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Research Article

The effect of OCT-2 inhibitor Daclatasvir on Metformin pharmacokinetics and pharmacodynamics at two dose levels: A Bayesian approach using Markov-Chain Monte Carlo simulations

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Abstract

Renal Organic Cation Transporter 2 (OCT2) plays a major role in metformin elimination. Daclatasvir, a Direct-Acting Antiviral (DAA), is an OCT2 inhibitor. Our study aimed to assess the potential interaction of daclatasvir with metformin pharmacokinetics and pharmacodynamics at two metformin doses. Twenty subjects were randomized in a two-period crossover study. The subjects received metformin 500 mg twice daily either alone (R1) or with daclatasvir 60 mg once daily (T1), followed by 1000 mg metformin twice daily either alone (R2) or with daclatasvir 60 mg once daily (T2). Metformin C_{max} was higher in T1 and T2 than in R1 and R2 by 12% and 11%, respectively, with a geometric mean ratio (GMR) of 1.12 (90% CI: 0.98-1.26) and 1.12 (90% CI: 0.86-1.36), respectively. Renal clearance (Clr) was lower in T1 and T2 compared to R1 and R2 by 15% and 11%, with a GMR of 0.85 (0.68-1.02) and 0.89 (0.69-1.09), respectively. The differences from baseline glucose level ($\Delta G_{\%}$) and the area under the $\Delta G_{\%}$-time curve (Δ AUG %) were higher for the 500 mg dose of metformin with daclatasvir ($p < 0.05$). Daclatasvir slightly altered the pharmacokinetics of metformin with a minor alteration of the pharmacodynamics of the 500 mg dose.

Keywords: *Metformin, Daclatasvir, OCT2, Pharmacokinetics, Pharmacodynamics, MCMC.*

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1. Introduction

Diabetes mellitus (DM) is a chronic metabolic condition characterized by persistent hyperglycemia that requires chronic management. It might be related to decreased insulin secretion, resistance to insulin's peripheral activities, or both. An increased risk of DM in chronic HCV patients was reported in several studies supporting an association between DM and HCV **[1, 2]**. The mechanism underlying this association remains unclear. However, the core protein of the hepatitis C virus (HCV) impairs insulin receptor substrate signaling, which ultimately alters the metabolic effect of insulin **[3]**. Metformin is the first-line pharmacologic treatment for the management of type 2 DM (T2DM) **[4, 5]**. However, when administered orally, metformin's half-life in plasma is only about 6 h, and, by 24 h, 90% is eliminated by the

kidneys. Organic cation transporter-2 (OCT2) - encoded in the SLC22A2 gene- is expressed at the basolateral membrane of the renal tubules, and is mainly responsible for the urinary elimination of metformin **[6-9]**.

Direct-acting antivirals (DAAs) metabolism is highly variable. DAAs are used in combinatorial therapeutic approaches. One of the newly approved DDAs with high-sustained virologic response (SVR) rates is daclatasvir **[10]**. Daclatasvir is a moderate inhibitor of many membrane transporters, including organic anion transporters-1B1 (OATP1B1) and organic cation transporters (OCTs) 1 and 2 **[11-12]**. Metformin is known to affect males and females differently, possibly due to the differential expression of OCT2 in the basolateral membrane of renal tubules **[13-15]**. Pre-clinical studies showed a higher expression of rOCT2 in the kidneys of males compared to females **[16]**. Another study addressing sex-based differences in renal cation transport found that rOCT2 is hormonally regulated, as higher levels of testosterone increased rOCT2 mRNA expression when administered in female rats while estradiol decreased rOCT2 mRNA expression when administered in male rats **[17]**. Genetic polymorphisms affect the function of OCTs in humans. Studies addressing OCTs genetic variants and polymorphism among different populations reported an alteration of the pharmacokinetics and pharmacodynamics of metformin **[18-21]**. Many studies suggest inter-ethnic variation in the frequency of OCT2 polymorphism **[22-24]**. Studies in different populations are required to address the effect of different variables, such as gender, genetic diversities, and interethnic variability on the efficacy and safety of metformin **[25-29]**. A study of healthy subjects from both genders reported a non-significant increase in exposure to metformin after co-administration with daclatasvir **[30]**. However, the reported small increase in metformin exposure might vary with gender, genetic variation, inter-ethnic variability, and dose **[31-33]**. Metformin uptake by OCT2 is concentration-dependent, which requires studying the effects of different dosing regimens on the OCT2-mediated interactions [34]. Therefore, the current study aimed to investigate the OCT-mediated interaction between two doses of metformin (500&1000 mg) and daclatasvir in healthy subjects.

2. METHODS

2.1. Study design and ethical considerations

The current study compromised a randomized, open-label, two-period, cross-over design (Figure 1). The study was registered at Clinicaltrials.gov (identifier number: NCT02574845). Ain Shams University Ethical Committee (approval number: 141) approved the study. The study was conducted following the Declaration of Helsinki and the International Council for Harmonization Guidelines for Good Clinical Practice. All subjects provided written informed consent before participation in our study. Adverse events were monitored and recorded in a case report for each subject.

2.2. Subjects

Twenty healthy adult male subjects participated in the study. We included subjects of age 18-55 years, with body mass index (BMI) between 18-30 kg/m^2, smoking frequency of fewer than 10 cigarettes per day, unremarkable physical examination, and normal laboratory tests. We excluded subjects with a history of renal, hepatic, gastrointestinal, autoimmune, endocrine, neurological, or cardiovascular disorders, treatment with any known enzyme or transporter-inducing/inhibiting agents before and during the study, and a history of hypersensitivity to the studied drugs or excipients. The subjects were randomized into two groups (R and T) **(Fig.**

1). In the first period, ten subjects in the R group received metformin 500 mg twice daily alone from day 1 to day 4 (R1) then received metformin 1000 mg twice daily alone from day 5 to day 7 (R2). The other ten subjects in the T group were given metformin 500 mg twice daily and daclatasvir 60 mg once daily from day 1 to day 4 (T1) then given metformin 1000 mg twice daily and daclatasvir 60 mg once daily from day 5 to day 7 (T2). Then, after a washout period of 7 days, a cross-over was performed in the second period of the study.

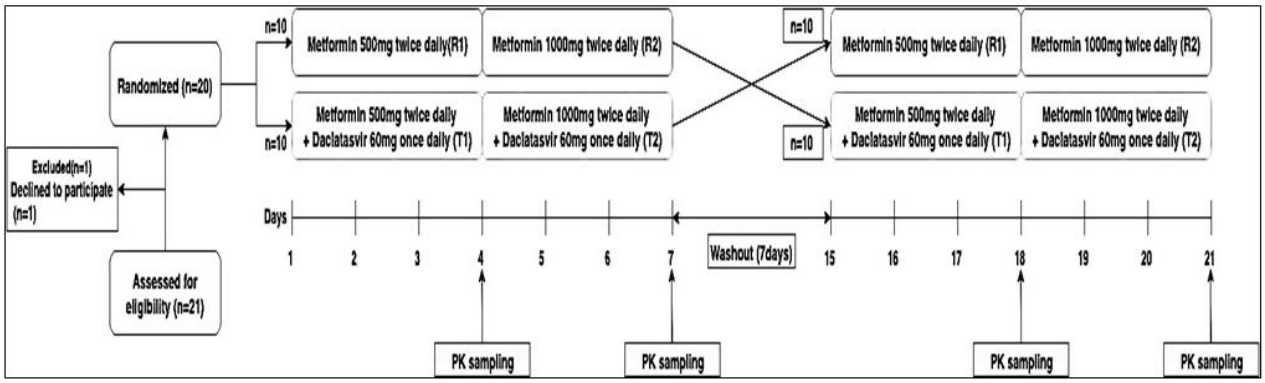

Fig.1. Flow chart of study design.

2.3. Dose administration and sample collection

The subjects administered their daily doses at the clinical site according to a scheduled dosing protocol on days 1, 2, 3, 5, and 6, and were accommodated at the clinical setting at 9 pm on days 3 and 6 of each study period. We instructed our subjects to fast for at least 8-10 hours before dose administration at 9 am and sample collection on days 4 and 7 and to limit tobacco and high-fat food intake throughout the entire duration of the study. We performed blood and urine collections and oral glucose tolerance tests (OGTTs) on days 4 and 7. Subjects were administered the doses with 200 mL of water. Registered nurses at our clinical setting collected blood samples from the subjects' forearm veins on days 4 and 7 at pre-dosing and 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, and 12 h post-dose. We centrifuged the collected blood samples at 4000 rpm for 5 min. We collected urine samples for metformin analysis from 0 to 12 h after drug administration. We stored plasma and urine samples at -80 ºC until required for the bio-analysis. We performed a 75-g OGTT at 2 h post-dosing (proposed t_{max} of metformin) and glucose concentration during the OGTT was measured pre-glucose ingestion (baseline glucose level) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, and 3 h after glucose ingestion using test strips and a digital device (ACCU-CHEK® Active, Roche Diagnostics, Mannheim, Germany).

2.4. Bio-analysis

Bioanalysis of plasma and urine samples was performed by high-performance liquid chromatography-tandem mass spectrometry (HPLC- MS/MS) using LC Agilent 1200 Series and Agilent 6410 Quadrupole Mass Spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA). A liquid-liquid extraction method was applied to samples by adding 3 mL or 2 mL diethyl ether-dichloromethane 70:30 V/V after alkalinization of 150µl plasma or 100 µL urine samples with 70 µL or 50 µL of 10 M sodium hydroxide, respectively, then vortex mixing for 2

min. After thorough mixing, the samples were centrifuged at 4000 rpm at 4 ºC for 8 min followed by evaporation of the clear supernatant to dryness under a vacuum at 45 ºC in a concentrator (Vacufuge® Plus, Eppendorf, Germany). The dry residue extract was reconstituted with 400 µL of the mobile phase (10 mM ammonium acetate pH 4.5 to acetonitrile 60:40 V/V) then centrifugation was applied to the reconstitute at 4200 rpm for 10 min at 4 ºC in a cooling centrifuge (Hermle Z 326K, Hermle Labortechnik GmbH, Wehingen, Germany). An aliquot of 200 µL was transferred into a vial insert, from which an aliquot of 5 µL was injected into the LC-MS/MS system with an isocratic mobile phase on a Hypersil gold C18 Column (50 mm x 4.6 mm, 5 um; Thermo Scientific Inc., Waltham, MA, USA). The transitions used for multiple reaction monitoring (MRM) mode on the mass spectrometer were130.1→ 60 for metformin.

2.5. Pharmacokinetics and OGTT

We calculated the pharmacokinetic parameters using a non-compartmental model by WinNonlin version 2.0 (Pharsight, Palo Alto, CA, USA). The pharmacokinetic parameters calculated from metformin plasma concentrations included the maximum plasma concentration (C_{max}) of metformin and area under the plasma concentration-time curve from time baseline to 12 h (AUC_{0-12}) and the elimination half-life in plasma (t1/2P). Metformin urine concentrations were used to determine the renal clearance of metformin (Clr) and the elimination half-life in urine (t1/2U). The percent difference between the baseline glucose level (Δ G %) and the area under the Δ G%-time curve (Δ AUG %) was determined from the OGTT and used to assess the metformin pharmacodynamics.

2.6. Statistical analysis

We performed the statistical analysis using SAS (SAS Institute Inc., Cary, NC, USA). Geometric mean ratios (GMRs) with two-sided, 90% confidence intervals (CIs) were calculated after the natural logarithmic transformation of the data. The 90% CIs of the GMRs outside of an equivalence range boundary of 80-125% were considered significant as per the Food and Drug Administration (FDA) guidance for Clinical Drug Interaction Studies **[35]**. A paired t-test was used to assess the percent change in glucose levels from baseline after the OGTT. Pooling the intra-subject coefficients of variation (CV_{intra}) from different studies yielded a pooled CV_{intra} of 0.17 **[36, 37]**. Calculated variances were weighted according to the sample size of each study before pooling. Based on the pooled CV_{intra}, a sample size of 18 subjects was deemed sufficient to detect a difference at 80% power with a 5% nominal level **[38]**; as such, 20 subjects were assessed for eligibility to account for any potential dropouts during the study. Effect size (Cohen's d) was calculated by dividing the mean difference by the pooled standard deviation to determine the magnitude of difference in exposure (AUC_{0-12}) of metformin with or without daclatasvir **[31, 32]**. An index of sensitivity was calculated based on the calculated CV_{intra} of exposure of metformin (AUC_{0-12}) in the present study to confirm an adequate sensitivity for the detection of small true differences **[39, 40]**. For further inference of study results, a Bayesian approach was applied using Markov-Chain Monte Carlo Simulation. (Appendix (A))

2.7.Markov-Chain Monte Carlo simulation

To estimate the effect of daclatasvir on metformin mean log AUC0-12 and renal clearance (Clr), we performed a Markov-chain Monte Carlo (MCMC) simulation using a Metropolis-Hastings algorithm with JAGS to estimate the posterior distribution on means. We assumed that the log of AUC0-12 and the log Clr follow a normal distribution:

The likelihood function, where y_{ik} represents the ith log AUC0-12 or Clr in each group, μ_k as the mean Log of each parameter and σ_k is the variance of each group

$$y_{ik} \sim N(\mu_k, \sigma_k)$$

We set a normal prior on means centered at zero with a precision of 0.01:

The prior treatment and control group meansμ_k. The τ is set to zero and φ as 0.01 (the variance is the reciprocal of precision)

$$\mu_k \sim N(\tau, {}^{1}/_{\varphi})$$

an exponential prior on the precision with a rate parameter λ of 0.5

The prior on σ_k. The λ is set to 0.5

$$\sigma_k \sim \exp(\lambda)$$

2.8. Precisions

Were assumed unequal among treatment and control groups and therefore separate identical priors were set on variances. Trace plots were used visually to check for algorithm convergence to the posterior distribution. We simulated 1×105 samples from the posterior distribution after a burn-in of 1000 iterations. We used the simulated means to calculate Monte-Carlo estimates of differences in means and 95% credible intervals for the means. Finally, we used the generated Monte-Carlo samples of means and variances to simulate 1×105 values for AUC0-12 and Clr. The samples were again used to estimate the difference between the observed AUC0-12 and Clr among groups.

3. Results

3.1 Subjects

All subjects completed the study (n = 20). All subjects had a mean age of 36 (± 9.5) years and a mean BMI of 24 (± 3.4) kg/m^2. The number and percentage of subjects with an AE are shown in **Table 1**. Physical examination, vital signs, and clinical laboratory tests evaluation did not reveal any co-morbid diseases. None of the reported AEs was serious or mandated drug discontinuation. AEs were more prevalent in the case of co-administration of metformin and daclatasvir.

Table 1. Most common adverse events (> 5.0%) during the study per treatment arm

Adverse Event	Metformin Monotherapy (R1 and R2) (n=20)	Metformin + Daclatasvir (T1 and T2) (n=20)
Abdominal Discomfort; n (%)	5 (25%)	8 (40%)
Diarrhea; n (%)	4 (20%)	7 (35%)
Nausea/Vomiting; n (%)	3 (15%)	4 (20%)
Flatulence; n (%)	3 (15%)	5 (25%)
Headache; n (%)	1 (5%)	2 (10%)

n (%), number of subjects (percentage).

3.2 Bioanalytical assay

The methods of analysis were linear over the range of 10 ng to 5000 ng and 2 µg to 200 µg with an accuracy range of 92% to 102% and 96% to 103% in plasma and urine, respectively. Adequate intra-day and inter-day precisions were determined for five replicates (n= 5) of quality-control samples with a relative standard deviation (RSD) of less than 5%, 7%, 6.5%, and 8% in plasma and urine, respectively. The lower limits of quantitation were 10 ng/mL in plasma and 2 ug/mL in urine.

3.3 Pharmacokinetics

A statistically significant increase in C_{max} occurred in T1 (metformin 500 mg + daclatasvir 60 mg) and T2 (metformin 1000 mg + daclatasvir 60 mg) by 12% and 11% with a geometric mean ratio (GMR) of 1.12 (90% confidence interval (CI): 0.98, 1.26) and 1.12 (90%CI: 0.86, 1.36), respectively. In terms of exposure, AUC_{0-12} of metformin increased in the presence of daclatasvir in T1 and T2 by 13%, and 4%, respectively **(Fig. 2)**. The Clr of metformin decreased by 15% and 11% in T1 and T2 relative to R1 and R2 with a significant GMR of 0.85 (0.68, 1.02) and 0.89 (0.69, 1.09), respectively. The calculated pharmacokinetic parameters in plasma and urine are presented in **Table 2 & 3**. The calculated effect size (Cohen's (d)) for the difference in exposure (AUC_{0-12}) of metformin with or without daclatasvir in the case of the 500 mg dose was 0.6 while a small effect size of 0.2 was calculated for the 1000 mg dose. The index for sensitivity based on the intrasubject variability in the exposure of metformin in the current study was 7.8 indicating a sensitive study for distinguishing true differences.

3.4. OGTT

Δ G% and Δ AUG% were significantly different for the 500 mg dose of metformin alone in R1 and metformin with daclatasvir in T1 ($p<0.05$), as shown in **Table 2**. No significant differences were observed between the 1000 mg dose of metformin alone (R2) or with daclatasvir (T2), as shown in **Table 3**. The maximum percent difference from baseline glucose level (Δ $G\%_{max}$) was lower in T1 and T2 (metformin + daclatasvir) than in R1 and R2 (metformin alone) by 12% and 5.6%, respectively **(Fig. 3)**. A relationship plot between metformin plasma concentration and its pharmacodynamics (anti-hyperglycemic effect) showed a counter-clockwise hysteresis **(Fig. 4)**.

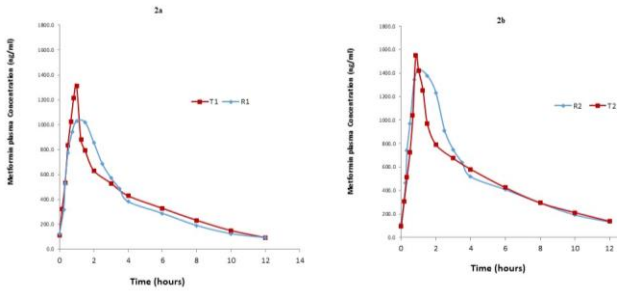

Fig. 2. Geometric Mean Plasma Concentration-time profile of Metformin 500 mg (2a) or 1000 mg (2b) alone (R1&R2) and with daclatasvir 60 mg (T1&T2)

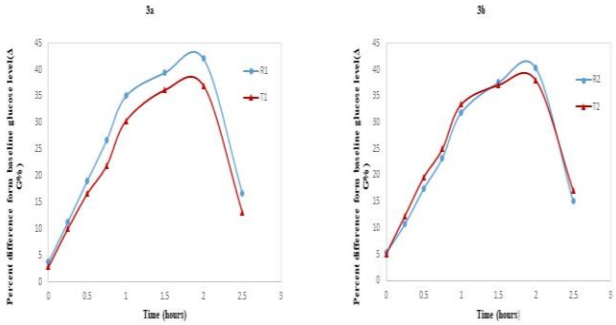

Fig. 3. Mean Percent change from baseline glucose level versus time curves after performing the oral glucose tolerance test (OGTT) for metformin 500 mg (3a) or 1000 mg (3b) alone (R1 &R2) and with daclatasvir (T1&T2)

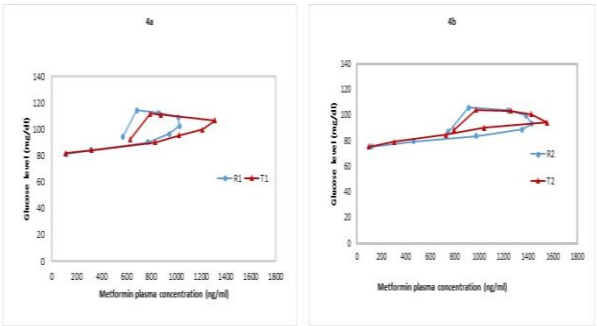

Fig. 4. Relationship between Metformin plasma concentration and pharmacodynamic response after oral glucose tolerance test for metformin 500 mg (4a) or 1000 mg (4b) alone and with daclatasvir showing counterclockwise hysteresis.

Table 2. Pharmacokinetics and OGTT parameters of metformin 500 mg either alone or with daclatasvir 60 mg

Parameter	Metformin 500 mg twice daily alone (R1)	Metformin 500 mg twice daily + Daclatasvir 60mg (T1)	GMR(90% CI)
C_{max} (ng/mL)	1669 (22)	1876 (25)	1.12 (0.98,1.26)*
$AUC_{0\text{-}12h}$ (ng/mL·h)	4703 (21)	5346 (19)	1.13(1.00,1.26)*
$T_{1/2plasma}$ (h) †	3.5 (2.5-5.1)	3.4(1.3-6.1)	-
Cl_r (mL/min)	676 (49)	579 (33)	0.85(0.68,1.02)*
$T_{1/2urine}$ (h) †	14.7(2.6-26.3)	16.2(7.1-27.3)	-
OGTT			**Arithmetic Mean difference(95%CI)(P-value)**
Δ G% (mg/dL)	21.6±15.2	18.6±13.6	2.9(1.5,4.3) (P=0.015)**
Δ AUG% (mg /dL.hr)	81.1±19.4	76.6±13.3	10.5(1.1,19.8) (P=0.029) **

Pharmacokinetic parameters are expressed as Geometric mean (coefficient of variation), OGTT pharmacodynamic parameters are expressed as arithmetic mean ± standard deviation

* T1/2plasma and T1/2urine; elimination half-life in plasma and urine respectively are expressed as median (Range), Cmax; the maximum plasma metformin concentration, AUC0-12; area under the plasma concentration-time curve from time zero up to 12 hours, AUC0-inf; area under the plasma concentration-time curve from time zero up to infinity, Clr; renal clearance, OGTT; Oral glucose tolerance test, Δ G%; percent difference from baseline glucose level, Δ AUG%; area under the Δ G%-time curve

* Significant GMR 90%CI falling outside equivalence boundary (0.8-1.25), **statistically significant $p < 0.05$

Table 3. Pharmacokinetics and OGTT parameters of metformin 1000 mg either alone or with daclatasvir 60 mg

Pharmacokinetic parameter	Metformin 1000mg twice daily alone(R2)	Metformin 1000mg twice daily +Daclatasvir 60mg(T2)	GMR(90%CI)
Cmax (ng/mL)	2108 (30)	2354 (49)	1.11(0.86,1.36)*
AUC0-12h (ng/mL·h)	6528 (31)	6824 (39)	1.04(0.81,1.27)*
T1/2plasma (h)†	3.4(1.5-6.9)	3.9(2.2-8.1)	-
Clr (mL/min)	555 (45)	497 (35)	0.89(0.69,1.09)*
T1/2urine (h)†	16.2(8.6-25.7)	15.3(10.7-25.1)	-
OGTT			**Arithmetic Mean difference(95%CI)(P-value)**
Δ G % (mg/dL)	20.1±14.1	20.8±13.7	-0.6(-1.8,0.5) (P=0.23)
Δ AUG% (mg /dL.hr)	75.7±15.2	77.1±15.4	-1.37(-7.7,5) (P = 0.6)

Pharmacokinetic parameters are expressed as Geometric mean (coefficient of variation), OGTT pharmacodynamic parameters are expressed as arithmetic mean ± standard deviation

† T1/2plasma and T1/2urine; elimination half-life in plasma and urine respectively are expressed as median (Range), Cmax; the maximum plasma metformin concentration, AUC0-12; area under the plasma concentration-time curve from time zero up to 12 hours, AUC0-inf; area under the plasma concentration-time curve from time zero up to infinity, Clr; renal clearance, OGTT; Oral glucose tolerance test, Δ G %; percent difference from baseline glucose level, Δ AUG%; area under the Δ G %;-time curve

* Significant GMR 90%CI falling outside equivalence boundary (0.8-1.25),

3.5. Markov-Chain Monte Carlo simulation

The algorithm converged to the posterior distribution without trends or autocorrelation **(Fig. 5 & 6)**. The mean metformin log AUC0-12 in subjects who received daclatasvir with metformin was 86% higher than in subjects who received metformin only **(Fig. 7)** and the mean metformin log renal clearance was 91% lower in subjects who received daclatasvir with metformin **(Fig. 8)**. The simulated AUC0-12 values were 7% higher for the metformin+daclatasvir group **(Fig. 9)** and the simulated renal clearance values were 9% lower than the metformin only group **(Fig. 10)**.

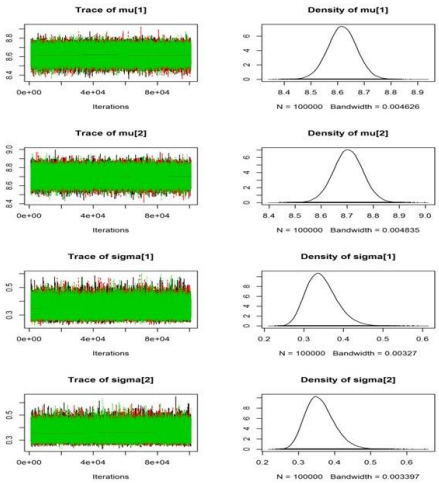

Fig. 5. Trace plots for mean log AUC 0-12 MCMC convergence diagnostics

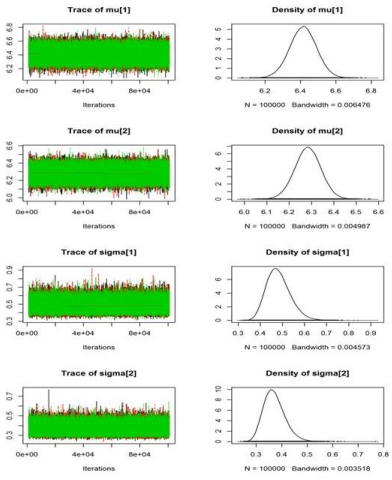

Fig. 6. Trace plots for mean log CLR MCMC convergence diagnostics

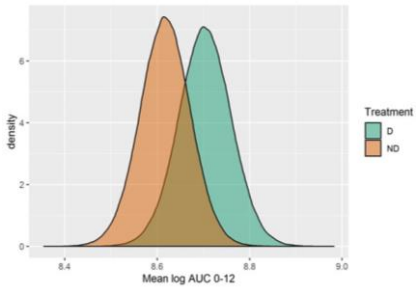

Fig. 7. Estimate the difference in mean log AUC0-12 between Metformin + Daclatasvir (D) and Metformin only (ND) groups

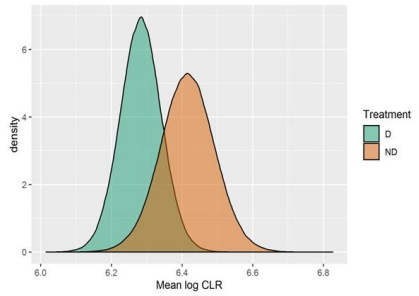

Fig. 8. Estimate the difference in mean log Clr between Metformin + Daclatasvir (D) and Metformin only (ND) groups

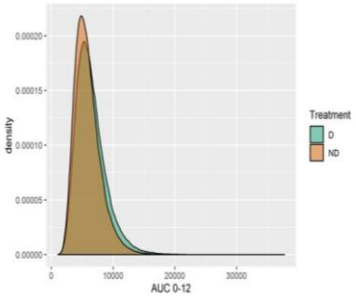

Fig. 9. Estimate the difference in AUC0-12 between Metformin + Daclatasvir (D) and Metformin only (ND) groups

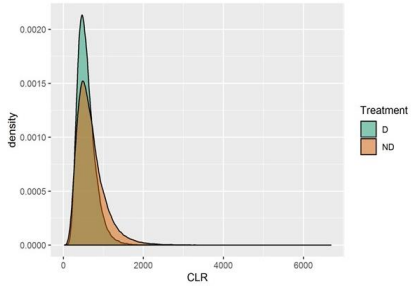

Fig. 10. Estimate difference in metformin Clr between Metformin +Daclatasvir (D) and Metformin only (ND) groups

4. Discussion

The Egyptian population has the highest prevalence of hepatitis C, enrollment of subjects from the Egyptian population is expected to be representative of this patient population **[1, 2]**. Metformin elimination is primarily facilitated by OCT2 in the kidneys and studies have shown that inhibitors of OCT2 can interact with metformin **[40-42]**. Daclatasvir was expected to alter the pharmacokinetics and pharmacodynamics of metformin by moderate inhibition of OCT2 **[12]**. Potential effects of Sex-based differences along with interethnic variations on the function of OCTs are important factors that should be considered in the context of transporter-mediated drug interaction studies **[13-16]**. The current study assessed the interaction between metformin and daclatasvir in healthy Egyptian men using a dosing regimen similar to that used in clinical practice. A decrease in the Clr of metformin was observed when daclatasvir (60 mg) was co-administered with both the 500 mg and 1000 mg doses of metformin. Interestingly, the reduction in renal clearance of metformin when combined with daclatasvir was slightly more pronounced with the 500 mg dose of metformin compared to the 1000 mg dose (Paired t-test; $P < 0.05$). The counterintuitive slight difference in renal clearance between the two doses of metformin could be attributed to the concentration-dependent inhibition of OCT2-mediated metformin transport, as previously described by Hacker et al. Besides, the increase in metformin's concentration in plasma with the higher dose (1000 mg) had overcome a weak inhibition (d=0.2) of OCT2 by daclatasvir probably due to an increase in the transporter-mediated uptake of metformin, especially with a metformin plasma concentration (0.018mM) far below the Michaelis-Menten constant (km) of metformin. OCT2 uptake of metformin was previously reported to be concentration-dependent in vitro (1.07 mM) **[30, 43-45]**. The calculated effect size (Cohen's (d)) for the magnitude of difference in exposure (AUC_{0-12}) of metformin with or without daclatasvir was 0.6 and 0.2 for the 500 mg dose and the 1000 mg dose respectively. These effect sizes suggest a moderate effect of daclatasvir on the exposure to the 500 mg dose of metformin and a small non-relevant effect on the exposure of the 1000 mg dose. Regarding the 500 mg dose, the calculated moderate effect size (d= 0.6) is consistent with the aforementioned more pronounced reduction of metformin's Clr with the 500 mg dose in the presence of daclatasvir.

In addition, the half-life of metformin in plasma and urine was not altered after co-administration of daclatasvir with the 1000 mg dose while a slight increase in the half-life of metformin in urine from 14.7 ho to 16.2 h was observed with the 500 mg dose. These comparable half-lives imply a modest, concentration-dependent inhibitory effect of daclatasvir on OCT2 and the involvement of other transporters in the clearance of metformin such as the multidrug and toxin extrusion protein (MATE), which are not inhibited by daclatasvir. The current study has revealed a slight increase in both C_{max} and AUC_{0-12} of the 500 mg and 1000 mg doses of metformin when co-administered with daclatasvir (60 mg). Smolders et al. reported a similar slight increase in the C_{max} and AUC_{0-12} of metformin (1000 mg) dose when co-administered with daclatasvir (60 mg) by 9% and 8%, respectively. Yet, this increase was not accompanied by a significant decrease in the Clr of metformin in their study. Unlike the 500-mg dose, the effect size (d= 0.2) calculated for the difference in exposure of the 1000-mg dose of metformin with daclatasvir was consistent with a small magnitude of difference for the same dose of metformin in smolders et al. In addition, the pharmacodynamic profile of the 1000 mg dose in the present study was comparable to that in smolders et al. However, the lower 500-mg dose of metformin yielded a moderate difference in

exposure (d= 0.6) with daclatasvir. This slight discrepancy could be explained by the concentration-dependent uptake of metformin by OCT2 in addition to interethnic and sex-based differences between the study populations as Smolders et al. included both male and female Caucasian subjects. Genetic variants and OCTs gene polymorphisms are associated with considerable inter-individual variability in the pharmacokinetics and pharmacodynamics response of metformin **[18-21]**. Regarding the OCT2 gene, there is increasing evidence of interethnic variation in the frequency of polymorphism **[22-24]**. Sex-based differences in terms of safety and tolerability were prominent in the occurrence of adverse drug reactions at a higher incidence in females than in males with metformin administration **[13]**. In terms of exposure, sex-based differences were eminent in a higher steady-state C_{max} and a lower C_{min} in females than males **[46]**. In a single-dose bioequivalence study, a significant difference in pharmacokinetic parameters of metformin was reported between males and females with a higher C_{max} in females **[47]**. Besides, Smolders et al. only studied the pharmacokinetics of a high dose of metformin (1000 mg) either alone or co-administrated with daclatasvir, which in the current study showed a smaller decrease in renal clearance than the lower dose of 500 mg as well as smaller effect size (d= 0.2) than the lower 500-mg dose (d=0.6). A calculated index of sensitivity of 7.8 based on a calculated intra-subject variability of 0.18 for exposure of metformin (AUC0-12) in the present study confirmed adequate study sensitivity for the detection of true differences **[38, 39]**. The daclatasvir-induced moderate alteration to metformin pharmacokinetics being more evident at the lower metformin dose (500 mg) was accompanied by a slightly enhanced glucose-lowering effect, observed through a statistically significantly different ΔG% and ΔAUG% ($p < 0.5$). The slight enhancement of the metformin effect is consistent with the reduction in renal clearance of metformin and the increase in metformin exposure. The higher (1000 mg) dose of metformin did not show the same alteration in the glucose-lowering effect when co-administered with daclatasvir. A possible explanation is that the renal clearance of the (1000 mg) metformin dose with daclatasvir was reduced to a lesser extent than the lower (500 mg) metformin dose with relatively higher bioavailability than the 500 mg dose. Besides, the slight enhancement of metformin's glucose-lowering effect with a 500 mg dose also suggests an extrahepatic weak inhibitory effect of daclatasvir on OCT1 in the gut accompanied by a local slight increase in metformin levels in the gut. This suggested gut-localized interaction with metformin transport by OCT1 could be a possible explanation for the enhancement of metformin's effect given the suggested role of the gut in metformin's mechanism of action **[48, 49]**. However, it is concluded from the study that this enhancement of metformin pharmacodynamic response is not clinically relevant. Moreover, the gut-localized interaction could be associated with the increased incidence of abdominal discomfort among the study subjects **(Table 1)**. A reduced OCT1 function with a decrease in metformin transport was reported to be associated with an increased incidence of gastric intolerance **[50, 51]**. An increase in the number of adverse events related to metformin was observed with daclatasvir. Therefore, we recommend close monitoring of patients for adverse events when combining metformin and daclatasvir. The relationship between metformin plasma concentration and its pharmacodynamics showed a counter-clockwise hysteresis, which indicates a delay in response. The hysteresis could be attributed to the complexity of the underlying mechanisms of the actions of metformin. An augmented effect of metformin action in the gut,

liver, and skeletal muscles can provide some explanation for the hysteresis. Also, the reported special flip-flop nature of metformin pharmacokinetics with the rate of absorption being slower than the elimination rate is consistent with a suggested role for the gut in the action of metformin **[9, 49, 52]**. The modest alteration of the pharmacokinetics and pharmacodynamics of metformin described in the present study is not considered clinically relevant. However, the study suggests that the inhibitory effect of daclatasvir on OCT1 in the gut could be associated with the increase in the occurrence of abdominal discomfort. The study compromises some limitations such as studying the interaction in healthy men with maintained homeostatic mechanisms in a fasting state only. In addition, the study did not determine the genetic polymorphisms of the OCT1 and OCT2 genes. It is recommended to furtherly investigate the described effects of daclatasvir on metformin pharmacokinetics and pharmacodynamics in patients with comorbid conditions and combined therapies rather than healthy subjects. In addition, a study of the effect of genetic polymorphisms of OCT 1 and 2 genes on the observed alterations is recommended. A close monitoring of adverse events, as well as precautionary monitoring of blood glucose levels with combined therapy of metformin and daclatasvir, is recommended.

Conclusion

Daclatasvir slightly altered the pharmacokinetics of metformin at two dosages (500 and 1000 mg) with a slight alteration of the lower 500 mg dose pharmacodynamics

Recommendations

A close monitoring of adverse events, as well as precautionary monitoring of blood glucose levels with combined therapy of metformin and daclatasvir, is recommended.

Declarations

Consent to publish

All authors have read and agreed to the published version of the manuscript

Ethics approval and consent to participate

The study is registered on ClinicalTrials.gov Identifier: NCT03686722 and approved by the ethics committee of Ain shams university with approval no: 141.

Availability of data and materials

All data generated or analyzed during this study are included in this published article in the main manuscript.

Competing interests

No competing interests were declared by the authors

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Authors' contributions

The manuscript was drafted and written by Mohamed Raslan and Lamia El Wakeel.

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