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PHARMACOGENETICS

Steady-state pharmacokinetics of metformin is independent of the *OCT1* genotype in healthy volunteers

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Abstract
Purpose The aim of the study was to determine the steady-state pharmacokinetics of metformin in healthy volunteers with different numbers of reduced-function alleles in the organic cation transporter 1 gene (*OCT1)*.
Methods The study was conducted as part of a randomized cross-over trial. Thirty-four healthy volunteers with known *OCT1* genotypes (12 with two wild-type alleles, 13 with one and 9 with two reduced-function alleles) were included. In one of the study periods, they were titrated to steady-state with 1 g metformin twice daily.
Results Neither $AUC_{0\text{-}12}$, C_{max} nor Cl_{renal} were statistically significantly affected by the number of reduced-function alleles (0, 1 or 2) in *OCT1*: ($AUC_{0\text{-}12}$: 0, 1, 2: 14, 13 and 14 h ng/L (P=0.61)); (C_{max}: 0, 1, 2: 2192, 1934 and 2233 ng/mL, (P=0.26)) and (Cl_{renal}: 0, 1, 2: 31, 28 and 30 L/h (P=0.57))
Conclusions In a cohort of healthy volunteers, we found no impact of different *OCT1* genotypes on metformin steady-state pharmacokinetics.

Keywords Metformin · OCT1 · Reduced-function alleles · Pharmacogenetics · Healthy volunteers

Introduction

For more than five decades, metformin has been the object of favourable safety observation and is a cornerstone in the treatment of type 2 diabetes (T2D). The drug has a very favourable safety/risk profile, including a reduction in morbidity and mortality for obese type 2 diabetic patients; it poses a minimal risk of hypoglycaemia and is furthermore a weight neutral drug [1]. Metformin lowers both basal and postprandial plasma glucose through a reduction in gluconeogenesis [2] and thus suppresses the hepatic glucose output in type 2 diabetic patients.

Metformin has a complex mechanism of action. Thus, there is experimental evidence that it inhibits complex 1 of the mitochondrial respiratory chain and hence decreases the intracellular ATP production [3–5]. Furthermore, it has recently been reported that metformin may also suppress gluconeogenesis by inhibiting the redox shuttle enzyme mitochondrial glycerophosphate dehydrogenase (mGPD) [6].

Due to the hydrophilic properties of metformin, its passage across cell membranes depends to a large extent on transporters which belong to the solute carrier (SLC) family. The driving force is the electrochemical gradient supplied by metformin itself [7]. The intestinal uptake of metformin is saturable and dose-dependent [8] and mediated by proton-activated plasma membrane monoamine transporter (PMAT, SLC29A4) [9] and organic cation transporter 1 (OCT1, SLC22A1) [10]. Metformin is not bound to plasma proteins or metabolized [11]. The hepatic uptake of metformin across the sinusoidal membranes is predominantly facilitated by OCT1. The OCT1 protein consists of 554 amino acids and

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comprises 12 α-helical transmembrane domains [12, 13]. Genetic variation in *OCT1* has been linked to altered pharmacokinetic and pharmacodynamic response to metformin [14–20]. In *OCT1*, the single nucleotide polymorphisms (SNPs) rs12208357 (R61C), rs34104736 (S189L), rs34130495 (G401S), rs34059508 (G465R) and rs72552763 (M420del) have been linked to reduced function both in vivo and in vitro [16–19]. In the proximal tubular cells of the kidney, the organic cation transporter 2 (OCT2, SLC22A2) and the H+/drug antiporters multidrug and toxin extrusion transporters 1 (MATE1, SLC47A1) and 2 (MATE2-K; SLC47A2) facilitate the excretion of metformin into the urine [7, 12, 21]. Genetic variations in *OCT2*, *MATE1* and *MATE2-K* have also been shown to affect metformin kinetics and response [18, 22–27]. Recently, the effect of genetic variations in transcription factors has been associated with change in metformin pharmacokinetics and dynamics [28].

The pharmacokinetics and pharmacodynamics of metformin are also influenced by drug–drug and drug–herbal medicine interactions ([29]; Stage et al. 2015 (under review)).

The purpose of the present study was to evaluate the steady-state pharmacokinetics of metformin in healthy fasting volunteers with different *OCT1* diplotypes (none, one or two reduced-function alleles). To our knowledge, this is the first prospective study in healthy volunteers with adequate power to evaluate the impact of reduced-function *OCT1* diplotypes on the kinetics of metformin.

Material and methods

Study participants

Healthy volunteers (12 women and 22 men) from the Pharmacogenomics Biobank of the University of Southern Denmark were included on the basis of their *OCT1* genotype [30]. All volunteers were healthy, neither pregnant nor breastfeeding, none took any medication or had a history of alcohol abuse. Before administration of study medication, all women were tested negative for pregnancy. The renal function was assessed by plasma creatinine, the hepatic function by plasma aminotransferase, and both were required to be within normal range. Informed consent was obtained from all volunteers included in the study.

The distribution of *OCT1* genotypes were rs12208357 (c.181 (C>T)), rs461473, rs34104736 (c.566 (C>T)), rs34130495 (c.1201 (G>A)), rs72552763 (c.1260 (GAT>del)), rs622342, rs34059508 (c.1393 (G>A)), *OCT2*: rs316019 (c.808 (G>T)), *MATE1*: rs2252281 (g.-66 (T>C)), rs2289669 and *MATE2*: rs34399035 (c.1177 (C>T)), rs12943590 (g.-130 (G>A)) are listed in Supplementary Table S1. The four known reduced-function alleles (RF) in *OCT1* (c.181 (C>T) rs12208357, c.1201 (G>A) rs34130495, c.1260 (GAT>del) rs72552763, c.1393 (G>A) rs34059508) resulted in five haplotypes H1–5 and eight diplotypes (Supplementary Table S2).

Study design

The study was designed as a randomized, cross-over trial with a washout period of at least 4 weeks between the phases. In both phases, the volunteers fasted for 42 h. This was done in order to include an evaluation of the pharmacodynamic effect of metformin on the glucose production in fasting healthy volunteers. The pharmacodynamic results are presented in a separate article (currently in preparation).

In one of the phases, the volunteers were titrated to steady-state with 1 g metformin orally twice daily. Thus, they were given tablets of metformin hydrochloride (Metformin "Actavis", Denmark, 500 mg) at 8:00 a.m. and 8:00 p.m. for 7 days (day 1: 500 mg a.m. and 500 mg p.m.; day 2: 500 mg a.m. and 1000 mg p.m.; day 3, 4, 5 and 6: 1000 mg a.m. and 1000 mg p.m.; day 7: 1000 mg a.m.). The volunteers were admitted to the Department of Endocrinology, Odense University Hospital, Denmark, at 4:00 p.m. after 20 h of fast (day 6). The next day at 8:00 a.m., after 36 h of fast, the experiment was initiated. The fast ended after 42 h. However, sampling for determination of plasma and urine metformin continued for an additional 6 h to 8 p.m. The blood samples for the metformin analysis were collected at timed intervals (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10 and 12 h); these were immediately centrifuged and kept at −20 °C until drug analysis. Urine was collected and stored at −20 °C until analysis.

Study procedures

The study was registered in the European Clinical Trial Database (EudraCT no.: 2011-001696-39) and approved by the Danish Health and Medicines Authority (J. no: 2011050747), the Danish Data Protection Agency (J. no. 2011-41-6231) and the Regional Committee on Biomedical Research Ethics of Southern Denmark (Project ID: S-20110082). The trial was registered at www.clinicaltrials.gov (NCT01400191). The study was conducted in accordance with the Helsinki Declaration and Good Clinical Practice (GCP) and monitored by the GCP unit, Odense University Hospital, Odense, Denmark.

Analytical methods

The plasma and urine concentrations of metformin were determined by a validated high-performance liquid chromatography method as previously described [31]. The interday and intraday precision did not exceed 7.5 % in plasma and 6.2 % in urine. The quantification limit was 5 ng/mL in plasma and 40 ng/mL in urine, respectively.

Genotyping

All SNPs except two were genotyped using TaqMan® real-time PCR predesigned assays or File-builder primers and probes and conducted on a StepOne Plus (Applied Biosystems, Foster city, CA, USA) in accordance with the manufacturer's protocol. The rs72552763 and rs34130495 were genotyped by sequencing. Assay numbers, sequences of the primers and probes used for genotyping are summarized in Supplementary Table S3.

Linkage disequilibrium, haplotype and diplotype inference

The software Haploview (version 4.2, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA) was used to estimate the haplotype frequencies and to visualize the structure of pairwise linkage disequilibrium (LD) between the SNPs in *OCT1*, *OCT2*, *MATE1* and *MATE2* in the study cohort. The diplotypes of the four reduced-function alleles in *OCT1* were inferred using the software package PHASE (Stephens, University of Washington, Seattle, WA, USA), version 2.1.1. [32, 33].

Pharmacokinetic data analysis

All pharmacokinetic parameters were calculated using non-compartmental methods (WinNonlin 6.2; Pharsight, Mountain View, CA, USA). The area under the plasma concentration–time curve of metformin was calculated using the Linear Up/Log Down method. The terminal elimination half-life of metformin was calculated as $t½=\ln 2/\lambda$, where λ is the terminal slope of the log plasma concentrations versus the time plot, calculated by linear regression. The time to maximum plasma concentration (t_{max}) and peak values' maximum plasma concentration (C_{max}) of metformin were read directly from the observed data. The renal clearance (CL_{renal}) was calculated as $Cl_{renal}=(\text{amount of metformin in the urine}_{0\text{-}12hr})/AUC_{0\text{-}12hr}$. The fraction of unbound metformin in plasma was set to 1. The individual glomerular filtration rates (GFR_i) were calculated using the estimated glomerular filtration rate (eGFR) according to the Modification of Diet in Renal Disease formula adjusted for the body surface area (BSA) [34]. Thus, $GFR_i=(eGFR\times BSA)/1.73\ m^2$. Hence, the apparent tubular secretion clearance (CL_{sec}) was calculated as $CL_{sec}=Cl_{renal}-GFR_i$.

Statistical analysis and considerations

The demographic data are presented as medians with 25th to 75th percentiles, while others are listed as means with 95 % confidence intervals unless otherwise specified. Statistical inferences of the pharmacokinetic results for *OCT1* diplotypes were analyzed using one-way analysis of variance. A p value lower than 0.05 was considered statistically significant. All statistical analyses were performed using STATA 11.0 (StataCorp, Texas, USA).

Sample size

The main outcome of the pharmacokinetic substudy is represented by differences in $AUC_{0\text{-}12}$ for metformin between volunteers homozygous and heterozygous for reduced-function alleles in *OCT1* (rs12208357, rs72552763, rs34130495 and rs34059508). Sample size was calculated for pharmacodynamic outcomes, and a specific a priori sample size calculation for this pharmacokinetic substudy was not performed. However, given a sample of 12 volunteers in the two groups, it can be estimated that a true difference of 30 % in $AUC_{0\text{-}12}$ can be detected, based on an interindividual coefficient of variance for $AUC_{0\text{-}12}$ of 25 %, and, given a two-sided level of significance of 0.05 and a power of 80 %.

Results

Thirty-seven, healthy Caucasian volunteers gave written informed consent to participate in the study, of which 34 completed the study (12 women and 22 men). Twelve had none, 13 had one and 9 had two reduced-function alleles in *OCT1*. Demographic characterization is shown in Table 1. No statistically significant differences in the baseline values were seen

Table 1 Demographic information at baseline

Demographic information	Median	25–75th percentile
Age at inclusion (years)	25	24–27
BMI (kg/m^2)	23	22.2–25.2
BSA (m^2)	2.0	1.8–2.1
Plasma creatinine (μmol/L)	79.5	71–85
Plasma alanine aminotransferase (U/L)	24	20–32
HDL (mmol/L)	1.4	1.2–1.7
LDL (mmol/L)	2.9	2.4–3.3
Triglyceride (mmol/L)	1	0.8–1.3
Total cholesterol (mmol/L)	4.7	4.3–5.6
GFR_i (mL/min)	107.9	91.1–119.6
HbA1c (%)	5.1	5–5.3
HbA1c (mmol/mol)	32	31–34
Gender *n*: women 12, men 22		

BMI body mass index=weight/height^2, *BSA* body surface area=weight$^{0.425}$ ×height$^{0.725}$ ×0.007184 [41]. *GFR_i* the individual glomerular filtration rates=(eGFR×BSA)/1.73 m^2 =(175×(plasma creatinine/88.4)$^{-1.154}$ ×(age)$^{-0.203}$ ×(0.742 if female)×(1.21 if Afro-American)×BSA)/1.73 m^2 [42].

between the different *OCT1* diplotypes. Three volunteers did not complete the study, two found 42 h of fasting too challenging and one was excluded due to early onset of diarrhoea.

The steady-state plasma concentration–time profiles of metformin after oral administration of a 1000 mg dose to healthy volunteers dependent on *OCT1* diplotype are illustrated in Fig. 1. Visually, volunteers homozygous for variant alleles (RF/RF) appeared to have the highest plasma concentration (C_{max}) and area under the plasma concentration–time curve ($AUC_{0\text{-}12}$). However, no statistically significant differences were seen between the different *OCT1* diplotypes for any of the pharmacokinetic parameters, not even after adjusting for the individual glomerular filtration rates (iGFRs) (Table 2). The pharmacokinetic parameters obtained are within the range of what has previously been reported [11, 19], and as expected, the renal clearance (CL_{renal}) correlated with the GFR (r^2=0.15, P=0.026).

When tested individually, none of the SNPs in *OCT2* (rs316019), *MATE1* (rs2289669, rs2252281) or *MATE2* (rs12943590) affected the pharmacokinetics of metformin. The renal clearance of metformin was not affected by gene–gene interactions between rs316019 and rs2252281 or between rs12943590 and rs2252281 (data not shown).

Discussion

In the present study, we were unable to show that difference in the numbers of *OCT1* reduced-function alleles affected the steady-state $AUC_{0\text{-}12}$ of metformin. Less metformin is expected to be transported into the hepatocytes in individuals with

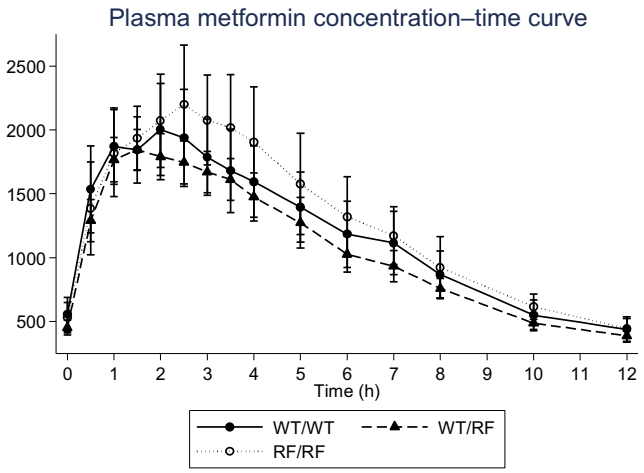

Fig. 1 The steady-state plasma concentration–time profiles of metformin after oral administration of a 1000 mg dose to healthy volunteers with the different *OCT1* diplotypes, reference genotype WT/WT alias homozygous for wild-type alleles (n=12), WT/RF alias heterozygous (n=13) and RF/RF alias homozygous for variant alleles (n=8). Each point represents the geometric mean value and the corresponding 95 % confidence interval

OCT1 reduced-function alleles. Hence, Shu et al. reported that healthy volunteers carrying one or two *OCT1* reduced-function alleles had higher AUC and C_{max} and lower oral volume of distribution (V_d/F) of metformin than non-carriers of OCT reduced-function alleles [17]. However, even when comparing the diplotypes most likely to be different (volunteers with none or two *OCT1* reduced-function alleles), we showed no differences in $AUC_{0\text{-}12}$ (2 %), C_{max} (2 %) or V_d/F. Furthermore, as illustrated in Fig. 1, there was no trend indicating a classic additive genotype to phenotype correlation for *OCT1* diplotypes. Thus, volunteers with two reduced-function alleles had higher $AUC_{0\text{-}12}$ and C_{max}, and volunteers with one reduced-function allele had lower $AUC_{0\text{-}12}$ and C_{max} compared to the wild-type group.

Tzvetkov et al. reported a positive correlation between the number of *OCT1* reduced-function alleles and renal clearance of metformin [18]. Given that OCT1 is localized in the apical side of the proximal and distal renal tubules, it has been suggested that OCT1 may be involved in the reabsorption of metformin from urine [18]. This study, as well as others, has not been able to reproduce this observation [17, 25].

In the present study, we were unable to show that difference in the numbers of *OCT1* reduced-function alleles affected metformin trough steady-state plasma concentration ($C_{ss,min}$). This is in contrast to our previous findings in a cohort of type 2 patients, where we found the trough value to decrease with an increasing number of reduced-function alleles [19]. In the former study, the trough values were measured at three separate occasions and in a larger cohort (n=159); thus, the finding in the present study could theoretically be a statistical type 2 error inflicted by the smaller sample size and number of observations. Opposite, but less likely, a type 1 error, due to random variation in the dataset, could have occurred in the first study. Danish diabetic patients do not appear to be genetically different from healthy Caucasians in general [19]. Disease itself may affect the tissue-specific expression of the transporters. Thus, in streptozotocin-induced diabetic rats, the renal expression of all *Octs* was reduced [35], and in hyperuric rats, the renal expression of *Oct2* was decreased [36]. Furthermore, cholestasis reduced Oct1 expression in rat livers but not in the kidneys [37]. In humans, there is a substantial interindividual variability in hepatic OCT expression. Thus, it has been shown that hepatic OCT1 mRNA and protein expression may vary 113- and 83-fold, respectively [38]. In the study by Nies et al., cholestasis was found to suppress the expression of OCT1. Hence, type 2 diabetes could theoretically affect the protein expression of OCT1 and make the patients more vulnerable to reduced-function alleles.

Other SNPs in *OCT1* (rs34130495, rs622342) and *MATE1* (rs2289669, rs8065082) have been reported to affect the distribution of metformin to peripheral compartments [39]. However, we did not observe an effect of these SNPs on V_d/F. Recently, we have performed a study with the purpose to

Table 2 The impact of *OCT1* diplotypes on metformin steady-state pharmacokinetics

Parameter	Reference WT/WT (n=12)	WT/RF (n=13)	RF/RF (n=9)	P	P_{adj}
T_{max} (h)	2.0 (0.5–3)	1.5 (1–3)	2.5 (1–3)	0.18[a]	
$T_{½}$ (h)	3.9 (3.4–4.5)	4.0 (3.5–4.6)	4.2 (3.6–4.9)	0.80	0.76
$C_{ss,max}$ (ng/mL)	2192 (1919–2502)	1934 (1703–2197)	2233 (1916–2603)	0.26	0.41
$C_{ss,min}$ (ng/mL)	408 (344–485)	378 (321–446)	422 (342–521)[b]	0.66	0.84
$AUC_{0\text{-}12}$ (h ng/L)	14 (12–16)	13 (11–14)	14 (12–17)[b]	0.61	0.86
CL/F (L/h)	74 (64–85)	80 (69–92)	69 (58–82)	0.42	0.58
CL_{renal} (L/h)	31 (27–36)	28 (24–32)	30 (25–36)	0.57	0.30
CL_{sec} (L/h)	25 (20–30)	21 (17–25)	24 (19–31)	0.40	0.29
V_d/F (L)	416 (332–521)	460 (370–570)	416 (321–540)	0.76	0.96
A_e (mg)	427 (359–500)	352 (300–412)	438 (362–530)	0.14	0.17

Steady-state pharmacokinetic parameters obtained from 34 healthy volunteers administered a dose of 1000 mg BID of metformin with statistical interference of the *OCT1* diplotypes. All data are presented as geometric means with 95 % confidence intervals except T_{max}, which is presented as median and range. Statistical inferences of the pharmacokinetic results and *OCT1* diplotypes were analyzed using one-way analysis of variance

A_e amount excreted in urine, $AUC_{0\text{-}12}$ area under the curve of plasma concentration–time of metformin, C_{max} maximal plasma concentration at steady-state, $C_{ss,min}$ minimum plasma concentration at steady-state, CL/F apparent total clearance after oral administration, CL_{renal} renal clearance, CL_{sec} tubular secretions clearance, P_{adj} P value adjusted for the individual glomerular filtration rates, *RF* reduced function, $T_{1/2}$ half-life, T_{max} time to the maximal plasma concentration, V_d/F apparent volume of distribution after oral administration of metformin, *WT* wild-type, the haplotype with only active alleles

[a] Kruskal–Wallis was used for statistics

[b] n=8

determine the intrapair similarity in trough steady-state plasma concentration of metformin in dizygotic and monozygotic twin pairs [40]. We found that the though value of metformin varied 5-fold but did not appear to be tightly genetically regulated. Furthermore, it has been demonstrated that the initial glycaemic response to metformin had a heritability of 34 % and that several variants scattered across the entire genome seemed to contribute to the effect modulation of metformin [41]. Taken together, this could indicate that the impact of *OCT1* reduced-function alleles on the pharmacokinetics of metformin in general is of minor clinical importance.

The impact on renal metformin clearance of the frequent *OCT2* SNP rs316019 (A270S) has been evaluated by several groups with conflicting results [18, 22–25], thus pinpointing the complexity of cross-evaluating genotype to phenotype effects in cohorts of different ethnicity. Furthermore, two promoter SNPs in *MATE1* and *MATE2* (rs2252281 (g.-66T>C) and rs12943590 (g.-130G>A)) have been shown to interact in vivo and change the pharmacokinetic/pharmacodynamic response to metformin [26]. Concurrently, other groups have described gene–gene interactions between SNPs in *OCT1* (rs622342) and *MATE1* (rs2289669) [27] and *OCT2* (rs316019) and *MATE2* (rs2252281) [25]. We could not demonstrate that these genetic variants affected the pharmacokinetics of metformin. However, the present study does not have sufficient power to evaluate these gene–gene interactions.

In 2014, Goswani et al. evaluated if genetic variation in specific transcription factor genes was associated with metformin pharmacokinetics and pharmacodynamics [28]. They found that several SNPs in specificity protein 1 (*SP1*) gene and a deletion in activating protein-2 repressor (*AP2-rep*) gene were associated with metformin pharmacokinetics and pharmacodynamics. Both SP1 and AP2-rep are important in the transcription modulation of *MATE1* [42, 43]. Further, they found that SNPs in peroxisome proliferator-activated receptor alpha (*PPARA*) gene and *HNF4A* were associated with metformin pharmacodynamics but not pharmacokinetics. Both PPAR-alpha [44] and HNF4A [45] have been suggested to increase the transcript levels of *OCT1*.

Hence, future studies on the clinical relevance of genetic variation in these transcription factors and epigenetic regulation of metformin pharmacokinetics/pharmacodynamics are warranted.

Conclusion

We found no impact of the different *OCT1* genotypes on metformin steady-state pharmacokinetics. Thus, the contribution of polymorphisms in *OCT1* to the observed clinical variation in metformin plasma concentrations appears to be of only minor importance.

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Author contributions MMC analyzed and interpreted the data. MMC, KH, OHN, PD, HBN and KB designed the study. TBS contributed to the data analysis. MMC drafted the manuscript. All authors critically assessed and reviewed the manuscript. All authors have made a final approval of the manuscript. MMC is the guarantor of this work and takes responsibility for the data integrity.

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