Published in final edited form as:

Clin Pharmacol Ther. 2012 November ; 92(5): 642–650. doi:10.1038/clpt.2012.106.

OATP1B1 Polymorphism as a Determinant of Erythromycin Disposition

CS Lancaster¹, GH Bruun², CJ Peer³, TS Mikkelsen⁴, TJ Corydon², AA Gibson¹, S Hu¹, SJ Orwick¹, RHJ Mathijssen⁴, WD Figg³, SD Baker¹, and A Sparreboom¹

¹Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, Tennessee, USA ²Department of Biomedicine, Aarhus University, Aarhus, Denmark ³Clinical Pharmacology Program, Office of the Clinical Director, National Cancer Institute, Bethesda, MD, USA ⁴Department of Pediatric Oncology and Hematology, Aarhus University Hospital, Skejby, Denmark ⁴Department of Medical Oncology, Erasmus MC – Daniel den Hoed Cancer Center, Rotterdam, the Netherlands.

Abstract

Previous studies have demonstrated that the pharmacokinetic profile of erythromycin, a probe for CYP3A4 activity, is affected by inhibitors or inducers of hepatic solute carriers. We hypothesized that these interactions are mediated by OATP1B1 (gene symbol, *SLCO1B1*), a polypeptide expressed on the basolateral surface of hepatocytes. Using stably transfected Flp-In T-REx 293 cells, erythromycin was found to be a substrate for OATP1B1*1A (wildtype) with a K_m of ~13 μ M, and its transport was reduced by ~50% in cells expressing OATP1B1*5 (V174A). Deficiency of the ortholog transporter Oatp1b2 in mice was associated with a 52% decrease in the metabolic rate of erythromycin ($P = 0.000043$). In line with these observations, in humans, the c.521T>C variant in *SLCO1B1* (rs4149056), encoding OATP1B1*5, was associated with a genotype-dosage dependent decline in erythromycin metabolism ($P = 0.0072$). These results suggest that impaired OATP1B1 function can alter erythromycin metabolism independently of changes in CYP3A4 activity.

Keywords

Erythromycin; OATP1B1; hepatic metabolism

INTRODUCTION

The therapeutic range for most cytotoxic anticancer agents is extremely narrow, and in most cases no information is available *a priori* on the intrinsic sensitivity of a patient's tumor to a

Correspondence: Alex Sparreboom, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, CCC, Room I5308. Phone: (901) 595-5346; Fax: (901) 595-3125; alex.sparreboom@stjude.org.

This work was previously presented, in part, at the Annual Meeting of the 113th American Society for Clinical Pharmacology and Therapeutics (ASCPT), held March 14-17, 2012, in National Harbor, MD. CS Lancaster was a recipient of ASCPT's 2012 David J. Goldstein Trainee Award in connection with this study.

CONFLICT OF INTEREST/DISCLOSURE

The authors declared no conflict of interest. None of the funding bodies had a role in the preparation of the manuscript.

AUTHOR CONTRIBUTIONS

CSL, RHJM, SDB, and AS wrote the manuscript; WDF, SDB, and AS designed research; CSL, GHB, CJP, TJC, TSM, AAG, SJO, and RHM performed research; GHB, TJC, and TSM contributed new reagents / analytical tools; CSL, GHB, CJP, RHM, WDF, SDB, and AS analyzed data.

particular agent, or the patient's tolerability of a given dose prior to therapy. Hence, currently applied dosing regimens for cytotoxic chemotherapy remain largely empirical. Since the effect of a therapeutic agent is generally a function of its concentration at the (molecular) site of action, it is obvious that a description of the spatial-temporal profile of the drug will be helpful, if not essential, in understanding and predicting normal tissue toxicity and optimizing tumor response. Because CYP3A4 and CYP3A5 (collectively referred to as CYP3A) are involved in the metabolism of the majority of all currently prescribed anticancer drugs, it has been hypothesized that phenotyping of an individual's constitutive CYP3A activity before chemotherapeutic treatment might assist in explaining pharmacokinetic variability, and subsequently utilize such information to individualize dosage.¹

The macrolide antibiotic erythromycin is the most widely probe in oncology used to assess phenotypic activity of hepatic CYP3A function.² The disposition properties of erythromycin, when applied as a phenotyping breath test, are highly variable and characterized by more than 50-fold differences in metabolic clearance between subjects.³ It has been assumed for several decades that a critical determinant of erythromycin's pharmacokinetic variability is associated with differential expression of polymorphic CYP3A enzymes in the liver. However, several published analyses indicate that the contribution of genetic variants in CYP3A4 or CYP3A5 to explaining pharmacokinetic variability of erythromycin is rather limited.^{3,4} A number of recent studies provided evidence that impaired function of several ATP-binding cassette transporters expressed on the bile canalicular membrane of hepatocytes, including ABCB1 (P-glycoprotein)^{5,6} and ABCC2 (MRP2),⁷ can also alter the metabolism of erythromycin independent of intrinsic changes in CYP3A activity.

The mechanism by which erythromycin is taken up into liver cells is still largely unknown. Previously reported drug-drug interaction studies have provided preliminary evidence that hepatocellular uptake of erythromycin may be partially regulated by OATP1B1 (formerly, OATPC; LST-1; OATP2), a polymorphic organic anion transporting polypeptide encoded by the *SLCO1B1* gene (formerly, *SLC21A6*). For example, rifampin, a known inhibitor of OATP1B-type carriers,⁸ can significantly inhibit erythromycin uptake and subsequent metabolism in freshly isolated rat hepatocytes,⁹ as well as impair erythromycin metabolism in human subjects undergoing an erythromycin breath test.¹⁰ Moreover, erythromycin can significantly increase the plasma levels of bromocriptine in humans¹¹ by a mechanism that involves, in part, inhibition of OATP1B1-mediated uptake of bromocriptine in the liver by erythromycin.¹² The aims of the current study were to (i) evaluate transport of erythromycin by OATP1B1 *in vitro*; (ii) determine erythromycin metabolism in mice that are knockout for the related transporter Oatp1b2; and (iii) assess the functional impact of germline variation in *SLCO1B1* on the metabolism of erythromycin in humans.

RESULTS

In vitro transport of erythromycin

To assess the possible transport of erythromycin by OATP1B1, *in vitro* uptake studies were performed in cells stably transfected with an empty vector, OATP1B1 or Oatp1b2. These experiments indicated that the OATP1B1 and Oatp1b2 proteins were able to take up estradiol-17 β -D-glucuronide, a known substrate used as a positive control (Figure 1A), as well as erythromycin (Figure 1B), and this process could be inhibited by rifampin (Figures 1A and 1B). The transport of erythromycin into the cells transfected with OATP1B1 was found to be saturable with a Michaelis-Menten constant (K_m) of $13.2 \pm 5.71 \mu\text{M}$, and a maximum velocity (V_{max}) of $58.3 \pm 13.2 \text{ pmol/mg/5 min}$ (Figure 1C). Compared to cell transfected with wildtype OATP1B1 (OATP1B1*1A), erythromycin transport was reduced

by more than 50% ($P < 0.05$) in cells expressing the OATP1B1*5 variant representing the *SLCO1B1* c.521T> variant (rs4149056; V174A) (Figure 1D). Altered erythromycin transport was not observed in cells transfected with the OATP1B1*1B [c.388A>G (N130D)] or OATP1B1*15 (N130D and V174A) variants (Figure 1D).

Erythromycin transport in Oatp1b2-knockout mice

We next evaluated the possible importance of this transporter for erythromycin in mice with a genetic deletion of the ortholog mouse transporter Oatp1b2 [Oatp1b2(−/−) mice], based on the hypothesis that a decrease in hepatocellular entry of erythromycin in Oatp1b2(−/−) mice would cause a subsequently diminished metabolism of the drug (Figure 2). Consistent with previous *ex vivo* data⁹ and *in vitro* data^{13,14} indicating that erythromycin is transported by rat Oatp1b2, deficiency of this transporter in mice was associated with a 52% decrease in the metabolic rate of erythromycin ($P = 0.000043$), as determined from the cumulative amount of exhaled ¹⁴CO₂ (Figure 3A). The respective concentration-time profiles of exhaled ¹⁴CO₂ over time in wildtype and Oatp1b2(−/−) mice (Figure 3A) is consistent with the hypothesis that the observed phenotype is linked with changes in erythromycin accumulation in the liver.

To rule out potentially altered, compensatory expression of enzymes and transporters in the liver of Oatp1b2(−/−) mice at baseline, microarrays were used to evaluate differential expression profiles of ATP-binding cassette transporters, P450 enzymes, and solute carriers of putative relevance for erythromycin. Compared to levels in liver of wildtype mice, transcripts of these genes were not statistically significantly altered in the Oatp1b2(−/−) mice (Figure 3C). Furthermore, there were no compensatory changes in the protein expression (Figure 3D) or activity (Figure 3E) of hepatic Cyp3a11, the main murine enzyme involved in the metabolism of erythromycin.

Association of *SLCO1B1* genotype with erythromycin transport in humans

To provide preliminary evidence for a possible role of OATP1B1 in the clinical pharmacology of erythromycin, an exploratory pharmacogenetic-association analysis was performed in human subjects with cancer undergoing an erythromycin breath test prior to the administration of chemotherapy. To this end, pharmacokinetic and pharmacogenetic data was obtained from 91 human subjects (38 females and 53 males) with a median age of 63 years (range, 40 to 83 years). The majority of the population was white (European American), and 20 patients were black (African American). The conventional erythromycin breath test parameter, the flux at 20 minutes (C₂₀) averaged 0.049 ± 0.020 %dose/min, with an 80-fold observed difference between the lowest and highest values. In line with previous findings,³ the observed C₂₀ values were higher in females than males (0.053 vs 0.042 %dose/min; $P = 0.00136$), and significantly correlated with body-surface area ($P = 0.0024$; $R^2 = 0.099$), and baseline serum levels of alanine-aminotransferase ($P = 0.063$; $R^2 = 0.048$) and α 1-acid glycoprotein ($P = 0.050$; $R^2 = 0.047$), but not with race ($P = 0.83$), age ($P = 0.93$) or other serum chemistry parameters ($P < 0.05$). Body-surface area, alanine-aminotransferase, and α 1-acid glycoprotein, but not sex, were retained in a subsequently performed multiple regression model ($P = 0.0009$; $R^2 = 0.197$).

Based on our *in vitro* studies, we focused specifically on the OATP1B1*5 variant (c.521T>C) for further *in vivo* evaluation. The relative frequency of this variant allele in our patient population was comparable with previously reported estimates.¹⁵ Moreover the distribution of this variant was in Hardy-Weinberg equilibrium ($P = 0.23$), and demographic characteristics at baseline were similar for individuals carrying 0, 1, or 2 variant alleles at the locus of interest (Table 1). As predicted based on its observed functional impact *in vitro*, the OATP1B1 c.521C substitution was found to be statistically significantly associated with

erythromycin metabolism in humans, in a gene-dosage dependent fashion (Figure 4A). The association was retained after eliminating subjects that carried two copies of this variant ($P=0.0171$).

Based on all data from all subjects, and after adjusting for the effects of factors found to be associated with erythromycin C_{20} in this dataset, namely body-surface area, alanine-aminotransferase, and α 1-acid glycoprotein, OATP1B1*5 genotype was found to be associated with C_{20} in a statistically significant fashion ($P=0.026$; final model, $R^2=0.274$). The association of OATP1B1*5 with erythromycin metabolism remained statistically significant after excluding the CYP3A5 expressors (Figure 4B), and after eliminating subjects that carried two copies of either the *SLCO1B3* 334T variant (Figure 4C) or the *ABCC2* -24T variant (Figure 4D), which were previously shown to be predisposed to altered erythromycin elimination.^{7,13} The OATP1B1*5-erythromycin association was also retained when the analysis was restricted to females ($P=0.0061$), despite the fact that none were homozygous variant at this locus.

DISCUSSION

The current study provides support for a growing body of evidence that solute carriers belonging to the family of organic anion transporting polypeptides can have a profound impact on the hepatic accumulation and systemic clearance of CYP3A4 substrates. Employing an array of *in vitro* transport assays, including intracellular accumulation studies in transfected mammalian cells, erythromycin was identified as a substrate for human OATP1B1. Our own previously reported transporter screen failed to observe transport of erythromycin by OATP1B1 when the transporter was over-expressed in *Xenopus oocytes*.¹³ This suggests that the interaction of erythromycin with this carrier might be dependent on cell context and/or is particularly sensitive to variation in the applied experimental conditions.

Based on sequence homology, shared basolateral localization in hepatocytes and overlapping substrate specificity,¹⁶ it has been suggested that in mice *Oatp1b2* fulfills the same function in the liver as OATP1B1 and OATP1B3 in humans. Based on this premise, we evaluated the metabolism of erythromycin in a mouse model with a genetic deletion of *Oatp1b2*. Possible limitations of this model are that these animals do not express a homologous protein and that, unlike in humans, mouse hepatocytes express multiple members of *Oatp1a*, a related subfamily of transporters that can potentially provide compensatory restoration of function when *Oatp1b2* is lost.¹⁷ Despite these limitations, the extent of erythromycin metabolism, as determined from the amount exhaled $^{14}\text{CO}_2$ following injection of [^{14}C -*N*-methyl]erythromycin, was substantially diminished in *Oatp1b2*(-/-) mice compared to wildtype mice. Gene expression profiling and *Cyp3a* protein expression measurements in liver samples excluded alterations in alternate transport mechanisms and/or metabolic pathways as possible causes of the impaired metabolic phenotype in *Oatp1b2*(-/-) mice. These findings suggest that *Oatp1b2*-mediated transport of erythromycin may be a critically important rate-limiting process in the elimination of this drug in mice. Nonetheless, considering the relatively low amino acid homology between OATP1B1 and *Oatp1b2* (about 64%) and between CYP3A4 and *Cyp3a11* (about 73%), additional investigation is required employing humanized models for these proteins to provide direct evidence for involvement of OATP1B1 in the hepatic uptake of erythromycin.

Using *in vitro* uptake studies, one functional variant in OATP1B1 (OATP1B1*5) was found to have a detrimental impact on erythromycin transport. This finding is consistent with previously studies showing substantially diminished transport activity of several OATP1B1 substrates by this particular variant when transfected into mammalian cells.¹⁸ The relevance

of this variant was also confirmed in our pharmacogenetic-association study performed in a group of predominantly white cancer patients undergoing an erythromycin breath test.

Previous studies have shown approximately 20 to 25% higher erythromycin metabolism in females than in males when using the erythromycin breath test.^{19–21} However, published studies on CO₂ output in different populations have revealed an approximately 20% lower rate of basal CO₂ production in females compared to males,²² which is consistent with the higher erythromycin metabolism in women. In line with these findings, we found that females had a 25.6% higher ¹⁴CO₂ production at 20 min following [¹⁴C-*N*-methyl]erythromycin administration than males in a univariate analysis, although this association was lost in a subsequent multiple regression analysis. Regardless of any apparent sex-related differences in intrinsic capacity to metabolize erythromycin, a similar degree of interindividual variation in exhaled ¹⁴CO₂ was observed in both female and male subjects.

The overall contribution of the OATP1B1*5 variant to explaining variation in the results obtained here with the erythromycin breath test was about 10.6%. This percentage is in the same order of magnitude as that observed previously for the same variant in the context of methotrexate clearance, namely between 9.3–11.3%.²³ It is possible that additional rare genetic variants or haplotypes in OATP1B1 of importance to the transport erythromycin in our population are yet to be discovered, as was recently demonstrated for methotrexate.¹⁵ Nonetheless, it is clear that the principal (genetic) contributors to the extensive interindividual variability associated with erythromycin metabolism currently remain elusive.

It has been proposed that the existence of multiple potentially redundant uptake transporters in the human liver with similar affinity for substrates may dictate that functional defects in all of these proteins are required to confer greatly altered disposition phenotypes such as those seen in a *Oatp1b2*(–/–) mice.¹⁷ In the case of erythromycin, currently available evidence indicates that its hepatocellular accumulation in humans is likely regulated, at least to some extent, by both OATP1B1 as well as the related transporter, OATP1B3.¹³ In this connection, it is noteworthy that the subject with the lowest recorded value of exhaled ¹⁴CO₂ at 20 min (namely, 0.00155 %dose/min, which is more than 30-fold lower than the observed average of 0.0488 %dose/min for the entire population) was also the only individual that carried known functional variants in both OATP1B1 and OATP1B3. While complete functional deficiency of either OATP1B1 or OATP1B3 has been recorded to occur at a reasonably high frequency in the human population,²⁴ deficiency of both transporters is extremely rare.²⁵ Nonetheless, the occurrence of multiple defective genetic variants in both of these transporters should be considered jointly in future studies, as such scenario may profoundly influence the metabolism of an agents like erythromycin, and could possibly help explain the existence of phenotypic outliers.

As mentioned earlier, the erythromycin-breath test has been extensively explored in oncology to predict the disposition properties of multiple anticancer agents that are metabolized by CYP3A, including gefitinib,²⁶ ifosfamide,²⁷ imatinib,²⁸ irinotecan,²⁹ docetaxel,¹ and vinorelbine.³⁰ In the context of our current findings, it is of note that the only agents for which statistically significant associations have been reported between breath test parameters and drug clearance, namely imatinib³¹ and docetaxel,³² are also the only drugs amongst those tested that are known substrates for OATP1B-type transporters. For docetaxel, the breath test-clearance associations have been confirmed in multiple independent studies involving patients receiving the drug once every week³³ or once every 3 weeks,^{4,34} as well as in subjects with liver function abnormalities³⁵ and in elderly patients.³⁶ In one recent report, however, no correlations were observed between breath test parameters and docetaxel clearance in 19 subjects.³⁷ It is important to point out that in the studied

patients, docetaxel clearance ranged by only 2.2-fold. The interindividual pharmacokinetic variability of docetaxel in this particular cohort of patients was thus substantially lower than what could be expected for a representative population, and this may have compromised detection of significant associations.

In conclusion, the current study suggests that a common germline variant in *SLCO1B1*, the gene encoding OATP1B1, is associated with altered metabolism of erythromycin. This observation supports prior evidence pointing to a critical contribution of hepatocellular uptake carriers in the *in vivo* handling of CYP3A4 probes, and provides a mechanistic explanation for previously reported drug-drug interaction studies involving erythromycin.^{10,11,38} Our results also suggest that the ability of erythromycin to predict the clearance of other CYP3A4 substrates will be compromised if differences in transporter affinities are not identified and fully taken into account. It logically follows that only once all factors contributing to the clearance of a particular CYP3A4 substrate drug are known, including non-metabolic determinants such as reliance on hepatic uptake transporters, should the clinical applicability and usefulness of erythromycin as a probe drug be considered.

METHODS

In vitro transport experiments

The generation, characterization, and maintenance of Flp-In T-Rex293 cells transfected with OATP1B1*1A (wildtype), OATP1B1*1B [c.388A>G (N130D); rs2306283], OATP1B1*5 [c.521T>C (V174A); rs4149056], or OATP1B1*15 (N130D, V174A) inserted into a pcDNA5/FRT vector has been reported previously.¹⁵ This model was selected as the cells contain a single stably integrated FRT site at a transcriptionally active genomic locus where targeted integration of a Flp-In expression vector ensures homogenous and high-level expression of multiple genetic variants. Mouse Oatp1b2 overexpressing HEK293 cells were created by stably transfecting a commercial cDNA cloned into a pDream2.1/MCS vector (GenScript, Piscataway, NJ). The radiolabeled compounds [6,7-³H(*N*)]estradiol-17 β -Dglucuronide (specific activity, 50.1 Ci/mmol) and [¹⁴C-*N*-methyl]erythromycin (specific activity, 55 mCi/mmol) were obtained from American Radiochemicals (St. Louis, MO). Uptake experiments were performed with these agents in the presence or absence of rifampin as described.³⁹

Animal experiments

Adult (8–12 week old) male Oatp1b2 knockout mice⁴⁰ and age-matched wildtype mice (Taconic, Germantown, NY), both on a DBA1/lacJ background, were housed in a temperature-controlled environment with a 12-hour light cycle, and given a standard diet and water *ad libitum*. Experiments were approved by the Institutional Animal Care and Use Committee of St. Jude Children's Research Hospital. Mice underwent an erythromycin breath test, as described.⁶ Briefly, mice received [¹⁴C-*N*-methyl]erythromycin (1 μ Ci/100 g; i.v.) in 2.5% dextrose, and were placed in a water-sealed polyurethane breath chamber with air continuously drawn through a vapor trap (acetone and dry ice), bubbled through an acidic methanol solution, and finally through 3 gas trapping washes containing 30 mL of gas trapping solution, composed of 27% (vol/vol) methanol, 41% toluene, 5% Emulsifier-safe, and 27% phenethylamine. Collection of breath was performed at 15, 30, 60, 90, and 120 min, and duplicate samples were analyzed by liquid scintillation counting. Values were used to calculate total ¹⁴CO₂ exhaled during the collection period.⁶ Experiments were performed in triplicate on 4 separate occasions.

Gene expression patterns in livers of wildtype mice and *Oatp1b2*($-/-$) mice were assessed on samples from 4 animals per group using the Mouse 430v2 GeneChip array (Affymetrix, Santa Clara, CA). Cyp3a11 protein expression in these same liver samples was assessed by Western blot,⁴¹ and liver microsomal Cyp3a activity was determined using midazolam, as described.⁷

Patient populations

Treatment protocols for the erythromycin breath test in the human subjects have been reported elsewhere.³ Briefly, patients with cancer with normal liver function received 0.04–0.07 mg of [¹⁴C-*N*-methyl]erythromycin (3–5 μ Ci) by i.v. bolus, and 8 serial breath samples were collected and analyzed as described.³ The test was performed at least 4 weeks after the last chemotherapy and at least one day before the next chemotherapy. Patients were not eligible for the study if, at the planned time of the test, they were taking phenytoin, carbamazepine, barbiturates, rifampin, phenobarbital, St. John's wort, ketoconazole, and/or other medications known to induce or inhibit CYP3A activity. The parameter C_{20} , representing the flux of exhaled ¹⁴CO₂ at 20 minutes after injection, was used as a surrogate for erythromycin clearance.⁴² Pharmacokinetic calculations were performed using Phoenix WinNonlin 6.1 (Pharsight, St. Louis, MO). Regarding liver function, appropriate criteria for eligibility in the context of the erythromycin breath test have been reported previously.^{3,35} These criteria include: (i) total bilirubin always $<1.5 \times$ upper limit of normal (ULN); (ii) elevations in transaminases were allowed as follows: aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) $<1.5 \times$ ULN if concurrent with alkaline phosphatase $<2.5 \times$ ULN, or AST and/or ALT 1.5 to $5 \times$ ULN if alkaline phosphatase $1.0 \times$ ULN, or AST/ALT $1.0 \times$ ULN with alkaline phosphatase 2.5 to $5 \times$ ULN, or isolated elevations in either AST or ALT or alkaline phosphatase (ie, without concurrent elevations of either AST and/or ALT $1.5 \times$ ULN or alkaline phosphatase $2.5 \times$ ULN). Written informed consent was obtained for all subjects, and the studies were approved by the local institutional review board, and Declaration of Helsinki recommendations for human subject biomedical research was followed.

Genotyping procedures

DNA was isolated from whole blood using MagnaPure LC (Roche Diagnostics GmbH), and then amplified.³ Variation in the *SLCO1B1* gene was assessed at the c.521C>T locus, which is associated with a V174A substitution, using direct nucleotide sequencing. This variant was selected on the basis of its relatively high allelic frequency and the known influence on functional properties of the encoded mutant proteins (OATP1B1*5).⁴³ In brief, exon 6 of *SLCO1B1* was amplified by PCR in a 25- μ L reaction containing 100 ng of gDNA, 1×5 PRIME Hotmaster Mix (5 PRIME, Gaithersburg, MD), and a final concentration of 200 nM of the forward primer (5'-TGTCAAAGTTTGCAAAGTGAA-3') and reverse primer (5'-GACAAAGGGAAAGTGATCATACAA-3'). This was done on an Eppendorf Mastercycler ep gradient S (Eppendorf, Hamburg, Germany), using a program consisting of denaturation at 94°C for 30 seconds, annealing at 61°C for 30 seconds, and extension at 68°C for 45 seconds for 35 cycles, with an initial heating at 94°C for 2 minutes, and a final extension at 68°C for 3 minutes. The PCR products were then run on a 1% agarose gel, stained with ethidium bromide and visualized under a UV lamp. Reactions that produced the appropriate 341bp PCR product were put through a clean-up using ExoSAP-IT (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. The clean PCR products were then sequenced with the same primers used in amplification using Big Dye Terminator (v3.1) Chemistry on an Applied Biosystem 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA). Forward and reverse sequences were aligned and analyzed using the software Sequencher (Gene Codes, Ann Arbor, MI).

Variations in the genes encoding CYP3A5 (rs776746, *CYP3A5* c.219-237G>A; *CYP3A5**3C), OATP1B3 (rs4149117; *SLCO1B3* c.334T>G; S112A) and ABCC2 (rs717620; *ABCC2* c.-24C>T; 5'UTR) were determined as described previously.^{3,7,13} Genotypes were called variant if they differed from the Refseq sequence for the genetic position of interest.

Statistical considerations

Due to the exploratory nature of the clinical studies no power calculations for sample size were performed *a priori*. All experiments were analyzed using one-way analysis of variance or a t-test. Associations between baseline patient characteristics and variant genotypes with erythromycin breath test results were evaluated by multiple regression analysis. To determine the impact of the variant genotype of interest after consideration of other factors, a model was first constructed incorporating all parameters determined to be jointly statistically significant other than genotype. Then, the genotype parameter was added to the model, and the statistical significance of the additional parameter determined. All *P* values are two-tailed, and the cutoff for statistical significance was set at *P* < 0.05. Statistical calculations were performed using NCSS 2004 (Number Cruncher Statistical Systems, Kaysville, UT).

Acknowledgments

We would like to thank Richard Kim and Jeffrey Stock for providing the Oatp1b2(−/−) mice, and Lubin Lan and Erin Schuetz for assistance with performing the erythromycin breath tests in mice. This work was supported in part by the American Lebanese Syrian Associated Charities (ALSAC), US Public Health Service Cancer Center Support Grant 3P30CA021765 (SDB), and NCI Grant 5R01CA151633-01 (AS). The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

REFERENCES

1. Opdam FL, Gelderblom H, Guchelaar HJ. Phenotyping drug disposition in oncology. *Cancer Treat Rev.* 2012
2. Watkins PB. Noninvasive tests of CYP3A enzymes. *Pharmacogenetics.* 1994; 4:171–184. [PubMed: 7987401]
3. Baker SD, et al. Factors affecting cytochrome P-450 3A activity in cancer patients. *Clin Cancer Res.* 2004; 10:8341–8350. [PubMed: 15623611]
4. Baker SD, et al. Pharmacogenetic pathway analysis of docetaxel elimination. *Clin Pharmacol Ther.* 2009; 85:155–163. [PubMed: 18509327]
5. Kurnik D, Wood AJ, Wilkinson GR. The erythromycin breath test reflects P-glycoprotein function independently of cytochrome P450 3A activity. *Clin Pharmacol Ther.* 2006; 80:228–234. [PubMed: 16952489]
6. Lan LB, Dalton JT, Schuetz EG. Mdr1 limits CYP3A metabolism in vivo. *Mol Pharmacol.* 2000; 58:863–869. [PubMed: 10999959]
7. Franke RM, et al. Effect of ABCC2 (MRP2) transport function on erythromycin metabolism. *Clin Pharmacol Ther.* 2011; 89:693–701. [PubMed: 21451505]
8. Fattinger K, Cattori V, Hagenbuch B, Meier PJ, Stieger B. Rifamycin SV and rifampicin exhibit differential inhibition of the hepatic rat organic anion transporting polypeptides, Oatp1 and Oatp2. *Hepatology.* 2000; 32:82–86. [PubMed: 10869292]
9. Lam JL, Okochi H, Huang Y, Benet LZ. In vitro and in vivo correlation of hepatic transporter effects on erythromycin metabolism: characterizing the importance of transporter-enzyme interplay. *Drug Metab Dispos.* 2006; 34:1336–1344. [PubMed: 16698890]
10. Frassetto LA, Poon S, Tsourounis C, Valera C, Benet LZ. Effects of uptake and efflux transporter inhibition on erythromycin breath test results. *Clin Pharmacol Ther.* 2007; 81:828–832. [PubMed: 17361125]

11. Nelson MV, Berchou RC, Kareti D, LeWitt PA. Pharmacokinetic evaluation of erythromycin and caffeine administered with bromocriptine. *Clin Pharmacol Ther.* 1990; 47:694–697. [PubMed: 2357864]
12. Lu WJ, Huang K, Lai ML, Huang JD. Erythromycin alters the pharmacokinetics of bromocriptine by inhibition of organic anion transporting polypeptide C-mediated uptake. *Clin Pharmacol Ther.* 2006; 80:421–422. [PubMed: 17015059]
13. Franke RM, Baker SD, Mathijssen RH, Schuetz EG, Sparreboom A. Influence of solute carriers on the pharmacokinetics of CYP3A4 probes. *Clin Pharmacol Ther.* 2008; 84:704–709. [PubMed: 18509328]
14. Franke RM, Carducci MA, Rudek MA, Baker SD, Sparreboom A. Castration-dependent pharmacokinetics of docetaxel in patients with prostate cancer. *J Clin Oncol.* 2010; 28:4562–4567. [PubMed: 20855838]
15. Ramsey LB, et al. Rare versus common variants in pharmacogenetics: SLCO1B1 variation and methotrexate disposition. *Genome Res.* 2012; 22:1–8. [PubMed: 22147369]
16. Roth M, Obaidat A, Hagenbuch B. OATPs, OATs and OCTs: the organic anion and cation transporters of the SLCO and SLC22A gene superfamilies. *Br J Pharmacol.* 2012; 165:1260–1287. [PubMed: 22013971]
17. Iusuf D, van de Steeg E, Schinkel AH. Functions of OATP1A and 1B transporters in vivo: insights from mouse models. *Trends Pharmacol Sci.* 2012; 33:100–108. [PubMed: 22130008]
18. Kameyama Y, Yamashita K, Kobayashi K, Hosokawa M, Chiba K. Functional characterization of SLCO1B1 (OATP-C) variants, SLCO1B1*5, SLCO1B1*15 and SLCO1B1*15+C1007G, by using transient expression systems of HeLa and HEK293 cells. *Pharmacogenet Genomics.* 2005; 15:513–522. [PubMed: 15970799]
19. Watkins PB, et al. Erythromycin breath test as an assay of glucocorticoid-inducible liver cytochromes P-450. Studies in rats and patients. *J Clin Invest.* 1989; 83:688–697. [PubMed: 2913056]
20. Hunt CM, Westerkam WR, Stave GM. Effect of age and gender on the activity of human hepatic CYP3A. *Biochem Pharmacol.* 1992; 44:275–283. [PubMed: 1642641]
21. Floyd MD, et al. Genotype-phenotype associations for common CYP3A4 and CYP3A5 variants in the basal and induced metabolism of midazolam in European- and African-American men and women. *Pharmacogenetics.* 2003; 13:595–606. [PubMed: 14515058]
22. Rivory LP, Slaviero KA, Hoskins JM, Clarke SJ. The erythromycin breath test for the prediction of drug clearance. *Clin Pharmacokinet.* 2001; 40:151–158. [PubMed: 11327195]
23. Trevino LR, et al. Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. *J Clin Oncol.* 2009; 27:5972–5978. [PubMed: 19901119]
24. Kim SR, et al. Genetic variations and frequencies of major haplotypes in SLCO1B1 encoding the transporter OATP1B1 in Japanese subjects: SLCO1B1*17 is more prevalent than *15. *Drug Metab Pharmacokinet.* 2007; 22:456–461. [PubMed: 18159134]
25. van de Steeg E, et al. Complete OATP1B1 and OATP1B3 deficiency causes human Rotor syndrome by interrupting conjugated bilirubin reuptake into the liver. *J Clin Invest.* 2012; 122:519–528. [PubMed: 22232210]
26. Chhun S, et al. Gefitinib-phenytoin interaction is not correlated with the C-erythromycin breath test in healthy male volunteers. *Br J Clin Pharmacol.* 2009; 68:226–237. [PubMed: 19694743]
27. Chugh R, et al. Assessment of ifosfamide pharmacokinetics, toxicity, and relation to CYP3A4 activity as measured by the erythromycin breath test in patients with sarcoma. *Cancer.* 2007; 109:2315–2322. [PubMed: 17464949]
28. Gurney H, et al. Imatinib disposition and ABCB1 (MDR1, P-glycoprotein) genotype. *Clin Pharmacol Ther.* 2007; 82:33–40. [PubMed: 17495881]
29. Mathijssen RH, et al. Prediction of irinotecan pharmacokinetics by use of cytochrome P450 3A4 phenotyping probes. *J Natl Cancer Inst.* 2004; 96:1585–1592. [PubMed: 15523087]
30. Schott AF, et al. Combination vinorelbine and capecitabine for metastatic breast cancer using a non-body surface area dosing scheme. *Cancer Chemother Pharmacol.* 2006; 58:129–135. [PubMed: 16283312]

31. Hu S, et al. Interaction of imatinib with human organic ion carriers. *Clin Cancer Res.* 2008; 14:3141–3148. [PubMed: 18483382]
32. Smith NF, Figg WD, Sparreboom A. Role of the liver-specific transporters OATP1B1 and OATP1B3 in governing drug elimination. *Expert Opin Drug Metab Toxicol.* 2005; 1:429–445. [PubMed: 16863454]
33. Slaviero KA, Clarke SJ, McLachlan AJ, Blair EY, Rivory LP. Population pharmacokinetics of weekly docetaxel in patients with advanced cancer. *Br J Clin Pharmacol.* 2004; 57:44–53. [PubMed: 14678339]
34. Hirth J, et al. The effect of an individual's cytochrome CYP3A4 activity on docetaxel clearance. *Clin Cancer Res.* 2000; 6:1255–1258. [PubMed: 10778948]
35. Hooker AC, et al. Population pharmacokinetic model for docetaxel in patients with varying degrees of liver function: incorporating cytochrome P4503A activity measurements. *Clin Pharmacol Ther.* 2008; 84:111–118. [PubMed: 18183036]
36. Hurria A, et al. Pharmacokinetics and toxicity of weekly docetaxel in older patients. *Clin Cancer Res.* 2006; 12:6100–6105. [PubMed: 17062686]
37. Michael M, et al. Docetaxel pharmacokinetics and its correlation with two in vivo probes for cytochrome P450 enzymes: the C(14)-erythromycin breath test and the antipyrine clearance test. *Cancer Chemother Pharmacol.* 2012; 69:125–135. [PubMed: 21626050]
38. Kovarik JM, et al. Effect of multiple-dose erythromycin on everolimus pharmacokinetics. *Eur J Clin Pharmacol.* 2005; 61:35–38. [PubMed: 15785960]
39. Bruno R, Vivier N, Veyrat-Follet C, Montay G, Rhodes GR. Population pharmacokinetics and pharmacokinetic-pharmacodynamic relationships for docetaxel. *Invest New Drugs.* 2001; 19:163–169. [PubMed: 11392450]
40. Zaher H, et al. Targeted disruption of murine organic anion-transporting polypeptide 1b2 (Oatp1b2/Slco1b2) significantly alters disposition of prototypical drug substrates pravastatin and rifampin. *Mol Pharmacol.* 2008; 74:320–329. [PubMed: 18413659]
41. Relling MV, McLeod HL, Bowman LC, Santana VM. Etoposide pharmacokinetics and pharmacodynamics after acute and chronic exposure to cisplatin. *Clin Pharmacol Ther.* 1994; 56:503–511. [PubMed: 7955814]
42. Rivory LP, et al. Optimizing the erythromycin breath test for use in cancer patients. *Clin Cancer Res.* 2000; 6:3480–3485. [PubMed: 10999732]
43. Franke RM, Gardner ER, Sparreboom A. Pharmacogenetics of drug transporters. *Curr Pharm Des.* 2010; 16:220–230. [PubMed: 19835554]

STUDY HIGHLIGHTS

What is the current knowledge on the topic?

Previous studies have demonstrated that the pharmacokinetic profile of erythromycin, a probe for CYP3A4 activity, is affected by inhibitors or inducers of hepatic solute carriers.

What question this study addressed?

Using a combinatorial approach involving cell-based model systems, transporter knockout animals, and human subjects with variable transporter activity, we here describe the identification of OATP1B1 as an important protein involved in the uptake of erythromycin into hepatocytes.

What this study adds to our knowledge?

This process was demonstrated to be sensitive to a chemical inhibitor and affected by a common reduced-function germline variant in *SLCO1B1*, the gene encoding OATP1B1.

How this might change clinical pharmacology and therapeutics?

Our findings support a growing body of evidence pointing to a critical contribution of transporters in the *in vivo* handling of CYP3A4 probes, and provide a plausible mechanistic explanation for previously reported drug-drug interaction studies.

Figure 1.

In vitro transport studies of erythromycin. (A) Transport of [6,7-³H(*N*)]estradiol-17β-Dglucuronide (E2-17β-glu), a positive control, by human OATP1B1 and mouse Oatp1b2 was evaluated with constructs transfected into mammalian cells (E2-17β-glu concentration, 0.1 μM; 15-min incubations) in the absence or presence of rifampin (200 μM; 15-min pre-incubation). (B) Transport of [¹⁴C-*N*-methyl]erythromycin (erythromycin) by human OATP1B1 and mouse Oatp1b2 was evaluated in transfected into cells (erythromycin concentration, 0.1 μM; 15-min incubations) in the absence or presence of rifampin (200 μM; 15-min pre-incubation). (C) Characterization of erythromycin concentration-dependent transport by human OATP1B1. (D) Transport of E2-17β-glu (0.1 μM; 15-min incubations) and erythromycin (concentration, 0.1 μM; 60-min incubations) was evaluated in Flp-In T-Rex293 cells transfected with OATP1B1*1A (wildtype), OATP1B1*1B [c.388A>G (N130D); rs2306283], OATP1B1*5 [c.521T>C (V174A); rs4149056], or OATP1B1*15 (N130D, V174A). All data represent the mean of at least 6 observations, and are expressed as the average percent of uptake values in cells transfected with an empty vector (VC). Error

bars represent the standard error. The star (*) denotes a significant difference from each corresponding VC ($P < 0.05$).

Figure 2.
Theoretical influence of *Oatp1b2*-knockout on the metabolism of [^{14}C -N-methyl] erythromycin (^{14}C -ER) in mice following an erythromycin breath test.

Figure 3. Loss of Oatp1b2 in mice alters erythromycin metabolism without impacting Cyp3a function. (A) Time course of cumulative exhaled $^{14}\text{CO}_2$ was evaluated in wildtype mice and Oatp1b2-deficient [Oatp1b2(-/-)] mice using 12 animals per group, representing triplicate observations from an experiment performed on 4 separate occasions. (B) Pairwise comparison of select genes encoding transporter or enzymes of known or suspected relevance to erythromycin was evaluated at baseline in livers of wildtype mice and Oatp1b2(-/-) mice using 5 animals per group. (C/D) Protein expression for Cyp3a11, normalized to β -actin in livers of wildtype mice and Oatp1b2(-/-) was evaluated using

Western blotting on samples from 4 animals per group. (E) Hepatic microsomal Cyp3a activity in the same livers, as determined by midazolam hydroxylation. All data represent mean (bars) and SEM (error bars).

Figure 4.

Erythromycin metabolism as a function of *SLCO1B1* genotype. (A) Data were obtained in 91 predominantly white cancer patients undergoing an erythromycin breath test (ERMBT). The same information is shown after eliminating subjects expressing CYP3A5 (B), and those individuals with either the *SLCO1B3* 334TT (C) or the *ABCC2* -24TT genotype (D). Each symbol represents an individual patient, and horizontal lines indicate median values. The *P*-value denotes a statistical comparison of the ERMBT results in the different genotype group, and a star (*) denotes a significant difference from each corresponding group with the reference *SLCO1B1* genotype ($P < 0.05$).

Table 1

Patient demographic data by *SLCO1B1* c.521T>C genotype^a

Variable	TT genotype	TC genotype	CC genotype	P-value
<i>Baseline screening</i>				
Number of evaluable patients	62	27	2	
White / Black	53 (85) / 9 (15)	26 (96) / 1 (4)	2 (100) / 0 (0)	0.28
Age (years)	61 (40–77)	65 (42–83)	75 (70–79)	0.22
Sex (male / female)	34 (55) / 28 (45)	17 (63) / 10 (37)	2 (100) / 0 (0)	0.37
Body-surface area (m ²)	1.85 (1.40–2.72)	2.01 (1.46–2.40)	1.91 (1.84–1.98)	0.14
ECOG performance status	1 (0–2)	1 (0–1)	1 (1–1)	0.83
<i>Primary tumor site</i>				
Breast	20 (32)	4 (15)	0 (0)	0.31
Prostate	15 (24)	8 (30)	2 (100)	
Lung	10 (16)	4 (15)	0 (0)	
Head and neck	5 (8)	2 (7)	0 (0)	
Miscellaneous	12 (20)	9 (33)	0 (0)	
<i>Pre-therapy chemistry</i>				
Total bilirubin (× ULN)	0.42 (0.17–1.25)	0.42 (0.17–1.25)	0.67 (0.42–0.92)	0.13
Aspartate aminotransferase (× ULN)	0.76 (0.28–4.70)	0.76 (0.30–3.87)	0.86 (0.76–0.97)	0.55
Alanine aminotransferase (× ULN)	0.60 (0.10–6.61)	0.53 (0.18–6.61)	1.14 (0.60–1.68)	0.67
Alkaline phosphatase (× ULN)	0.84 (0.36–8.69)	0.90 (0.34–8.69)	2.22 (0.55–3.88)	0.18
α ₁ -acid glycoprotein (mg/dL)	133 (60–257)	142 (63–257)	141 (79–202)	0.23

^aContinuous data are given as median with range in parenthesis, and categorical data as number of patients with percentage of the total population in parenthesis.

Abbreviations: ECOG, Eastern Cooperative Oncology Group; ULN, upper limit of institutional normal.