Published in final edited form as:
Clin Pharmacol Ther. 2008 February ; 83(2): 273–280. doi:10.1038/sj.clpt.6100275.

Effect of Genetic Variation in the Organic Cation Transporter 1, OCT1, on Metformin Pharmacokinetics

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Abstract

The goal of this study was to determine the effects of genetic variation in the organic cation transporter 1, OCT1, on the pharmacokinetics of the antidiabetic drug, metformin. Twenty healthy volunteers with known OCT1 genotype agreed to participate in the study. Each subject received two oral doses of metformin followed by collection of blood and urine samples. OCT1 genotypes had a significant (P<0.05) effect on metformin pharmacokinetics, with a higher area under the plasma concentration–time curve (AUC), higher maximal plasma concentration (C_{max}), and lower oral volume of distribution (V/F) in the individuals carrying a reduced function *OCT1* allele (R61C, G401S, 420del, or G465R). The effect of OCT1 on metformin pharmacokinetics in mice was less than in humans possibly reflecting species differences in hepatic expression level of the transporter. Our studies suggest that *OCT1* genotype is a determinant of metformin pharmacokinetics.

Introduction

Response to a drug is determined by its pharmacokinetic properties. Until recently, pharmacokinetic studies have focused largely on drug metabolizing enzymes. However, it has become increasingly clear that membrane transporters are also important determinants of pharmacokinetics.[1,2,3,4,5] The organic cation transporter, OCT1, an influx transporter encoded by *SLC22A1*, interacts with a variety of structurally diverse compounds including clinically used drugs such as the anticancer drug oxaliplatin,[2] the antidiabetic drug metformin,[3,4] and the antihypertensive drug pindolol.[5] However, the role of OCT1 in pharmacokinetics remains to be determined.

The widely used antidiabetic agent metformin has been well characterized as a substrate of organic cation transporters, including OCT1 and its paralog, OCT2, a transporter expressed in abundance in the kidney.[3,4] Metformin is eliminated from the body primarily by the kidney without any significant metabolism *in vivo*.[6] Following intravenous doses, *Oct1*−/− and *Oct1*

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Conflict of Interest
Steven A Sheardown and Lin Yue are employees of the GlaxoSmithKline Company. The other authors declared no conflict of interest.

+/+ mice have similar systemic pharmacokinetic properties.[3] However, the role of OCT1 in the pharmacokinetics of metformin following an oral dose is not known. This is particularly important as the gastrointestinal absorption of metformin is incomplete in humans, and slow absorption is the rate-limiting factor in metformin disposition.[6] Human OCT1 has a wide tissue distribution, but is abundantly expressed in the liver with much lower levels in other tissues, including intestine and skeletal muscle.[7] Metformin appears to ameliorate hyperglycemia by reducing hepatic glucose production and gastrointestinal glucose absorption and by improving glucose utilization in skeletal muscle.[8] The coincident tissue expression patterns of OCT1 with the sites of action of metformin suggest that OCT1 is a determinant of the pharmacodynamics of metformin.

We and others have demonstrated that human *OCT1* exhibits polymorphic variation in the coding region.[9,10,11] A number of non-synonymous polymorphisms of OCT1 have been found to have reduced transport for model OCT1 substrates or metformin.[11,12] Several reduced function variants are common in human populations. For example, in European Americans, OCT1-420del has an allele frequency of 18.5% and OCT1-R61C has a frequency of 7.2%.[11] In addition, several reduced function or nonfunctional variants have been identified with ethnic-specific allele frequencies of greater than 1% (*e.g.*, OCT1-P341L, OCT1-G401S, and OCT1-G465R). The polymorphisms of *OCT1* provide a genetic tool to study the *in vivo* role of OCT1 in pharmacokinetics and pharmacodynamics in humans. Further, these polymorphisms may contribute to the variation in response to drugs.

We have recently described the effect of genetic variation in *OCT1* on metformin action in a group of healthy volunteers.[12] In this study, we have analyzed the effect of genetic variation in *OCT1* on the pharmacokinetics of metformin in the same group of volunteers (Table 1).[12] Further, we have determined the pharmacokinetics of oral metformin in *Oct1*−/− and *Oct1*+/+ mice. We report here, for the first time, that OCT1 activity, which is determined by genotype in humans, affects metformin pharmacokinetics.

Results

OCT1 genotyping

We genotyped 208 Caucasian DNA samples from the SOPHIE (Study of Pharmacogenetics in Ethnically Diverse Populations, see Methods) cohort. Of the 208 samples, 30 (14.4%) were heterozygous for OCT1-R61C, 9 (4.3%) heterozygous for OCT1-G401S, and 10 (4.8%) heterozygous for OCT1-G465R. One subject (0.005%) was homozygous for OCT1-R61C. Interestingly, no subject was found to have more than one of the three polymorphisms. The observed frequencies were in Hardy–Weinberg equilibrium and comparable to those observed previously.[9,11]

Our clinical study was initially based on the previous cellular characterization[11] and designed to assess the effects of OCT1-R61C, OCT1-G401S, and OCT1-G465R on metformin pharmacokinetics and pharmacodynamics in white American subjects.[12] The previous reduced function OCT1-P341L was not identified in white American subjects and had normal transport function for metformin in our cellular studies, whereas OCT1-S14F, S189L, and S220V, which exhibited reduced metformin uptake, have very low allele frequencies in white American subjects. We thus did not genotype the subjects for OCT1-P341L, S14F, S189L, and S220V. We recruited 10 volunteers who carried any of OCT1-R61C, G401S, and G465R variant alleles and 10 volunteers who did not from the SOPHIE project. However, in further cellular studies, we identified another common OCT1 variant (420del) that exhibited a reduced uptake of metformin.[12] Therefore, all of the participants in this clinical study were also genotyped for OCT1-420del. Of the 20 volunteers, five (25%) volunteers were found to be OCT1-420del heterozygotes. Three of the five volunteers were also heterozygous for G465R. The

characteristics and genotypes of each subject were reported in the Supplementary Material in our previous report[12] and are re-summarized in Table 2. In our clinical studies, subjects were placed into one of two groups based on their *OCT1* genotypes: OCT1-variant group and OCT1-reference group (Tables 1 and 2). The subjects in the OCT1-variant group carried at least one of the four polymorphisms (OCT1-R61C, G401S, 420del, and OCT1-G465R), whereas those in the OCT1-reference group had the reference allele at all the four positions in the *OCT1* gene.

Effects of OCT1 genotype on metformin clinical pharmacokinetics

The healthy subjects with known OCT1 genotypes received two separate oral doses (1,850 mg total) of metformin to achieve pharmacological effects (Table 1).[12] For determination of metformin pharmacokinetics, the plasma concentrations of metformin were measured for 24h after the second dose. OCT1 genotype had a significant effect on the pharmacokinetics of metformin (Figures 1, 2 and Table 3). The plasma concentrations of metformin tended to be higher in the individuals in the OCT1-variant group than those in the OCT1-reference group. The concentrations at most of the sampling time points (1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, and 10h) were significantly higher in the individuals in the OCT1-variant group than in those in the OCT1-reference group ($P<0.05$). There was no difference in the time (T_{max}) to the maximal plasma concentration (C_{max}). However, individuals who carried a variant *OCT1* allele had a significantly higher C_{max} than those who did not ($P=0.004$). The area under the plasma concentration–time curve (AUC) of metformin was significantly greater in the OCT1-variant group than that in the OCT1-reference group (Figure 2, Table 3, AUC_A, $P=0.01$). The difference was also very significant after we normalized the results to a single dose of 1,000mg by excluding the contribution from the residual metformin from the first dose (see Methods, Figure 2, Table 3, AUC_B, $P=0.004$). The oral volume of distribution (V/F) was significantly lower in individuals with the variant *OCT1* alleles compared to those in the OCT1-reference group (54% lower, $P=0.022$). Interestingly, whereas individuals in the OCT1-reference group had a significantly higher oral clearance (CL/F, 53% higher, $P=0.005$), the renal clearance (CL_R) was similar between the two *OCT1* genotype groups ($P=0.795$). The variant group tended to excrete more metformin in the urine than the reference group. The fraction excreted in the urine ($f_{e,u}$), which was normalized to the second dose of 1,000mg, was larger in the variant group than in the reference group. However, the difference was not significant because of large variability (28 versus 19%, $P=0.19$, Table 3). No other pharmacokinetic parameters were found to be significantly different between the two genotype groups.

Metformin pharmacokinetics in *Oct1−/−* and *Oct1+/+* mice

The *Oct1*−/− ($n=6$) and *Oct1*+/+ mice ($n=5$) were dosed with 15mg/kg metformin through oral gavage. The 24-h plasma concentration–time profile was similar in *Oct1*−/− and *Oct1*+/+ mice. However, consistent with those of healthy subjects, the blood metformin concentrations tended to be higher in *Oct1*−/− mice than in *Oct1*+/+ mice. This was obvious at the absorption phase (0–1.0h) of metformin (Figure 3). *Oct1*−/−mice thus had a greater metformin AUC during the absorption phase ($P=0.05$, Table 4). *Oct1*−/− mice also tended to have a greater metformin AUC from 0 to 24h and a smaller oral volume of distribution; however, the differences were not significant between the two genotypes of mice ($P=0.07$ and 0.09 for AUC and V/F, respectively, Table 4).

The blood concentration-time curves of metformin after an oral dose in *Oct1*+/+ mice (n=5) and *Oct1*−/−mice (n=6). The mice were given an oral dose of metformin (15mg/kg containing 0.2mCi/kg of ^{14}14C-metformin), approximating the single dose of 1,000mg in humans. Data represent mean±SE. (**a**) The blood concentration-time curve from 0 to 24 h. (**b**) The blood concentration-time curve for the first 4h after metformin administration. The difference in AUC of the absorption phase (0–1h) is significant between *Oct1*+/+ and *Oct1*−/− mice (3.170.25 versus 4.070.33μg h/ml, $P=0.05$).

Discussion

This study provides evidence that OCT1 genotype/activity affects the pharmacokinetics of metformin. In particular, the pharmacokinetic properties of metformin, including AUC, *V*/*F*, and C_{max}, were significantly different between the individuals who carried one of the reduced function alleles, OCT1-R61C, G401S, 420del, or G465R and those who carried only OCT1-reference alleles. Similar trends were observed in *Oct1*−/− and *Oct1*+/+ mice. This study is the first to show that polymorphisms in *OCT1* contribute to variation in the pharmacokinetics of metformin.

Human OCT1 is mainly expressed in the liver.[7,13,14] Hepatic uptake of metformin is dramatically reduced in *Oct1*−/− mice compared to *Oct1*+/+ mice after metformin administration.[3,12] The effects of *OCT1* genotype on the pharmacokinetics of metformin, as evidenced by the smaller oral volume of distribution in the individuals carrying the reduced function alleles of *OCT1*, may be explained by differences in the distribution of the drug to the liver. Our results suggest that the liver is a critical organ responsible for the volume of distribution of metformin in human body. Metformin is not metabolized in the body.[6] We expect that for OCT1 substrates that are extensively metabolized in the liver, OCT1 may be a critical determinant of their access to drug-metabolizing enzymes, and therefore, the transporter activity may have a large effect on their clearance.

It is noteworthy that variation in the CL_R of metformin also has a strong genetic component.[15] In a previous study, genetic variation in *OCT2*, the *OCT1* paralog expressed in abundance in the kidney, was found to alter metformin uptake kinetics *in vitro*.[16] Metformin is a better substrate for OCT2 in comparison to OCT1.[17] Further clinical studies are warranted to examine metformin disposition among individuals with different *OCT2* genotypes.

The CL_R of metformin was similar between the individuals who carried the OCT1 variants and those who carried only reference alleles of *OCT1*. Interestingly, however, the CL/*F* was significantly lower in the individuals who carried the variant alleles in comparison to those who only carried the reference alleles. In addition, the variant group tended to excrete more metformin in the urine, as indicated by the larger $f_{e,u}$. As individuals with OCT1-variant alleles may take up less metformin in the liver, the lower CL/*F* and larger $f_{e,u}$ may be explained, in part, by a lower biliary excretion of metformin. It has been reported that the biliary excretion of the model organic cation, tetraethylammonium, was reduced to about half in *Oct1*−/− mice compared to *Oct1*+/+ mice.[18] OCT1 is also expressed on the basolateral membrane of the intestine,[7] and therefore, intestinal clearance of metformin may be reduced in individuals with reduced function variants of OCT1. Changes in enterohepatic recycling may also occur in individuals with OCT1-variant alleles. Significantly reduced distribution of metformin in the intestine has been observed in *Oct1*−/− mice in comparison to *Oct1*+/+ mice that followed intravenous doses.[3] However, human OCT1 is mainly expressed in the liver with minor expression in the intestine.[7,13,14] Further studies are still required to understand fully the mechanisms for the lower CL/*F* in individuals who carry reduced function OCT1-variant alleles.

Variation in bioavailability and volume of distribution is a major source of variation in the pharmacokinetics of metformin. The bioavailability of metformin, which decreases with increasing doses, is only approximately 30–60% with a large individual variability.[19,20,21,22] The apparent volume of distribution of metformin ranges from about 60 to 300l in different studies.[6,19,20,21] Our results indicate that *OCT1* genotype contributes to the variation in the pharmacokinetics of metformin. In particular, individuals with OCT1 variants had a smaller oral volume of distribution in comparison to those in the OCT1-reference group. As discussed above, this may be explained by a much lower hepatic uptake of metformin in the individuals

carrying an OCT1-variant allele. Renal excretion has been reported to be the major route of metformin clearance.[19,20,21,22] The bioavailability of metformin, if estimated from the $f_{e,u}$, seems to be lower for individuals with only reference alleles (20%) and comparable for those with the variant alleles (28%), when compared to those reported by others (32 and 33%).[20, 21] Therefore, the difference in metformin pharmacokinetics between the two genotype groups might be, at least in part, due to the difference in bioavailability. However, because our data suggest that a significant non-renal clearance (at least in the individuals with only reference alleles) contributes significantly to the total clearance of metformin, the bioavailability actually could not be calculated from the $f_{e,u}$ in this study. Further studies using intravenous dosing are needed to measure metformin bioavailability in individuals with different *OCT1* genotypes and estimate the contribution of non-renal clearance to total clearance to a more accurate extent.

We previously reported that individuals carrying the reduced function variants of OCT1 and *Oct1*−/− mice had a significantly lower pharmacodynamic response to metformin (Table 1).[12] In this report, the individuals carrying OCT1 reduced function variants had higher metformin concentrations in the blood and higher AUCs, suggesting that they may have a larger pharmacodynamic response to metformin instead. The paradox is explained by the observation that OCT1 is predominantly expressed in the liver,[7] and that the liver is a major target organ for metformin action.[8] Many drug transporters, expressed in tissues such as liver, intestine, and kidney, are important in drug absorption and disposition, and have been increasingly recognized as determinants of pharmacokinetics.[1] On the other hand, when expressed in target tissues and cells, a drug transporter may be a direct determinant of pharmacodynamics by controlling tissue-specific drug levels. The factors such as genetic variation affecting the activity of such a transporter may significantly alter the pharmacodynamics without changing the systemic pharmacokinetics of a drug.

Very recently, Shikata *et al.*[23] observed no remarkable differences in the prevalence of OCT1 and OCT2 polymorphisms between diabetic patients classified as responders and non-responders to metformin treatment. There are a number of possible reasons why that study and ours seemingly reach different conclusions. Importantly, the studies were conducted in different ethnic groups and involved different polymorphisms. In particular, the function-altering *OCT1* polymorphisms in our study were not identified by Shikata *et al.*,[23] who focused on more common coding and intronic region variants. Further, the studies employed very different study approaches. We used a genotype to phenotype approach in contrast to their approach of phenotype to genotype. Although the number of subjects in each study was small, the approach of phenotype to genotype may particularly require a large sample size to obtain significant results. Other factors such as subject characteristics and metformin efficacy measurement may also cause the difference. Giving the limits of our studies, particularly our small sample size and our use of healthy subjects under very controlled experimental conditions, a prospective, larger-scale replication in diabetic patients is necessary to determine whether *OCT1* genotype plays a role in clinical pharmacokinetics and pharmacodynamics of metformin.

The effect of OCT1 activity on metformin pharmacokinetics was less pronounced in mice than in healthy individuals. We did not detect statistically significant differences in the pharmacokinetic properties of metformin between *Oct1*−/− and *Oct1*+/+ mice (with the exception of AUC in the absorption phase), suggesting species differences in the role of OCT1 in metformin disposition. The greater effect of OCT1 on metformin pharmacokinetics in humans may be explained by a greater role OCT1 on metformin pharmacokinetics in humans versus mice. This may be due to greater expression levels of OCT1 in the human liver in comparison to mouse liver. In humans, OCT1 is predominantly expressed in the liver, with much low levels in other tissues.[7,13,14] In rodents, OCT1 is expressed in equal abundance in the liver, kidney, and small intestine.[24,25] It is also possible that the contribution of OCT1 to

metformin disposition in mice may be less because of the presence of functionally redundant transporters or that expression levels of OCT2, which has a greater affinity for metformin, are increased in the liver and intestine of *Oct1*−/− mice in comparison to wild-type mice.

In conclusion, the pharmacokinetics of metformin is significantly affected by OCT1 activity or genotype in healthy subjects. The effect of *OCT1* genotype on the pharmacokinetics of other OCT1 substrates, in particular those metabolized in the liver, is likely to be clinically significant and warrants further study.

Methods

Healthy human volunteers

Healthy human volunteers in this study were recruited from those who participated in another study, SOPHIE. Subjects in the SOPHIE cohort range between the ages of 18 and 40 years and have been evaluated to be healthy on the basis of medical history provided by a study questionnaire. All SOPHIE participants have signed prior consent for genetic testing and have given permission to be contacted about their willingness to participate in related research studies. To be eligible for this study, subjects should not be taking any medications other than vitamins. Individuals with anemia (hemoglobin <12g/dl), elevated liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltransferase) to greater than double the respective normal value, or elevated creatinine concentrations (men ≥1.5mg/dl, women ≥1.4mg/dl) were excluded. Women of childbearing age were asked to provide a urine sample to confirm a negative pregnancy test before the study.

No data are currently available regarding the effect of ethnicity on metformin disposition and response. In the previous study using the OCT1 model substrate, 1-methyl-4-phenylpyridinium, we found that the common variants of OCT1 with reduced or no function have ethnic-specific allele frequencies (1–8%).[11] Interestingly, those variants occur primarily in Caucasians. Therefore, we first genotyped Caucasian DNA samples in SOPHIE for three common reduced or none function variants: OCT1-R61C, OCT1-G401S, or OCT1-G465R (see below for the genotyping methods). Our subsequent recruitment from SOPHIE was limited to healthy male or female Caucasian volunteers who had any of the three variants. During the study, the participants were also genotyped for OCT1-420del. For clarity in this study, the group of individuals who carried any of the four polymorphisms, OCT1-R61C, OCT1-G401S, OCT1-420del, and OCT1-G465R, are referred to as the OCT1-variant group, and those who had the reference allele at all four positions are in the OCT1-reference group.

Genotyping

OCT1-R61C and OCT1-G465R were genotyped by a TaqMan assay. The reaction mixture consisted of 1 μl of 2× TaqMan Master Mix, 0.05 μl of Assay Mix (yielding a final primer concentration of 900nM and final probe concentration of 200nM), 0.95 μl of double-distilled water, and 2ng of DNA pre-dried in the plate. The cycling conditions were as follows: 92°C for 10min, 50 cycles of 92°C for 15s and 60°C for 1min, and a 4°C terminal hold. The reaction was run on ABI 7900HT.

OCT1-G401S and OCT1-420del were genotyped by sequencing the exons. The polymerase chain reaction (PCR) mixture consisted of 0.2 μl of 10× PlatTaq PCR buffer, 0.2 μl of 2 μM dNTP mix, 0.06 μl of 50mM $MgCl_2$, 0.02 μl of 5U/μl Platinum Taq (Invitrogen, Carlsbad, CA), 0.4 μl of 5M betaine, 0.04 μl of 100% dimethyl sulfoxide, 1.08 μl of double-distilled water, and 10ng of genomic DNA pre-dried in the plate. The PCR cycling conditions were as follows: 95°C for 5min; 10 touchdown cycles of 94°C for 20s, 61°C (−0.5°C each cycle) for 20s, and 72°C for 45s; 35 cycles of 94°C for 20s, 56°C for 20s, and 72°C for 45s; 72°C for

10min; and a 4°C terminal hold. The PCR products were cleaned up by adding 0.25 μl of 1U/μl SAP (shrimp alkaline phosphatase), 0.025 μl of 10U/μl exonuclease I, and 1.725 μl of double-distilled water to the 2 μl PCR product for a 4 μl final volume. The cleanup conditions were as follows: 37°C for 60min, 95°C for 15min, and a 4°C terminal hold. The sequencing mixture consisted of 0.5 μl of BigDyev3.1, 0.75 μl of 5× sequencing dilution buffer, 2 μl of 1 μM primer, 0.75 μl of double-distilled water, and 1 μl of PCR product. The mixture was cycled as: 94°C for 1min; 30 cycles of 94°C for 10s, 55°C for 5s, and 60°C for 4min; and 4°C for the terminal hold. The sequencing products were cleaned up using Millipore Montage, run on ABI 3730XL, and analyzed with Sequencher Version 4.5. The primers for genotyping are summarized in Table 5.

Clinical study procedures

The study protocol was reviewed and approved by the Committee on Human Research at University of California at San Francisco (UCSF). After informed consent was obtained, healthy individuals with known OCT1 genotypes were recruited into this open label study. The general study design has been described elsewhere.[12] Subjects were admitted to the General Clinical Research Center (GCRC) at San Francisco General Hospital (SFGH), and remained at this center for the duration of the study. Subjects were dosed with 850mg metformin (Caraco Pharmaceutical Laboratories, Detroit, MI) in the evening followed by a dose of 1,000mg on the morning of the second day. The pharmacodynamic response in the individuals has been reported previously.[12]

For metformin pharmacokinetics, blood samples were collected at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12, and 24h after the second metformin dose for determination of plasma metformin concentrations. Standardized meals were provided starting 5h after metformin administration (at noon). Following the metformin dose, volunteers were asked to drink 8oz of water every 4h to maintain urine flow and pH. Urine samples were collected between the following time points: 0–2, 2–4, 4–8, 8–12, and 12–24h after the second metformin dose. The volume and pH of urine were recorded for each interval, and 20ml of the urine was then stored at −20°C for analysis of metformin content.

Analytical methods for metformin

Metformin concentrations in plasma and urine were assayed by a highly specific and sensitive liquid chromatography–tandem mass spectrometry method. To prepare the samples for liquid chromatography–tandem mass spectrometry analysis, an aliquot of the clinical plasma or urine samples was mixed with acetonitrile in the presence or absence of the internal standard, propranolol. The mixture was vortexed for 1min and then centrifuged for 10min at 3,000r.p.m. An aliquot of the supernatant was transferred to an autosampler vial, and 3 μl was injected onto the column in a 4°C autosampler. The mobile phase consisted of 80% acetonitrile, 20% double-distilled water, 0.5% 2M ammonium acetate aqueous solution, and 0.05% acetic acid (v/v). The quantification limit was 10ng/ml for plasma and 100ng/ml for urine. Both the intra-day and inter-day coefficients of analysis variation were less than 10%.

Clinical pharmacokinetics

The concentration–time profile of metformin was evaluated by non-compartmental analysis (WinNonlin 4.0, Pharsight Corporation, Mountain View, CA). The subjects received two doses of metformin to maximize the pharmacodynamic effects. The second dose (1,000mg) was received 12h after the first dose (850mg). Metformin pharmacokinetics was reported to be linear with increasing doses up to 2,500mg.[22] In this study, we calculated the pharmacokinetics of metformin from the plasma and urine concentrations after the second dose, considering the contribution of residual metformin from the first dose. We assumed that the AUC is proportional to doses. Participants and treatment periods with missing or unreasonable urine

volumes were excluded from the statistical analysis. C_{max} and T_{max} were directly determined from the plasma concentration–time profile. The elimination rate constant (k_e) was estimated from the slope of the best-fit line determined by linear regression analysis of the log-linear part of the concentration–time curve. The elimination half-life ($T_{1/2}$) was calculated by the equation $T_{1/2}=\ln 2/k_e$. The AUC after the second dose (AUC_A) was calculated by the linear trapezoidal rule for the rising phase of the plasma concentration–time curve, by the log-linear trapezoidal rule for the descending phase, and extrapolation to infinity calculated as division of the last measured concentration by k_e. The contribution of residual metformin from the first dose was estimated from the plasma concentration at time 0. The residual AUC (AUC_R) was calculated by division of the concentration at time 0 by k_e. Thus, the AUC for the second dose (AUC_B) was calculated by subtraction of AUC_R from AUC_A. CL_R was calculated for metformin by dividing A_e (metformin excreted in the urine from 0 to 24h) by $AUC_{0–24h}$. Metformin apparent oral clearance (CL_{oral}) was calculated by dividing the second dose by AUC_B. The apparent oral volume of distribution (V_{oral}) was calculated by the equation $V_{oral}=CL_{oral}/k_e$. We assume that A_e is proportional to AUC. Thus, the A_e corresponding to the second dose of 1,000mg was estimated as: real $A_{e,0–24h}$ times ($1-AUC_R/AUC_A$). The fraction of metformin excreted in the urine ($f_{e,u}$) was approximately estimated by dividing the corresponding A_e by the second dose.

Mice

Oct1−/− mice were generated as described elsewhere.[12] The animals were 10 weeks of *Oct1* −/− and *Oct1*+/+ (wild-type) mice, with comparable mixed genetic background (on average 97% FVB, 1.5% 129/OLA, and 1.5% C57BL6). All animals were housed in a virus-free, temperature-controlled facility on a 12-h light dark–cycle. They received standard mouse food and water *ad libitum*. The experiments on mice were approved by the Institutional Animal Care and Use Committee of University of California at San Francisco.

Metformin pharmacokinetics in mice

Ten-week-old male mice were fasted for 12h, then given an oral gavage dose of 15mg/kg metformin in saline with 0.2μCi/g of ^{14}C-metformin, and placed in metabolic cages for 24h. The food was reintroduced 4h after metformin treatment. Blood samples were collected before metformin treatment (0h) and at 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10, 12, and 24h after metformin treatment by tail bleeding into heparinized microhematocrit capillary tubes (Fisher, Pittsburgh, PA). Urine and feces were collected from tubes attached to the cages. Metformin in blood, fecal homogenates, and urine was measured by scintillation counting. The pharmacokinetic parameters were obtained by non-compartmental analysis using WinNonlin 4.0 (Pharsight Corporation, Mountain View, CA).

Statistical analysis

Unless indicated, the data are presented as mean±SD. The pharmacokinetic variables between the reference and mutant genotypes were compared using two-tailed Student's *t*-test. The level of statistical significance was set at $P<0.05$.

Acknowledgments

This work was supported by grants from the National Institutes of Health (NIH, GM36780 and GM61390). This work was also made possible in part by core services provided by the General Clinical Research Center (GCRC) at San Francisco General Hospital (SFGH), funded by the National Center for Research Resources (NIH MO1-RR00083-44). We thank Dr Morrie Schambelan and the staffs of GCRC at SFGH for their excellent assistance in our clinical study. We thank Dr Thomas J Urban for his technical help. Data are available at http://www.pharmgkb.org.

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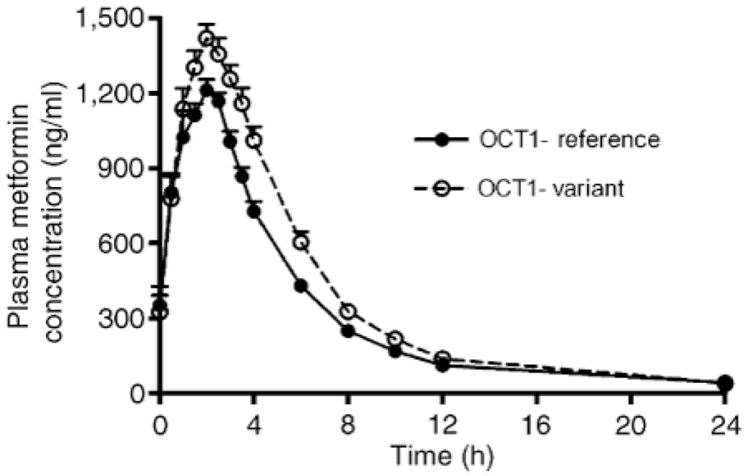

Figure 1.

The plasma concentration-time curves of metformin after oral administration in healthy individuals who carry an OCT1-variant allele (n=12) and those who carry only OCT1-reference alleles (n=8). The individuals were given two doses of metformin. The first dose of 850mg was given at 1800hours on study day 1 and the second dose of 1,000mg at 0600hours on study day 2. Blood samples for the pharmacokinetic analysis were drawn up to 24h after the second dose. The plasma metformin concentration-time curves after the second dose are shown. Data represent mean±SE.

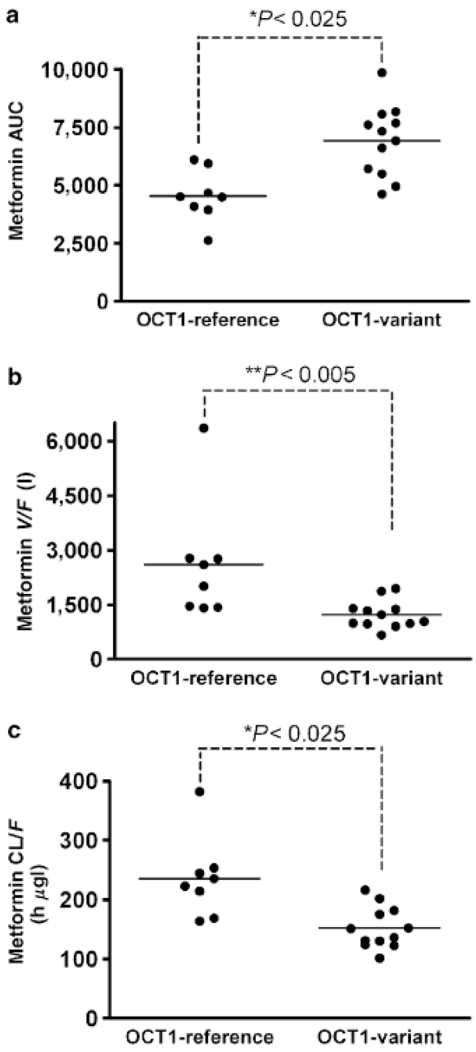

Figure 2.

The effect of OCT1 polymorphisms on the pharmacokinetic parameters of oral metformin. (**a**) AUC_B (AUC normalized to the second dose of 1,000mg); (**b**) V/F (oral volume of distribution; volume of distribution divided by oral bioavailability); (**c**) CL/F (oral clearance; clearance divided by oral bioavailability). The lines represent mean values for the two groups. *P<0.025, **P<0.005, difference between the two genotype groups, unpaired Student's t-test.

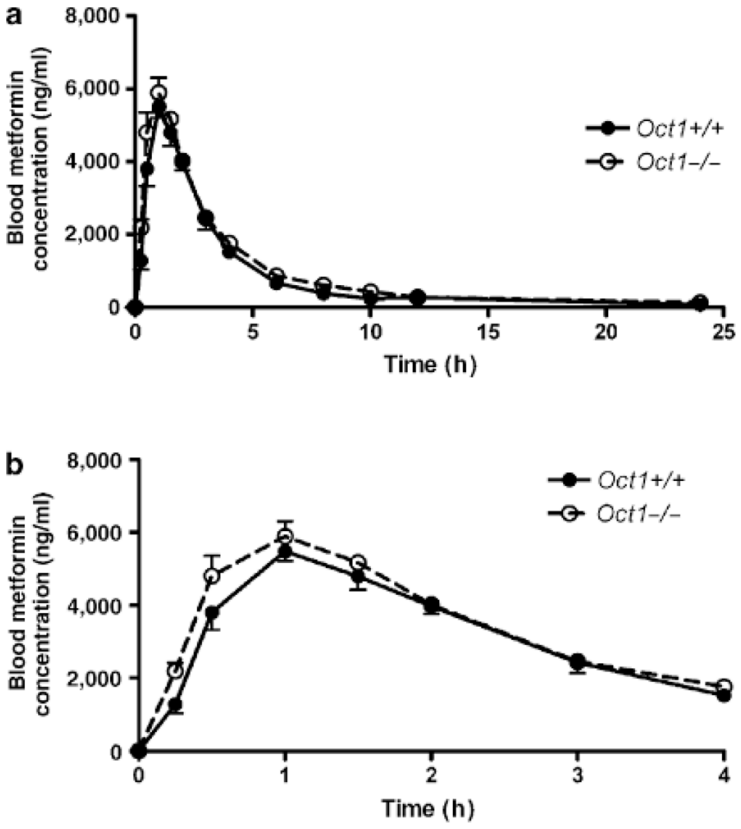

Figure 3.

The blood concentration-time curves of metformin after an oral dose in *Oct1*+/+ mice (n=5) and *Oct1*−/− mice (n=6). The mice were given an oral dose of metformin (15mg/kg containing 0.2mCi/kg of ^{14}14C-metformin), approximating the single dose of 1,000mg in humans. Data represent mean±SE. (**a**) The blood concentration-time curve from 0 to 24 h. (**b**) The blood concentration-time curve for the first 4h after metformin administration. The difference in AUC of the absorption phase (0–1h) is significant between *Oct1*+/+ and *Oct1*−/− mice (3.170.25 versus 4.070.33μg h/ml, *P*=0.05).

Table 1

The effect of OCT1 polymorphisms on glucose-lowering effects of metformin following OGTTs in healthy subjects(OCT1-reference n=8, variant n=12)[12]

	Glucose AUC (min mg/dl)		C_{30min} (mg/dl)		C_{max} (mg/dl)	
Genotype Group	**Without Met**	**With Met**	**Without Met**	**With Met**	**Without Met**	**With Met**
Reference	19,800±1,500	18,300 ± 1,600[a]	137±23.3	113 ± 10.3[a]	145 ± 18.0	124±16.5[a]
Variant	19,800±2,500	21,400 ± 2,300[a,b]	143±20.9	127±15.8[a,b]	155 ± 25.4	144±17.4[b]

Glucose AUC, the area under the time–plasma glucose concentrations curve; C_{30min}, the plasma glucose concentration 30 min after glucose administration; C_{max}, the maximal plasma glucose concentration after glucose administration; OCT1, organic cation transporter 1; OGTT, oral glucose tolerance test. OGTT (75 g glucose) was conducted on each individual twice. One was without metformin treatment (baseline OGTT). The individuals were then given two doses of metformin (total 1,850 mg). The second OGTT was conducted under metformin treatment. Study details were described in ref. [12].

[a] $P<0.05$ vs without metformin treatment (paired Student's t-test).

[b] $P<0.05$ vs OCT1-reference (unpaired Student's t-test).

Clin Pharmacol Ther. Author manuscript; available in PMC 2010 November 9.

Table 2

The characteristics and *OCT1* genotype of healthy volunteers who participated in the clinical study[12]

Subject no.	Polymorphism	Body weight[a] (kg)	Height[a] (cm)	Age[a] (year)	Gender
1	Reference	82.1	186	28	M
3	Reference	58.7	169	36	F
4	Reference	67	175	25	M
7	Reference	64.7	162	40	F
9	Reference	82.2	185	36	M
10	Reference	76.6	179	31	M
12	Reference	68.2	177	27	F
16	Reference	52.2	171	27	F
Subtotal		68.9±10.8	176±8.1	31.3±5.4	4M, 4F
2	R61C	102	188	35	M
6	G401S	80	184	34	M
8	R61C[b]	97.1	186.3	27	M
11	R61C	60.9	163	34	F
13	420del	67.9	173	35	F
14	G465R, 420del	66.3	160	26	F
15	G465R, 420del	74.2	159	26	F
17	R61C	75.1	186	27	M
18	G174S,[c] 420del	79.1	168	40	F
19	G465R, 420del	62.2	162	32	F
20	G401S	69	183	25	M
21	G401S	55.4	157	19	F
Subtotal		74.1±14.0	173±12.3	30.0±5.9	6M, 6F

F, female; M, male; MPP^+, 1-methyl-4-phenylpyridinium; OCT1, organic cation transporter 1.

[a] Data are represented as mean±SD.

[b] Homozygote for R61C.

[c] Found in a volunteer after resequencing, and not determined on the cellular phenotype of OCT1 function (MPP^+ and metformin uptake).

Table 3

Metformin pharmacokinetic parameters from healthy individuals who only carry OCT1-reference alleles (OCT1-reference) and those who carry an OCT1 variant allele.

	OCT1-reference (n=8)[a]		OCT1-variant (n=12)[a]	
	Mean	**SD**	**Mean**	**SD**
$T_{1,2}$ (h)	7.3	2.3	5.8	1.2
T_{max} (h)	1.9	0.52	2.2	0.72
C_{max} (μg/ml)	1.3	0.10	15*	0.19
AUC_A (h μg/l)	7,700	970	9,200**	1,200
AUC_B (h μg/l)	4,500	1,200	6,900*	1,600
V/F (l)	2,600	1,800	1,200**	400
CL/F (l/h)	240	73	150*	37
CL_R (l/h)	40	16	38	21
$f_{e,u}$ (%)	19	8.8	28	16

AUC_A, area under the curve of plasma concentration–time of metformin; AUC_B, AUC normalized to the second dose of 1,000 mg C_{max}, maximal plasma concentration; CL/F, oral clearance (clearance over oral bioavailability); CL_R, renal clearance; $f_{e,u}$, the fraction excreted in the urine; $T_{1/2}$, half-life; T_{max}, time to the maximal plasma concentration; V/F, oral volume of distribution (volume of distribution divided by oral bioavailability). The residual contribution of the first metformin dose was considered during the calculation of the pharmacokinetics parameters (see Methods for details). The individuals were given two doses of metformin. The first dose of 850 mg was given at 1800 hours on study day 1 and the second dose of 1,000mg at 0600 hours on study day 2. The first blood sample (0 h) was drawn immediately before the second dose. Blood and urine samples for the pharmacokinetic analysis were then collected up to 24h. Blood and urine collection was complete for all participants, except that an incomplete urine collection was obtained in two individuals (one OCT1-reference and one OCT1-variant).

[a] The individual with incomplete urine collection was excluded when calculating CL_R and $f_{e,u}$

* P< 0.025 compared with the OCT1-reference.

** P< 0.005 compared with the OCT1-reference.

Table 4

Metformin pharmacokinetic parameters from *Oct1*+/+ and *Oct1*−/− mice.

	0ct1+/+ (*n*=5)		*Octl*−/− (*n*=6)	
	Mean	**SD**	**Mean**	**SD**
$T_{1/2}$ (h)	5.5	2.0	5.4	1.7
$AUC_{0–1\ h}$ (μg h/ml)	3.1	0.57	4.0*	0.73
$AUC_{0–24h}$ (μg h/ml)	19.0	1.9	21.8	2.5
V/F (ml)	356	200	187	75
CL/*F* (ml/h)	29.7	6.0	23.6	4.5
Urine recovery (%)	60	7	50	13
Feces recovery (%)	30	10	29	3

AUC, area under the curve of blood concentration–time of metformin; CL/*F*, oral clearance (clearance over oral bioavailability); $T_{1/2}$, half-life; *V/F*, oral volume of distribution (volume of distribution over oral bioavailability). The mice were given an oral dose of metformin (15mg/kg containing 0.2mCi/kg of ^{14}C-metformin), approximating the single dose of 1,000mg in humans. The radioactivity in blood was determined and converted to mass amounts.

*P=0.05 compared with *Oct1*+/+/ mice

Table 5

Genotyping primers for OCT1-R61C, OCT1-G465R, OCT1-G401S, and OCT1-420del

Variant	Primers (5′→3′)
R61C	Forward: GCCTTTGCGCCCATCTG Reverse: CTCCGCAGGGCTCCAG VIC probe: CACAGCGCTGGCTC FAM probe: CACAGCACTGGCTC
G465R	Forward: GTGAATCACAGAATTATCGTATTTTTTGTCCT Reverse: GTCACACAGGGAGGAACACA VIC probe: TCAGGAACCTCGGAGTGA FAM probe: CAGGAACCTCAGAGTGA
G401S	Forward: TTTCTTCAGTCTCTGACTCATGC
420del	Reverse: TCCCCACACTTCGATTGC