Clin Cancer Res; 17(4); 937–46. © 2010 AACR.

Introduction

Drug development is frequently discontinued, or stringent restrictions are imposed when treatment-associated QT prolongation is observed (1). The potential for drugs to lengthen the QT interval is now under major scrutiny by the Food and Drug Administration as QT interval prolongation is considered a surrogate for torsades de pointes (2). Pharmacogenetic studies have showed that genotypic differences in metabolizing enzymes are associated with the risk of QT interval changes and the development of arrhythmias through alteration in pharmacokinetics and pharmacodynamics (3–5), although to date, involvement of drug transporters remains unexplored.

One such drug transporter, ABCB1 (P-glycoprotein, MDR1), has been shown to be protective in several tissues including heart (6–9), where it effluxes both endogenous and exogenous substrates away from these tissues back into the systemic circulation (10). ABCB1, expressed in the cardiac endothelium, may be a component of a proposed blood–heart barrier that protects cardiac tissues from damage induced from endogenous and exogenous substances. Meissner and colleagues showed that ABCB1 is variably expressed in the cardiac endothelium based on two allelic variants (i.e., 2677G>T/A and 3435C>T) that have significant representation in many world populations (11); this study showed increased expression associated with the variant alleles. Later, it was shown that 1236C>T, 2677G>T/A, and 3435C>T SNPs were related to altered ABCB1 protein folding with reduced efflux for some substrates (12). We have previously reported that...
electrocardiograms (ECG) of patients treated with romidepsin often show measurable increases in QT interval when measured using the Bazett correction (13). In addition, we have shown that the above ABCB1 polymorphisms were not related to the plasma pharmacokinetics of romidepsin (14); however, given that romidepsin concentrations still may be increased locally within cardiac tissues, QTc prolongation could be altered by differential efflux due to allelic differences in ABCB1 protein expression and function (12, 15–18).

Using the histone deacetylase inhibitor romidepsin (formerly FR901228 or FK228, NSc630176) as a model substrate, this study aims to determine whether ABCB1 is a constituent of a blood–heart barrier in which the transporter limits intracardiac concentrations of QT-prolonging substrate drugs (13, 19–21). First, we confirmed previous reports that romidepsin is an ABCB1 substrate (22–24) and determined the transport characteristics of wild-type and variant ABCB1 toward romidepsin. Secondly, to test the hypothesis that ABCB1 limits the local intracardiac concentration of romidepsin with ensuing effects on QT prolongation, romidepsin was administered to mice lacking the ABCB1-type P-glycoprotein. Both intracardiac and serum concentrations of romidepsin were measured, and QT prolongation was assessed. Finally, to test the hypothesis that allelic variation in ABCB1 is clinically associated with QT prolongation, we evaluated the consequences of the ABCB1 1236C>T, 2677G>T/A, and 3435C>T variants on QTc in 115 patients with cancer, following administration of romidepsin.

Materials and Methods

Transport of romidepsin by ABCB1

Romidepsin was obtained as a gift from Gloucester Pharmaceuticals. LLC-PK1 cells were transfected with ABCB1 variants containing the exon 12 (1236T), the exon 21 (2677T), and the exon 26 wobble single nucleotide polymorphism (SNP; 3435T). Membrane ABCB1 protein content was ascertained via flow cytometry using previously published methods (25). Transport assays were carried out in each transfectant as described (26), and romidepsin concentration was evaluated by mass spectrometry using previously published methods (27) and calculations (28). Transfection methods and transport assays are described in greater detail in the Supplementary Methods S1.

Study animals and drug administration

Pharmacokinetics and ECG studies were done using female FVB.129P2-Abcb1atm1Bor-Abcb1atm1Bor N12 double knockout mice obtained from Taconic Farms. All mice were administered a single bolus of romidepsin either through the tail vein (3.6 mg/kg dissolved in 100 µL of 80:20 propylene glycol:ethanol) or the vehicle only.

Pharmacokinetic analysis in mice

Plasma and intracardiac romidepsin concentration was determined 5, 15, and 30 minutes, and 1, 2, 4, 8, 16, and 24 hours after drug injection (n = 3 per time point). Romidepsin concentrations in plasma and tissue samples were determined by previously published methods (27). More detailed information about sample collection can be found in the Supplementary Methods S1.

Transmitter implantation and ECG data collection in mice

The mice were divided into four groups: group 1 consisted of Abcb1a1b−/− mice receiving 3.6 mg/kg romidepsin; group 2 consisted of Abcb1a1b−/− mice receiving vehicle control; group 3 consisted of wild-type FVB mice receiving 3.6 mg/kg romidepsin; and group 4 consisted of wild-type FVB mice receiving vehicle control (n = 3 for each group). QTc correction methods in mice have been previously reported (29). Further information about ECG monitoring and data collection can be found in the Supplementary Methods S1 published online.

Statistical considerations for mouse experiments

The plasma and intracardiac area under the curve (AUC) were compared between wild-type and Abcb1a1b knockout mice using Bailer’s method for destructive sampling (30). For the analysis of ECG, the original data sets were filtered to remove extreme outliers. From each mouse’s QT and RR interval lengths, means were calculated at 6-minute intervals (10 intervals per hour) using data obtained within 30 minutes of each interval. The deviations of all the observed values from these means were calculated, and the observations with deviations greater than the 95th percentile of the distribution or less than the 5th percentile were dropped from the analyses. For the QT intervals, these percentiles translated into deviations above or below the mean (~0.9 milliseconds), and for the RR intervals, deviations of magnitude greater than about 6.5 milliseconds above the mean or 5.7 milliseconds below it were dropped. The cutoffs corresponded to approximately 1.5 standard deviations of their distributions. For the outcomes derived from the QTc values, comparisons between groups were assessed using
the Wilcoxon rank-sum test. Because this test has low power when the groups are small, the results of the t test with Satterthwaite’s approximate degrees of freedom are also provided.

**Patient population**

We evaluated data from patients with T-cell lymphoma participating on a phase II clinical trial of romidepsin (n = 61). Eligibility criteria were reported previously (13). A separate cohort of patients was included in the analysis consisting of: (a) patients participating on the same multi-institutional trial as the aforementioned cohort who were treated at institutions other than the NCI (n = 42; ref. 13) and (b) patients treated on the single-agent phase I clinical trial of romidepsin previously conducted at the NCI (n = 12; ref. 21). For the latter cohort, the common eligibility criteria were as described in the phase II population, except that patients with malignancies other than T-cell lymphoma were also eligible. All patients also received premedication with the antiemetic ondansetron or granisetron. Overall, samples were available from 115 patients who treated on the phase II clinical trial of romidepsin previously conducted at the NCI (21). For the latter cohort, the common eligibility criteria were as described in the phase II population, except that patients with malignancies other than T-cell lymphoma were also eligible. All patients also received premedication with the antiemetic ondansetron or granisetron. Overall, samples were available from 115 patients (n = 12 from the phase I trial and n = 103 from phase II trials; see Table 1). All patients were enrolled on protocols approved by the Institutional Review Board, and genotyping was approved as well.

**Phenotypic evaluation of clinical data**

Electrocardiograms were obtained immediately before romidepsin administration and 4 hours after the start of romidepsin administration (at the end of infusion). Only a subset of the original cohort had evaluable QTc data (n = 83). It should be noted that although ondansetron is known to prolong the QTc interval, this is transient and no effect of ondansetron treatment on QTc interval would be expected at the end of the 4-hour romidepsin infusion when study ECGs were ascertained (31). More detailed information about determination of ΔQTcB (change in QTc with Bazett correction relative to a single baseline), ΔQTcBMB (change in QTc with Bazett correction relative to multiple baselines), ΔQTcF, and ΔQTcB is provided in Supplementary Results S2.

**Genotyping analysis**

Variants in the ABCB1 gene were analyzed according to previous methodologies (14), and ABCB1 diplotypes were computed as described previously (17). Genotype frequency analysis of Hardy–Weinberg equilibrium was conducted using Helix Tree Software v4.4.1 (Golden Helix Inc.). The linkage between each pair of SNPs was determined in terms of the classical D’ statistic.

**Statistical considerations in the clinical data**

All data are reported as mean values with 95% confidence intervals (CI), unless specified otherwise. Changes in QTc interval from baseline (ΔQTc) as well as drug clearance based on genotype or haplotype were evaluated using the Fisher–Freeman–Halton test. Because of limited numbers of observations, subsequent analyses were based on grouping patients on the basis of the number of reference alleles in multiple loci; with these, resulting two group statistical comparisons being evaluated using an exact Wilcoxon rank-sum test. Repeated-measures ANOVA was used to evaluate differences in ΔQTcB and ΔQTcF versus genotype over the course of repeated administrations of romidepsin. The Jonckheere–Terpstra trend test was employed in comparisons between trends in doses, number of variant alleles in genotype, and haplotype categories (32). Differences between laboratory values and body composition versus genotype were evaluated using the Kruskal–Wallis test. Comparisons between the various ΔQTc data sets (i.e., ΔQTcB, ΔQTcBMB, ΔQTcF, and ΔQTcF) were evaluated using the Spearman rank correlation method. All P values are two-tailed, and those obtained from nonparametric tests are from exact calculation, not asymptotic approximation. Given the exploratory nature of this study, P values were not corrected for the large number of tests done. Thus, P values less than 0.01 were considered significant whereas all others more than 0.01 and less than 0.05 were considered to be notable trends.

**Results**

**Transport of romidepsin by ABCB1**

We first confirmed that romidepsin is a substrate of ABCB1, and determined the transport characteristics of wild-type and variant ABCB1 toward romidepsin. Consistent with previous reports (22–24), we found that romidepsin (2 μmol/L) is recognized by wild-type ABCB1 "CGC" (Fig. 1A). We also noted that ABCB1 variant at the 1236, 2677, and 3435 loci ("TTT") has reduced...
transport capability (Fig. 1B), as indicated by an increase in romidepsin transport in the basolateral to apical direction in LLC-PK1 cell monolayers. The B→A/A→B ratio for wild-type ABCB1 “CGC” was 12.10 (95% CI = 10.87–13.32) whereas the ratio was significantly reduced at 5.846 (95% CI = 4.495–7.196, \( P < 0.001 \)) for the TTT variant. Transport of romidepsin in the basolateral to apical direction by the ABCB1 “TTT” variant was significantly impaired compared with wild-type ABCB1 (\( P = 0.028 \); Fig. 1C); the difference in transport was in spite of “TTT” variant cells expressing more ABCB1 at the membrane than wild-type cells (Supplementary Fig. 1A and B). Transport was not evaluated in mock transfected cells as there was an approximate 1,000-fold increase in membrane-associated ABCB1 resulting from the transfection (Supplementary Fig. 1C).

**Plasma and intracardiac pharmacokinetics in mice**

To test the hypothesis that ABCB1 expression impacts either plasma or intracardiac levels of romidepsin, drug was administered to mice lacking the ABCB1-type P-glycoprotein (\( \text{Abcb1a/1b}^{−/−} \) mice) and romidepsin concentrations in the plasma and heart were monitored over 24 hours. Female mice were selected because females may express more murine ABCB1 homologues and were thus expected to have a greater difference in \( \text{Abcb1a/1b}^{−/−} \) expression compared with female mice that lack the ABCB1-type P-glycoprotein (33). The mean plasma AUC\(_{0−2}\) for \( \text{Abcb1a/1b}^{−/−} \) knockout mice versus wild-type mice (291.76 ng h/mL vs. 255.64 ng h/mL, respectively) was not statistically different (\( P = 0.11 \); Bailer’s method, \( Z \) test; Fig. 2A). However, when romidepsin exposure (AUC\(_{0−2}\)) was evaluated in heart tissue, a significant difference was observed (\( P = 0.0026 \); Bailer’s method, \( Z \) test). Mean heart AUC\(_{0−2}\) was 35% higher for \( \text{Abcb1a/1b}^{−/−} \) knockout mice than wild-type mice (0.23 ng h/mg vs. 0.17 ng h/mg, respectively; Fig. 2B). Similar, albeit nonsignificant, results were obtained using a ratio of the cardiac and plasma AUC\(_{0−2}\); however, this was primarily due to factoring in plasma

![Image](image-url)
AUC data that were not significantly different (Supplementary Results S1). These results suggested that there could be a difference in the ECG effects resulting from exposure to romidepsin.

**Electrocardiogram phenotype of knockout versus normal mice receiving romidepsin**

To test the hypothesis that Abcb1 expression could alter ECG measurements of the QT interval, Abcb1a1/b knockout mice were administered romidepsin and monitored over 72 hours via a surgically implantable ECG transmitter. For the analysis of the \( \Delta QTc \) values, the filtered values (e.g., \( \Delta QTc \pm 1SD \)) were averaged in 2-hour intervals from 0 to 72 hours for each mouse (see Fig. 3), and the results are identified by the midpoints of the intervals. The mean time taken to achieve peak \( \Delta QTc \) (i.e., \( \Delta QTc_{\text{max}} \)) was 16 hours in knockout mice and 31 hours in wild-type mice. This difference has \( P \) equal to 0.01 by the exact Wilcoxon rank-sum test, the smallest possible two-sided \( P \) value in a comparison of \( N = 3 \) versus \( N = 3 \). After a variance stabilizing transformation, the difference reaches significance by the \( t \) test (\( P = 0.02 \)). There is no apparent difference between the peak values (2.2–5.2 milliseconds in knockout mice vs. 1.8–2.7 milliseconds in wild-type mice, \( P = 1.0 \) by the Wilcoxon test). Therefore, although we did not observe a difference in \( \Delta QTc_{\text{max}} \), a decrease in the time taken to achieve \( \Delta QTc_{\text{max}} \) was noted in Abcb1a1/b knockout mice receiving romidepsin.

**Relationship between dose and \( \Delta QTc \) in multiple \( \Delta QTc \) data sets obtained from patients**

All methods of calculating \( \Delta QTc \) data obtained in this study were highly correlated with only one exception (Supplementary Results S1). Therefore, we only utilized the \( \Delta QTc_{\text{BMB}} \) and the \( \Delta QTc_{\text{MB}} \) observations in subsequent analyses because more data were available and the different methods used to measure QTc were statistically similar. There was no association between dose and \( \Delta QTc_{\text{BMB}} \), although a weak trend was noted in which mean \( \Delta QTc_{\text{BMB}} \) increased with increasing dose (mean \( \Delta QTc_{\text{BMB}} \) (milliseconds) = –5.0, 11.9, 15.8, 22.2, and 29.0 for doses (mg/m\(^2\)) = 12.7, 14, 17.8, 18, and 24.9, respectively with \( n = 1, 71, 6, 4, \) and 1; \( P = 0.11 \); exact Jonckheere–Terpstra test for trend). Similarly, there was no association between dose and \( \Delta QTc_{\text{BMB}} \) (\( P = 0.43 \)); thus, comparisons between genotype, and \( \Delta QTc_{\text{MB}} \) and \( \Delta QTc_{\text{BMB}} \) were made by combining patients receiving different doses.

**Genotype versus \( \Delta QTc \) relationships following infusion of romidepsin in romidepsin-naïve patients**

As our \( \text{in vitro} \) and \( \text{in vivo} \) data indicated that variability in ABCB1 expression and function may alter the measurement of ECGs, we undertook to genotype of patients treated with romidepsin, in which ECG data were available, in order to test the hypothesis that \( \Delta QTc \) varied as a function of ABCB1 genotype. A summary of the patients and treatments evaluated, and genotype variation is included in Tables 1 and 2, respectively. No association between genotype and \( \Delta QTc \) versus romidepsin pharmacokinetics or laboratory test values was found in humans (Supplementary Results S1).

The initial analysis consisted of all patients for which both \( \Delta QTc_{\text{BMB}} \) and genotyping data were available (mean = 8 milliseconds; \( n = 78 \)). The ABCB1 2677G>T polymorphism was associated with interindividual variation in \( \Delta QTc_{\text{BMB}} \) (\( P = 0.017 \); uncorrected) when individuals carrying 2677A alleles were excluded. Patients carrying two copies of variant alleles at the ABCB1 2677G>T/A SNP were found to have the lowest \( \Delta QTc_{\text{BMB}} \) (5 milliseconds; 95% CI = –3–13 milliseconds; \( n = 14 \)) as compared with heterozygous patients (13 milliseconds; 95% CI = 7–18 milliseconds; \( n = 33 \)) and patients carrying 2677GG genotypes (18 milliseconds; 95% CI = 12–23; \( n = 25 \); Fig. 4A). Although the ABCB1 1236C>T SNP was marginally associated with \( \Delta QTc_{\text{BMB}} \) (\( P = 0.042 \)), perhaps by virtue of its strong linkage to the 2677G>T/A SNP, the 3435C>T SNP was not related.

Functional analyses have shown that these polymorphisms have at least an additive effect on altering protein folding and function (12, 16); thus, we compared diplo- types consisting of the ABCB1 1236–2677–3435 variants (diplo- types 1–5), 1236 to 2677 variants (diplo- types 6–10), and the 2677 to 3435 variants (diplo- types 11–15; Supplementary Table S3) in order to evaluate the effects
of multiple polymorphisms on phenotype as was done previously (17). Consistent with these observations (12, 16), considering the SNPs in the context of a haplotype increased the significance of the associations explored above with diplotype 1 to 5, 6 to 10, and 11 to 15 being associated with ΔQTcBMB (P = 0.011, P = 0.020, and P = 0.010, respectively). Diplotypes 1 to 5 were associated with ΔQTcBMB with means equal to 22, 12, 12, 12, and 3 milliseconds, respectively (Fig. 4B). Within diplotype 6 to 10, individuals carrying diplotype 6 had the highest mean ΔQTcBMB (20 milliseconds; 95% CI = 13–27, n = 19), whereas diplotype 10 had the lowest mean (6 milliseconds; 95% CI = −4–15; n = 12). Diplotypes 7 to 9 had roughly equal means (overall 12 milliseconds; 95% CI = 7–16; n = 44; Fig. 4C). Diplotypes 11 to 15 were also associated with ΔQTcBMB with a similar trend in means (ΔQTcBMB = 19, 13, 14, 10, and 3 milliseconds, respectively; Fig. 4D). No ABCB1 SNP or diplotype was associated with ΔQTcBMB values, whereas those carrying the 2677TT genotype had the lowest ΔQTcBMB values, whereas those carrying the 2677TT genotype had the lowest ΔQTcBMB values that not all samples yielded sequencing data or showed PCR amplification.

Table 2. Genotype and allele frequencies of the studied variants

<table>
<thead>
<tr>
<th>Allelic varianta</th>
<th>Effectb</th>
<th>Nc</th>
<th>Genotype frequenciesd</th>
<th>Allele frequenciesa</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Wt</td>
<td>Het</td>
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<tr>
<td>Caucasians (N = 90)</td>
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<tr>
<td>ABCB1 1236C&gt;T</td>
<td>G411G</td>
<td>89</td>
<td>28 (31.4%)</td>
<td>41 (46.1%)</td>
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<tr>
<td>ABCB1 2677G&gt;T</td>
<td>A893S</td>
<td>87</td>
<td>23 (26.4%)</td>
<td>43 (49.4%)</td>
</tr>
<tr>
<td>ABCB1 2677G&gt;A</td>
<td>A893T</td>
<td>87</td>
<td>23 (26.4%)</td>
<td>2 GA (2.3%)</td>
</tr>
<tr>
<td>ABCB1 3435C&gt;T</td>
<td>I1145I</td>
<td>90</td>
<td>20 (22.2%)</td>
<td>43 (47.8%)</td>
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<td>African Americans (N = 19)</td>
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<tr>
<td>ABCB1 1236C&gt;T</td>
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<td>19</td>
<td>8 (42.1%)</td>
<td>6 (31.6%)</td>
</tr>
<tr>
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<td>A893S</td>
<td>19</td>
<td>11 (57.9%)</td>
<td>5 (26.3%)</td>
</tr>
<tr>
<td>ABCB1 2677G&gt;A</td>
<td>A893T</td>
<td>19</td>
<td>11 (57.9%)</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td>ABCB1 3435C&gt;T</td>
<td>I1145I</td>
<td>19</td>
<td>11 (57.9%)</td>
<td>2 (10.5%)</td>
</tr>
<tr>
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<td>6</td>
<td>0(%)</td>
<td>5 (83.3%)</td>
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<td>6</td>
<td>1 (16.7%)</td>
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<tr>
<td>ABCB1 2677G&gt;A</td>
<td>A893T</td>
<td>6</td>
<td>1 (16.7%)</td>
<td>1 (16.7%)</td>
</tr>
</tbody>
</table>

Abbreviations: Wt, homozygous wild-type allele patient; Het, heterozygous patient; Var, homozygous variant patient.

aNumber represents position in nucleotide sequence.
bNumber represents amino acid codon.
cGenotype data were not available in all patients as not all samples yielded sufficient DNA or PCR amplified.
dNumber represent number of patients with percentage in parenthesis; the difference in the total number of patients is due to the fact that not all samples yielded sequencing data or showed PCR amplification.
eHardy–Weinberg notation for allele frequencies (p, frequency for wild-type allele and q, frequency for variant allele).
fThe 2677G>T/A polymorphism is triallelic and 2 different SNPs are therefore presented.
gThree Hispanics and 3 Asians were included as “other” because accurate allele frequency information could not be obtained with so few individuals from these populations.

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increase the likelihood of an association with ABCB1 alleles. Taken together, the data suggest that individuals carrying wild-type alleles at ABCB1 have an increased likelihood of developing greater ΔQTc following romidepsin compared with individuals with variant genotypes.

No relationship was found between ABCB1 genotype and ΔQTcF or ΔQTcB over the course of repeated infusions (Supplementary Results S1). Thus, it seems that alterations in romidepsin-related QTc prolongation due to allelic variation in ABCB1 may only be important following the first administration of romidepsin.

Discussion

This study suggests that ABCB1 is involved in a previously unexplored blood–tissue barrier in the cardiac endothelium in which it limits drug exposure in the heart. Interestingly, the observed associations with cardiac repolarization were found to be independent of an influence of ABCB1 on romidepsin plasma pharmacokinetic parameters in both mice and humans. Mice lacking the ABCB1-type P-glycoprotein had a greater intracardiac exposure to romidepsin. Although there was no difference in the QTcmax, this difference in exposure may have altered cardiac repolarization because the time to maximum QTc was different between wild-type and knockout (31 vs. 16 hours). These data suggest that ABCB1 is a component of a blood–tissue barrier within the cardiac endothelium. Consistent with an impact of ABCB1 on cardiac exposure to romidepsin, we also show differences in the QT interval associated with common inherited variants in the ABCB1 gene. Unexpectedly, the variant alleles understood to be associated with reduced function of the ABCB1 transporter (12, 16) were in this study associated with a reduced impact of romidepsin on the QT interval. Taken together, these results are consistent with a hypothesis that patients carrying variant ABCB1 alleles have increased cardiac ABCB1 gene expression (11), which in turn limits the exposure of cardiac tissue to romidepsin.

The human ether-a-go-go related gene (hERG) encodes the α-subunit of the rapid delayed rectifier current I_{K1} in the heart, which contributes prominently to terminal repolarization in human ventricular myocytes. Romidepsin has been shown to alter terminal cardiac repolarization by inhibiting the hERG protein, potentially by acetylation (34). Maximal QTc prolongation was delayed following romidepsin administration in mice despite the rapid distribution and clearance of the drug, demonstrating that romidepsin has a delayed, rather than acute, effect on ΔQTc in mice, similar to the effect observed in humans (13). This is inconsistent with direct hERG/I_{K1} blockade (29, 35, 36); rather, it is likely that the delay in QTc prolongation is
related to an intracellular mechanism whereby romidepsin modulates certain pathways related to the hERG protein, such as by blocking hERG shuttling to the membrane by hsp90 as has been observed in several other drug treatments (i.e., fluoxetine, pantamidine, probucil, geldenamycin, radicicol, and celastrol; reviewed in ref. 37). Nonetheless, removal of Abcb1a/1b from the genome seems to be responsible for decreasing the time taken to ΔQTcmax, most likely by increasing the overall exposure of the heart to romidepsin in mice. It is expected that this occurs because Abcb1a/1b is important for effluxing romidepsin from the heart back into the systemic circulation, thereby protecting the heart from QTc changes. On the other hand, using whole heart homogenate, the exposure increase between wild-type and Abcb1a/1b−/− mice was relatively modest (~1.35-fold increase in knockouts); however, it is unclear to what degree cardiac exposure to romidepsin must increase in order to see an effect on QTc. Moreover, romidepsin exposure may have been higher in cardiac myocytes of knockout mice, or the actual distribution altered, than was apparent from whole heart homogenate as Abcb1 in cardiac endothelial cells would primarily limit drug penetration into highly vascularized cardiac muscle tissue.

Meissner and colleagues showed that human individuals carrying the variant ABCB1 2677TT genotype have increased ABCB1 mRNA levels in cardiac endothelial cells. Although, the results of Meissner and colleagues contrast with the current understanding of ABCB1 polymorphic variation (i.e., wild-type alleles are most often related to higher expression in other tissues; ref. 38), the current data obtained with romidepsin are consistent with Meissner and colleagues as individuals with higher copy numbers of variant ABCB1 2677TT alleles exhibited reduced lengthening of the QT-interval following romidepsin, compared with patients carrying increasing numbers of ABCB1 wild-type alleles. The association between ΔQTc is strongest when all three of the studied alleles are considered together in a haplotype, consistent with functional studies of ABCB1 allelic variation (12, 16). Meissner and colleagues did not evaluate haplotypes in their study of mRNA expression. Because we determined that ABCB1 variant alleles confer a phenotype with lowered transport efficiency, the data suggest that gene expression in the cardiac endothelium may be a more important factor than altered protein folding and function induced by the nonsynonymous 2677G>T/A (A893S/T) SNP, or the synonymous 1226C>T and 3435C>T transitions (12, 16).

The increase in time taken to achieve ΔQTcmax in mice suggests that the human data may result from ABCB1 expression status modulating an early or later ΔQTcmax and not necessarily a greater magnitude of ΔQTc induced by romidepsin. This study found that ABCB1 alleles have an effect after 4 hours such that individuals carrying wild-type alleles, in which ABCB1 expression is presumably low in the cardiac endothelium (11), also have a larger ΔQTc at that time point. We did not find a relationship between ABCB1 SNPs and ΔQTc at 24 hours or during subsequent administrations of the drug. However, because QTc was not monitored continuously in clinical trials, we were unable to assess ΔQTcmax in patients, and future studies in humans must evaluate QTc prolongation with more resolution in order to determine whether or not ABCB1 expression is responsible for a greater magnitude or a delayed ΔQTcmax induced by romidepsin in humans. Moreover, romidepsin may induce ABCB1 in humans (23) thereby facilitating its own elimination from the heart; this is possibly the reason that the difference in ΔQTc based on genotype is only apparent following the first administration, but not subsequent administrations of the drug. It also remains unclear whether other ABCB1 substrate drugs that prolong the QT interval (risperidone, clozapine, and potentially tamoxifen) are also limited from penetrating the heart tissue in a similar fashion, and whether genetic variation influences their intracardiac concentration. A single, small study in patients treated with doxorubicin seems to indicate that cardiomyopathy is more prevalent in patients with low ABCB1 mRNA and protein expression in cardiac endothelium (8). Thus, further research is required to ascertain the clinical importance of ABCB1 expression on substrate drugs that mediate QT prolongation and cardioxicity.

This study contains several limitations and inconsistencies with the literature. First, the functional impact of allelic variation in ABCB1 gene expression presented herein adds to a body of literature that has been controversial. Many investigations have showed that in liver, intestine, and blood cells, the ABCB1 2677G>T/A and 3435C>T wild-type alleles actually predict higher ABCB1 expression relative to variant alleles (39–42). However, functional studies have not consistently confirmed an impact of this higher gene expression on systemic drug exposure, with investigators reporting both increased and decreased drug clearance associated with these alleles. Nonetheless, our results are consistent with the observations set forth by Meissner and colleagues in human cardiac endothelial cells; cardiac expression of ABCB1 may be regulated in a tissue-dependent fashion (11). To date, no clinically significant QT prolongation has been observed in romidepsin clinical trials (13). Third, this study did not evaluate the pharmacokinetics of reduced romidepsin and the active metabolite, and we are not able to conclude whether or not exposure to the active metabolite is greater. Thus, our results should be considered exploratory and in need of further validation.

In conclusion, this study provides evidence that QTc interval changes following treatment with the ABCB1 model substrate romidepsin might be linked to the expression of the ABCB1 drug transporter in the cardiac endothelium. We believe that this is the first demonstration that ABCB1 expression alters QT prolongation both in preclinical and clinical settings. This study is also the first to suggest that ABCB1 allelic variation may serve as a marker for prolonged QT-interval mediated by ABCB1 substrate drugs. These results warrant further evaluation for both romidepsin, and for other ABCB1 substrates that have the potential to cause cardiac side effects.
Disclosure of Potential Conflicts of Interest

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organization imply endorsement by the U.S. Government. None of the authors disclose potential conflicts of interest.

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References


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