Metformin and daclatasvir: absence of a pharmacokinetic–pharmacodynamic drug interaction in healthy volunteers

Running head: Interaction study between metformin and daclatasvir

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Summary

AIM

The aim of this study was to evaluate the effect of the proposed organic cation transporter (OCT) inhibitor daclatasvir on the pharmacokinetics and pharmacodynamics of the OCT substrate metformin.

METHODS

This was an open-label, two-period, randomized, crossover trial in 20 healthy subjects. Treatment A consisted of metformin and treatment B consisted of metformin+daclatasvir. Pharmacokinetic curves were recorded at steady state. Geometric mean ratios (GMRs) with 90% confidence intervals (CIs) were calculated for metformin area under the concentration-time curve from 0 to 12 hours ($AUC_{0–12}$), maximum plasma concentration (C_{max}) and final plasma concentration (C_{last}). An oral glucose tolerance test was performed, measuring insulin, glucose and lactate levels.

RESULTS

The GMRs (90% CI) of metformin $AUC_{0–12}$, C_{max} and C_{last} (B versus A) were 109% (102–116%), 108% (101–116%) and 112% (103–122%). The GM $AUC_{0–2}$ for insulin, glucose and lactate during treatment A and B were 84 and 90 $h \cdot mE/L$, 13.6 and 13.4 $h \cdot mmol/L$ and 3.4 and 3.5 $h \cdot mmol/L$, respectively.

CONCLUSIONS

Bioequivalence analysis showed that daclatasvir does not influence the pharmacokinetics of metformin in healthy subjects. Pharmacodynamic parameters were also comparable between treatments.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Several drug interactions with daclatasvir were previously studied; however, the drug–drug interaction between daclatasvir and metformin has not been evaluated, neither *in vivo* nor *in vitro*.
- Metformin is a substrate of drug transporters OCT1 and OCT2, which are possibly inhibited by daclatasvir.

WHAT THIS STUDY ADDS

- This study showed that there is no drug–drug interaction between daclatasvir and metformin.
- This study showed that daclatasvir and metformin can be combined, but physicians are recommended to monitor for (altered) adverse events during treatment.

Introduction

Chronic hepatitis C virus (HCV) infection is associated with insulin resistance, which might cause type 2 diabetes mellitus (T2DM) [1]. It is estimated that 150–170 million people are infected with HCV worldwide and 422 million people were living with T2DM in 2014 [2, 3]. In addition, both conditions have a high impact on international healthcare, because of the high morbidity and mortality rates of T2DM and HCV [4, 5].

The association between HCV and insulin resistance/T2DM has been studied extensively [6, 7]. Compared with controls, there is an increased prevalence of diabetes mellitus (DM) in HCV patients [8]. T2DM is two- to threefold more prevalent in HCV-infected patients compared with hepatitis-B-infected individuals [1]. Insulin resistance itself causes liver disease [1], and in combination with HCV, patients have an increased risk of developing cirrhosis and hepatocellular carcinoma [8, 9].

Furthermore, insulin resistance in HCV patients is correlated with reduced efficacy of HCV treatment, and viral clearance is associated with improved insulin sensitivity [1, 10]. In the literature, there is consensus about the relationship between HCV and insulin resistance/T2DM; however, the mechanisms behind this relationship are still under debate [7].

Metformin is a biguanide used for the treatment of T2DM, since it has, e.g. the ability to lower the blood glucose concentrations in T2DM patients. In western counties, metformin is the first choice in treatment of T2DM. Metformin is not metabolized, but it is a substrate of several membrane transporters, namely plasma membrane monoamine transporter (PMAT), organic cation transporter (OCT) 1, 2 and 3 and multidrug and toxin extrusion protein (MATE) 1 and 2K. The oral absorption and hepatic uptake of metformin is mediated by PMAT, OCT1 and OCT3. However, the involvement of the OCTs in intestinal absorption remains controversial [11, 12]. Metformin is excreted renally through glomerular filtration (protein binding is negligible) and active tubular secretion [11]. Tubular secretion is facilitated by uptake into the tubular cells via OCT2 and excretion into the urine via MATE1 and MATE2K [13]. Tubular reabsorption might be facilitated by OCT1 and PMAT [11, 14, 15]. Since the renal clearance of metformin is higher than creatinine clearance, it is deduced that tubular secretion plays an important role in its excretion [16].

Drug interactions influencing metformin pharmacokinetics (PK) are a result of inhibition or induction of the previously mentioned drug transporters. The human immunodeficiency virus (HIV) integrase inhibitor dolutegravir increases the metformin exposure by 79%, probably via inhibition of OCT2 [17]. Rifampicin, an OCT inducer, causes increased renal clearance and tubular secretion of metformin [18].

Similarly, a potential interaction may exist between NS5A-inhibitor daclatasvir and metformin. Daclatasvir is used for the treatment of HCV infection in combination with sofosbuvir and is licensed

for the treatment of genotype 1, 3 and 4 [19]. It is metabolized by CYP3A4 and is a substrate of P-gp. It does not influence drug-metabolizing enzymes, but, at least *in vitro*, it seems to inhibit the activity of several drug transporters, such as P-gp, OCT1 and OCT2, organic anion transporting polypeptide 1B1 and breast cancer resistance protein [19]. However, the clinical relevance of OCT1 and OCT2 inhibition was unknown at the time of this study.

Our hypothesis is that daclatasvir could decrease metformin tubular excretion, through inhibition of OCT2, and therefore causes increased plasma concentrations and increased glucose-lowering activity. Inhibition of OCT1 in the liver could also lead to increased plasma concentrations of metformin. The proposed *in vivo* PK interaction and the net pharmacodynamic (PD) effect are unknown, and therefore we conducted a PK–PD study to evaluate the potential drug–drug interaction between daclatasvir and metformin.

Methods

Study design

This was an open-label, two-period, randomized, crossover trial in healthy subjects. Subjects were randomized in treatment sequences AB and BA. Treatment A (reference) consisted of 500 mg metformin twice daily (BID) on Day 1 and 2 (Metformin HCL Actavis 500 mg). The dose was increased to 1000 mg BID on Days 3 to 8. This gradual dose step-up was chosen to limit adverse events (AEs), as subjects used metformin without food for 8 days.

Treatment B (test) consisted of 500 mg metformin BID (Day 1–2) and 1000 mg metformin BID (Days 3–8). From Day 1 to Day 8, 60 mg daclatasvir once daily was added (Daklinza®, Bristol-Myers Squibb [20]). Between treatments there was a wash-out period of 13 days.

To study metformin and daclatasvir exposure, at Day 8 of treatment (steady state), blood samples for a PK curve were obtained up to 12 hours and 24 hours after intake of metformin and daclatasvir, respectively. Secondly, to study metformin excretion, 12-hour urine was collected for the determination of metformin renal clearance.

The PD of metformin was studied using an oral glucose tolerance test (OGTT), which was also performed at Day 8 of treatment. During this 2-hour test, venous blood was withdrawn to determine the plasma concentrations of glucose, lactate and insulin.

Study participants

Healthy males and females were included in this study. Subjects eligible for inclusion were 18–55 years old and had a body mass index (BMI) of 18–36 kg/m^2. Subjects had to be in good age-appropriate health condition (physical examination, electrocardiography and biochemical, haematologic and urinalysis testing).No concomitant medication was allowed, except for acetaminophen <2000 mg/day. Main exclusion criteria were a positive HIV, hepatitis B or HCV test, pregnancy and estimated glomerular filtration rate (eGFR) <60 mL/min.

Dosing and adherence

During study visits at Days 1, 2, 3 5, and 8, medication was administered at 8 AM supervised by the study personnel. In between study visits, subjects took the medication at home, and adherence was assessed as follows: (1) tablets were counted by the trial nurses; (2) Medication Event Monitoring System (MEMS) caps (Aardex Ltd, Zug, Switzerland) were used to monitor the opening of the metformin-containing bottles; and (3) subjects were instructed to record the time of medication intake (and any AE) in a diary.

PK sampling and oral glucose tolerance test

The study was conducted at the Clinical Research Centre Nijmegen in the Radboud university medical center, Nijmegen, the Netherlands.

At steady state (Day 8), blood samples were withdrawn to measure the plasma concentrations of metformin (A and B) and daclatasvir (B). Drugs were taken concomitantly after an overnight fast, and blood was withdrawn in EDTA tubes at t = 0 (predose), 0.5, 1, 1.5, 1.9, 2.5, 3, 4, 6, 8, 10 and 12 hours after metformin intake. During treatment B (daclatasvir), an additional sample was collected at 24 hours. Blood samples were stored in a refrigerator until centrifuged (5 minutes at 1900g). Plasma was transferred into polypropylene tubes and stored at –40◦C until bioanalysis. To study metformin excretion at steady state and to assess the renal clearance of metformin, urine was collected for 12 hours at intervals of 4 hours. Prior to the start of collection, morning urine was voided before the administration of metformin. Participants were asked to drink 200 mL water every 4 hours. Volume and pH of urine were noted, and urine was stored by –40◦C until further bioanalysis.

For the OGTT, the participants were instructed to avoid strenuous exercise and to follow a carbohydrate-controlled diet (at least 200–250 g carbohydrates per day) for 3 days prior to Day 8. The OGTT was performed after an overnight fast for at least 14 hours. At 10 AM, the subjects drank 75 g glucose. Following the glucose intake, venous blood was withdrawn at t = 0 (predose), 30, 60, 90 and 120 minutes to determine the plasma concentrations of glucose, lactate and insulin.
Data were collected using Castor EDC (Castor Electronic Data Capture, Ciwit BV, Amsterdam, the Netherlands).

Bioanalytical methods

Metformin and daclatasvir were analyzed in the laboratory of the Department of Pharmacy of the Radboud university medical center, Nijmegen, the Netherlands. Metformin in plasma and urine were

determined using two different validated ultra-performance liquid chromatography (UPLC) assays with ultraviolet (UV) detection (236 nm).

Metformin was extracted from 200 µL plasma using 80µL 4M sodium hydroxide and 3mL 1-butanol/ (n-) hexane (50:50, v/v). This solution was vortexed for 1 minute at 1600 rpm and centrifuged for 5 minutes at 1900 g. The aqueous phase was frozen for 1 minute by –40°C before the organic phase was poured into a vial. Metformin was then back–extracted from the organic phase by adding 200µL 0.1% phosphoric acid. This solution was mixed for 1 minute at 1600 rpm and centrifuging for 5 minutes at 1900 g.

Metformin was extracted from 20 µL urine following the same procedures after adding 200 µL blank plasma. After back-extraction, 100 µL of the water phase was diluted with 900 µL water before injection.

Chromatography was performed using an Acquity UPLC HSS T3 analytical column (1.8 µm, 2.1 × 100 mm; Waters, Milford, MA, USA) with a mobile phase of 0.02M phosphate buffer, pH 3.23. The flow rate was set on 0.6mL/min. After every injection, the column was rinsed with a combination of eluent and acetonitrile (50:50, v/v) before equilibrating back to the initial eluent.

Accuracy across five metformin quality-control samples measured in three runs (n = 15) over 2 days ranged from 101 to 103% in plasma and 98 to 101% in urine. Interday precision ranged from 0.0 to 2.4% in plasma and 0.0 to 3.9% in urine (n = 15). Intraday precision ranged from 1.2 to 5.8% in plasma and 2.3 to 8.9% in urine (n = 5). For metformin in plasma, the calibration range was 0.01–5.00 mg/L and for urine the range was 2.0–2100 mg/L.

Daclatasvir was measured using a validated UPLC method with UV detection (314 nm). Daclatasvir was extracted from 100 µL plasma using 200 µL acetonitril/methanol (50:50, v/v) with 0.1% formic acid. This solution was vortexed for 5 minutes at 2500 rpm and centrifuged for 5 minutes at 1910 g. The supernatant (170µL) was poured into a vial and centrifuged for 5 minutes at 1910 g; 10 µL was then injected onto an Acquity UPLC BEH C18 analytical column (1.7 µm, 2.1 × 50 mm; Waters, Milford, MA, USA). The flow rate was set to 0.550 mL/min, and daclatasvir was eluted by using a gradient 0.05M phosphate buffer and 0.05M phosphate buffer/acetonitrile 30/70 v/v.

Accuracy across five daclatasvir quality-control samples measured in three runs over 2 days ranged from 98 to 107%. Interday precision ranged from 0.0 to 1.3% and intraday precision ranged from 1.3 to 6.0%. The calibration range of the method was 0.03–10 mg/L.

Insulin samples were collected in lithium–heparinized tubes and determined at the clinical chemistry laboratory of Radboud university medical center, Nijmegen, the Netherlands (random access analyzer, Roche E170 modular immunoassay, Roche Diagnostics International Ltd, Rotkreuz, Switzerland). Glucose and lactate (blood gas tube Provent 4646E, lithium–heparin coating) were determined directly after sampling, using a glucose enzymatic–amperometric method (Biosen C-line GP, EKF-diagnostic GmbH, Barleben, Germany).

Pharmacokinetic analysis

A non-compartmental approach was used (WinNonlin/Phoenix version 6.3, Pharsight Corporation, St. Louis, MO, USA) to assess the area under the time curve from 0 to 12 hours ($AUC_{0–12}$) and 12-hour plasma concentration (C_{12}) for metformin and from 0 to 24 hours ($AUC_{0–24}$) and 24-hour plasma concentration (C_{24}) for daclatasvir. In addition, maximum plasma concentration (C_{max}), time to reach C_{max} and apparent elimination half-life of metformin and daclatasvir were determined. Metformin renal clearance was calculated by dividing the total amount metformin excreted (0–12 hours) by the

$AUC_{0–12}$. The secretion of metformin was calculated by subtracting the metformin clearance with the creatinine clearance, which was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula ($eGFR = 141 \times \min[S_{cr}/\kappa, 1]^{\alpha} \times \max[S_{cr}/\kappa, 1]^{-1.209} \times 0.993^{Age} \times 1.018$ [if female] × 1.159 [if black]). In addition, the geometric mean ratios (GMRs) with 95% confidence intervals (CIs) (treatment B versus A) for metformin secretion and eGFR were calculated.

Pharmacodynamic analysis

The plasma concentrations of glucose, lactate and insulin were used to calculate the area under the concentration-time curve from 0 to 2 h ($AUC_{0–2}$) for which WinNonlin/Phoenix was used. The insulin and glucose concentrations were also used to calculate the homeostatic model assessment insulin resistance (HOMA-IR) score, which is used to quantify insulin resistance (HOMA-IR = [glucose × insulin]/22.5).

Statistical analysis

The $AUC_{0–12}$ values of metformin for treatment A and B were compared using the bioequivalence approach, which is recommended by the European Medicines Agency (EMA) to evaluate PK drug interactions [21]. GMRs with 90% CIs of $AUC_{0–12}$, C_{max} and C_{12} were calculated for metformin, comparing treatment B and treatment A. We used a linear mixed-effect model with fixed parameters to calculate the GMR with 90% CI. Fixed parameters were treatment, period, sequence and subjects within sequence according to EMA guidelines [21].

For bioequivalence between treatment A and B, the $AUC_{0–12}$ GMR with 90% CI should fall within the range of 80–125%.

Based on a previously observed inter-subject coefficient of variation (CV%) of 22% for metformin $AUC_{0–12}$ [22], we expected the intra-subject CV% to be lower: 15%. For the sample-size calculation, we used a power calculation in SAS® 9.2 (paired t-test for lognormal distribution for showing

equivalence). For 80% power to prove bioequivalence, a sample size of 17 subjects should be included in the study. To account for possible drop-outs, 20 subjects were to be included. Metformin renal clearance was log-transformed and compared between treatments using a paired t-test.

Glucose, lactate and insulin AUC_{0-2} values were log-transformed and compared between treatments using a paired t-test. All statistical analyses were performed in IBM SPSS Statistics, version 22.

Safety and tolerability

During all study visits, AEs and laboratory safety (biochemistry and haematology) were monitored by the study nurses and physicians. AEs were graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events ('DAIDS AE GradingTable'); version 1.0, December 2004, clarification August 2009 [23]).

Ethics

The trial was approved by the Investigational Review Board of Radboud university medical center, Nijmegen, the Netherlands. The trial was conducted in accordance with Good Clinical Practice and the Declaration of Helsinki and registered at ClinicalTrials.gov (NCT02565862). All participants signed informed consent forms before screening evaluations.

Results

Baseline characteristics

Twenty subjects (nine male) were enrolled, and all subjects completed the study. All subjects were Caucasian; their median (range) age was 47.5 (20–55) years and the median (range) BMI was 26.6

(22.9–36.0) kg/m^2. The subjects were in normal health, based on medical history, physical examination, vital signs and biochemical and haematology data.

In general, adherence to the study medication was good, as proven by pill count, monitoring of the MEMS caps and the registration in the diary. Two subjects took a double dose of daclatasvir, and three subjects forgot one or two tablets of 500 mg metformin. These deviations did not lead to exclusion of any of the study participants.

Pharmacokinetics of metformin and daclatasvir

Steady-state geometric mean (GM) concentration–time curves of metformin are shown in Figure 1a and the PK parameters are shown in Table 2. One subject vomited during treatment A; therefore, the results of 19 subjects are presented. The GMR with 90% CI of the metformin $AUC_{0–12}$, C_{max} and C_{12} of metformin with and without daclatasvir (B versus A) were 109% (102–116%), 108% (101–116%) and 112% (103–122%), respectively. Since the CIs of all parameters fell within the range of 80–125%, absence of an interaction was confirmed.

Urine was collected to estimate renal metformin clearance. Treatment B included 19 subjects, because urine was not correctly stored for one subject. The GM (range) renal clearance of metformin for treatment A was 351 (148–646) mL/min and for treatment B, it was 333 (166–537) mL/min (p = 0.504). The GM (range) for metformin secretion during treatment A and B was 275 (25–538) mL/min and 269 (91–445) mL/min (p = 0.3822), respectively. The GMR (95% CI) for metformin secretion (B versus A) was 98% (70–137%) and 98% (95–101%) for eGFR (Figure 2).

The GM concentration–time curve of daclatasvir and PK parameters are shown in Figure 1b and Table 2, respectively, combined with references values [24]. The GMs with geometric coefficient of variation (GCV%) $AUC_{0–24}$, C_{max} and C_{24} values of daclatasvir were 18.38 (44) h·mg/L, 1.85 (40) mg/L and 0.30 (63) mg/L.

Pharmacodynamics of metformin

The OGTT was used to study PD endpoints (insulin, lactate, glucose). Treatment A contains data from 19 subjects, since one subject was not able to tolerate the glucose drink during treatment A. The subject vomited and was excluded from the analysis.

GM (GCV%) for AUC_{0-2} for insulin for treatment A and B were 86 (49) and 87 (54) h·mE/L, respectively (p = 0.430). The glucose and lactate GM (GCV%) AUC_{0-2} values during treatment A and B were 13.7 (10) and 13.4 (14) h·mmol/L and 3.4 (15) and 3.4 (18) h·mmol/L, respectively (p = 0.919; p = 0.779, respectively) (Figure 3). In Figure 4, we show the AUC_{0-2} ratios (treatment B/treatment A) per subject for glucose, insulin and lactate.

The HOMA-IR score was calculated for the individual subjects (treatment A and B), showing the variation of insulin resistance in the study population. The HOMA-IR varied from 0.73 to 4.81 during treatment A and 0.94 to 4.19 during treatment B.

Safety and tolerability

A total of 129 AEs were reported during the trial, varying from three to 11 AEs per subject. No serious adverse events (SAEs) were reported. Only one AE was graded as grade 3 (elevated amylase). The majority of the AEs were 'probably' related to the use of study medication (55%) and were reported during the combined treatment of metformin and daclatasvir (59%). Six AEs (four subjects) were reported directly after the intake of the study medication.

Most commonly reported AEs were diarrhoea (n = 26), stomach ache/stomach cramps (n = 15), nausea (n = 11), headache (n = 10) and fatigue (n = 9). The gastrointestinal AEs are most likely caused by metformin. Subjects recovered from all AEs after the end of treatment. AEs reported (≥5%) per treatment are shown in Table 3.

Discussion

We studied the potential interaction between the NS5A-inhibitor daclatasvir and the biguanide metformin in healthy volunteers. We hypothesized that the exposure to metformin could possibly be increased due to OCT1 and/or OCT2 inhibition by daclatasvir, with altered glucose plasma concentrations as a result.

The results of the PK analysis did not support this hypothesis: no interaction was observed when metformin was administered with daclatasvir. In addition, there was no difference in metformin renal secretion between treatments. Therefore, we concluded that daclatasvir does not affect systemic exposure to metformin. Similarly, the PD analysis showed no difference between treatments, so we concluded absence of a PD interaction between daclatasvir and metformin.

The apparent absence of an effect of daclatasvir on metformin PK is confirmed by *in vitro* studies that we carried out later. Comparing the maximum therapeutic concentration of daclatasvir (C_{max}) of 1.85 mg/L in this study with the reported half maximum inhibitory concentration (IC_{50}) showed that the *in vivo* unbound C_{max} was indeed lower than the *in vitro* data, as daclatasvir is highly bound to plasma proteins (99%). The IC_{50} of daclatasvir for OCT2 was 7.3 µM [25] and for OCT1 it was 1.4 µM [26], representing plasma concentrations of ~5.4 mg/L and ~1.0 mg/L, respectively, of unbound daclatasvir. We should note that, at the site of action (intestine, hepatocyte), the daclatasvir concentration might be different than the used C_{max}, as this is the plasma concentration after systemic absorption. Daclatasvir concentrations could be higher in the intestine and portal vein, possibly inhibiting OCTs. This could be an explanation for the statistically significantly increased metformin plasma concentration when combined with daclatasvir (GMR and CI >100%). We argue that this increase is not clinically relevant for patients with normal metformin clearance, but it might be clinically relevant in special populations with reduced metformin clearance, such as patients with renal impairment.

In daily practice, metformin is administered with food. In this trial, we deviated from this recommendation, as subjects had to fast overnight for the execution of the OGTT. The systemic exposure of metformin is decreased in a fed state (C_{max}: 40%; AUC: 25%) [27]. In our study, C_{max} and AUC were elevated when compared with a previous study in healthy volunteers where metformin was taken with food: C_{max} 1.32 mg/L and AUC_{0-24} 20.5 h·mg/L [28]. The high number of metformin-related AEs could be explained by these increased metformin exposures. Secondly, intake of metformin without food could possibly cause additional AEs[27].

We used an OGTT to study the PD effect of metformin on the glucose regulation with and without daclatasvir. The OGTT was conducted because the PK drug interaction was only clinically relevant when also the glucose regulation (PD) would be altered. Secondly, we did not want to exclude the possibility that there was a PD effect without a PK effect. In this study, we showed that both PK and PD were related, as neither the systemic metformin concentrations nor OGTT results were affected by daclatasvir. The relation between the OGTT and metformin PD was shown previously in healthy volunteers, whereas the blood glucose levels were not altered [18, 29, 30].

Daclatasvir PK was studied only in treatment group B, in the presence of metformin; therefore, the PK of daclatasvir was compared with literature in Table 2. Daclatasvir was not studied separately, as metformin was thought not to influence any drug enzymes or transporters and therefore we did not expect that metformin would influence daclatasvir PK [31]. Daclatasvir exposure was increased compared with reference values as shown in Figure 1b [24]. In our study, subjects took daclatasvir fasted, whereas daclatasvir was taken with food in the reference study. This could be an explanation for the elevated daclatasvir plasma concentrations, because food decreases daclatasvir AUC by 23% and C_{max} by 28% [20]. However, daclatasvir plasma concentrations were somewhat higher than we would expect based on the food effect alone. Daclatasvir PK is increased solely by CYP3A4 and/or P-gp inhibitors, and metformin is neither of these. It could be that metformin induces other

unidentified drug transporters or drug-metabolizing enzymes that contribute to the metabolism or distribution of daclatasvir [32]. Another explanation could be that the fasted healthy volunteers in our study had better absorption of daclatasvir, possibly caused by a more acidic gastric pH, increasing the solubility of daclatasvir.

No unexpected AEs or SAEs were reported in this study. The study medication was overall well tolerated; however, almost all subjects reported diarrhoea and or stomach ache/cramps, which were related to the use of metformin. One subject did not tolerate the glucose solution, but overall the OGTT was well tolerated by the fasted participants. However, we must comment that the number of AEs was 76 with combined treatment of daclatasvir and metformin, versus 53 when metformin was given alone. This could be caused by the relatively high daclatasvir plasma concentrations combined with the small increase of metformin plasma concentrations. Therefore, our recommendation is that daclatasvir and metformin can be combined, but physicians are recommended to monitor for (altered) AEs during treatment.

Limitations of our study were that daclatasvir PK was not studied separately and that we included healthy, Caucasian subjects who might not completely reflect the HCV/T2DM patient population that will use these drugs. Therefore, we included subjects with a wide range of age, BMI and insulin resistance (HOMA-IR).
We did not determine OCT genotypes, since all the PK curves of the subjects were in the same concentration range, we observed a low inter-subject variability for metformin and the sample size was limited.

In conclusion, the establishment of bioequivalence in this study showed that daclatasvir did not influence the PK of metformin in healthy subjects. PD parameters were also comparable between treatments. An increased number of AEs was reported when daclatasvir was combined with

metformin; however, no unexpected AEs were reported in this study. We recommend monitoring for altered AEs during treatment when daclatasvir and metformin arecombined in HCV-infected patients with T2DM.

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Conflict of Interest

E.J. Smolders, A. Colbers, C.T.M.M. de Kanter, K. Velthoven-Graafland, L.T. Wolberink, N. van Ewijk-Beneken Kolmer, J.P.H. Drenth, R.E. Aarnoutse and C.J. Tack declare that they have no conflicts of interest that are directly relevant to the content of this manuscript. D.M. Burger is a member of advisory boards of AbbVie, Bristol-Myers Squibb, Gilead, Janssen, Merck and ViiV Healthcare. He received sponsorship and research grants from Bristol-Myers Squibb, Janssen, Merck and ViiV Healthcare. However, this did not influence the content of this manuscript.

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Author contributions

E.J.S.: design and execution of the trial, analysis of data, manuscript preparation. A.C.: design of the trial, analysis of data, manuscript preparation and study supervision. C.T.M.M.K.: critical revision of the manuscript. K.V.-G.: development and validation of the bio-analytical method of daclatasvir and supervision of sample analysis. L.T.W.: sample analysis of daclatasvir and metformin. N.E.-B.K.: development and validation the bio-analytical method of metformin and supervision of sample analysis. J.P.H.D.: critical revision of the manuscript. R.E.A.: critical revision of the manuscript. C.J.T.: design of the trial, critical revision of the manuscript, interpretation of results. D.M.B.: design of the trial, interpretation of results, critical revision of the manuscript, study supervision.

References

1. Hammerstad SS, Grock SF, Lee HJ, Hasham A, Sundaram N, Tomer Y. Diabetes and Hepatitis C: A Two-Way Association. Front Endocrinol (Lausanne) 2015; 6: 134.
2. World Health Organization. Global Report on Diabetes: executive summary. 2016.
3. World Health Organization. Hepatitis C: WHO fact sheet No. 164. 2014; Accessed: 2016: http://www.who.int/mediacentre/factsheets/fs164/en/.
4. Lauer GM, Walker BD. Hepatitis C virus infection. N Engl J Med 2001; 345: 41-52.
5. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001; 414: 782-7.
6. Badar S, Khubaib B, Idrees M, Hussain A, Awan Z, Butt S*, et al.* Association of Hepatitis C Virus With Insulin Resistance: Evidences From Animal Studies and Clinical Studies. Hep Month 2012; 12: 11-15.
7. Torres DM, Harrison SA. Hepatitis C virus and insulin resistance/diabetes mellitus. Gastroenterol Hepatol (N Y) 2008; 4: 568-70.
8. Antonelli A, Ferrari SM, Giuggioli D, Di Domenicantonio A, Ruffilli I, Corrado A*, et al.* Hepatitis C virus infection and type 1 and type 2 diabetes mellitus. World J Diabetes 2014; 5: 586-600.
9. Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG*, et al.* Insulin resistance is associated with chronic hepatitis C and virus infection fibrosis progression. Gastroenterology 2003; 125: 1695-704.
10. Arase Y, Suzuki F, Suzuki Y, Akuta N, Kobayashi M, Kawamura Y*, et al.* Sustained virological response reduces incidence of onset of type 2 diabetes in chronic hepatitis C. Hepatology 2009; 49: 739-44.
11. Gong L, Goswami S, Giacomini KM, Altman RB, Klein TE. Metformin pathways: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 2012; 22: 820-27.
12. Muller J, Lips KS, Metzner L, Neubert RH, Koepsell H, Brandsch M. Drug specificity and intestinal membrane localization of human organic cation transporters (OCT). Biochem Pharmacol 2005; 70: 1851-60.

13. Wang L, Weinshilboum R. Metformin Pharmacogenomics: Biomarkers to Mechanisms. Diabetes 2014; 63: 2609-10.
14. Tzvetkov MV, Vormfelde SV, Balen D, Meineke I, Schmidt T, Sehrt D*, et al.* The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin. Clin Pharmacol Ther 2009; 86: 299-306.
15. Xia L, Engel K, Zhou M, Wang J. Membrane localization and pH-dependent transport of a newly cloned organic cation transporter (PMAT) in kidney cells. Am J Physiol Renal Physiol 2007; 292: F682-90.
16. Tucker GT, Casey C, Phillips PJ, Connor H, Ward JD, Woods HF. Metformin kinetics in healthy subjects and in patients with diabetes mellitus. British Journal of Clinical Pharmacology 1981; 12: 235-46.
17. Zong J, Borland J, Jerva F, Wynne B, Choukour M, Song I. The effect of dolutegravir on the pharmacokinetics of metformin in healthy subjects. J Int AIDS Soc 2014; 17: 19584.
18. Cho SK, Yoon JS, Lee MG, Lee DH, Lim LA, Park K*, et al.* Rifampin Enhances the Glucose-Lowering Effect of Metformin and Increases OCT1 mRNA Levels in Healthy Participants. Clin Pharmacol Ther 2011; 89: 416-21.
19. EMA. Daklinza: Summary of Product Characteristics. 2015; Accessed: 2015: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/003768/WC500172848.pdf.
20. EMA. Daklinza Product Information. 2014; Accessed: 2016: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/003768/WC500172848.pdf.
21. EMA. Guideline on the investigation of bioequivalence. 2010; Accessed: http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2010/01/WC500070039.pdf.
22. Najib N, Idkaidek N, Beshtawi M, Bader M, Admour I, Alam SM*, et al.* Bioequivalence evaluation of two brands of metformin 500 mg tablets (Dialon & Glucophage)--in healthy human volunteers. Biopharm Drug Dispos 2002; 23: 301-6.
23. Division of Aids. Division of Aids table for grading the severity of adult and pediatric adverse events; Version 1.0. December, 2004. In.
24. Gandhi Y, Adamczyk R, Wang R, Stonier M, Kandoussi H, Hesney M*, et al.* Assessment of Drug-Drug Interactions Between Daclatasvir and Darunavir/Ritonavir or Lopinavir/Ritonavir [abstract 80]. In: 16th International Workshop on Clinical Pharmacology of HIV and Hepatitis Therapy Washington, DC, 2015.
25. FDA. Daklinza: Clinical Pharmacology and Biopharmaceutics review(s). 2015; Accessed: 2016: http://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/206843Orig1s001,s003ClinPharmR.pdf.
26. Ministry of Health LaWJ. Daklinza Tablets 60 mg. 2014; Accessed: 2016: https://www.pmda.go.jp/files/000209021.pdf.
27. EMA. Metformin Actavis: Product Information. 2013; Accessed: 2016: http://www.medicines.org.uk/emc/medicine/23244/SPC.
28. Timmins P, Donahue S, Meeker J, Marathe P. Steady-State Pharmacokinetics of a Novel Extended-Release Metformin Formulation. Clin Pharmacokinet 2005; 44: 721-29.
29. Cho SK, Kim CO, Park ES, Chung J-Y. Verapamil decreases the glucose-lowering effect of metformin in healthy volunteers. Br J Clin Pharmacol 2014; 78: 1426-32.

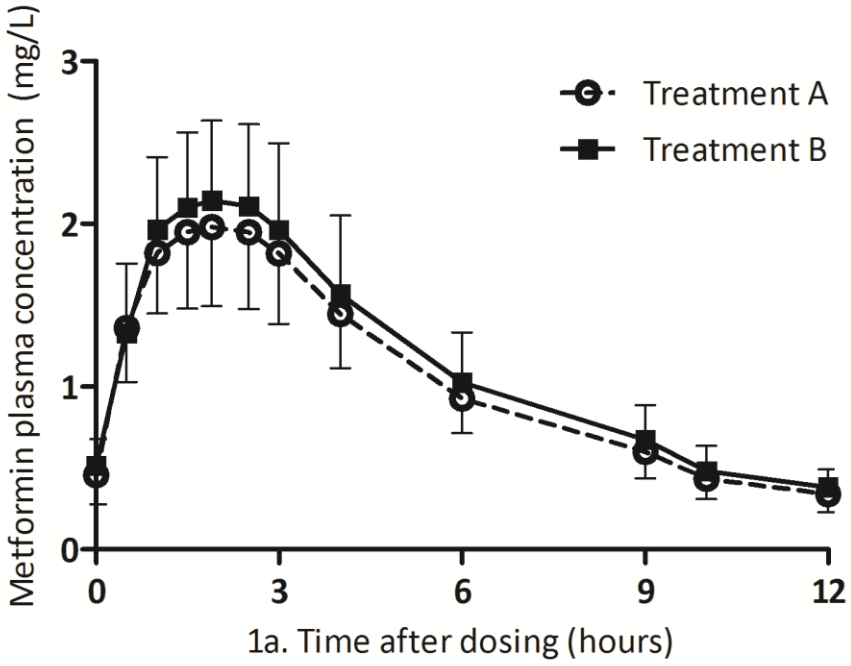
30. Stage TB, Pedersen RS, Damkier P, Christensen MMH, Feddersen S, Larsen JT*, et al.* Intake of St John's wort improves the glucose tolerance in healthy subjects who ingest metformin compared with metformin alone. Br J Clin Pharmacol 2015; 79: 298-306.
31. Stage TB, Brosen K, Christensen MM. A Comprehensive Review of Drug-Drug Interactions with Metformin. Clin Pharmacokinet 2015; 54: 811-24.
32. Stopfer P, Giessmann T, Hohl K, Sharma A, Ishiguro N, Taub ME*, et al.* Pharmacokinetic Evaluation of a Drug Transporter Cocktail Consisting of Digoxin, Furosemide, Metformin, and Rosuvastatin. Clinical Pharmacology and Therapeutics 2016; 100: 259-67.
33. Alexander SP, Fabbro D, Kelly E, Marrion N, Peters JA, Benson HE*, et al.* The Concise Guide to PHARMACOLOGY 2015/16: Enzymes. Br J Pharmacol 2015; 172: 6024-109.
34. Alexander SP, Kelly E, Marrion N, Peters JA, Benson HE, Faccenda E*, et al.* The Concise Guide to PHARMACOLOGY 2015/16: Transporters. Br J Pharmacol 2015; 172: 6110-202.
35. Southan C, Sharman JL, Benson HE, Faccenda E, Pawson AJ, Alexander SP*, et al.* The IUPHAR/BPS Guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands. Nucleic Acids Res 2016; 44: D1054-68.

Table of links

TARGETS
Enzymes [33]
CYP3A4
Transporters [34]
BCRP
MATE1
MATE2K
OATP1B1
OCT1
OCT2
OCT3
P-gp
PMAT

LIGANDS
Sofosbuvir
Dolutegravir
Rifampicin
Metformin

These Tables list key protein targets and ligands in this article that are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [35], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16[33, 34].



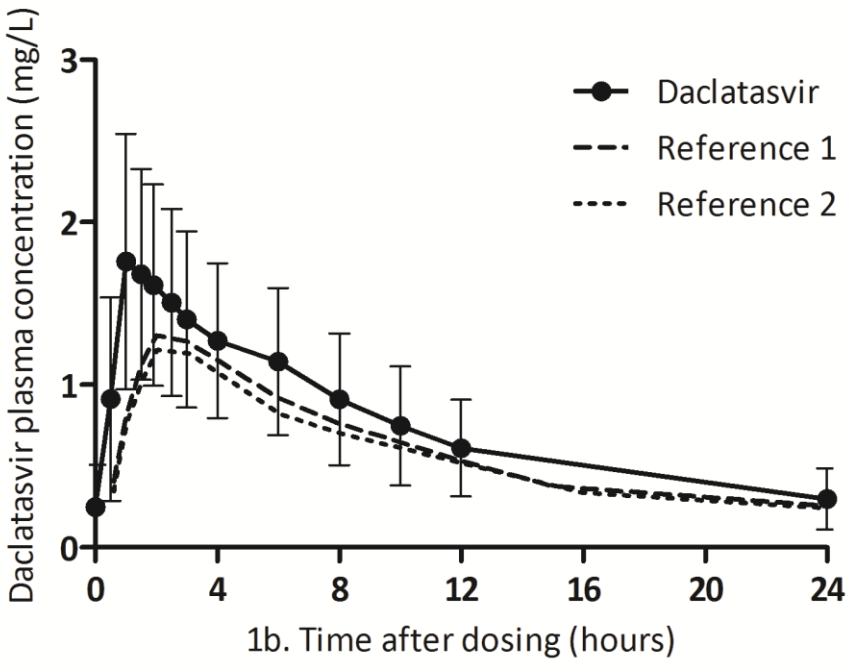


Figure 1 Pharmacokinetic curves of metformin for both treatments (1a). Pharmacokinetic curve of daclatasvir (and reference) (1b). Data shown are geometric means with geometric coefficient of variation. Reference curves for daclatasvir were adapted from Gandhi et al. [24]. Treatment A: data of 19 subjects were used. Treatment B: data of 20 subjects were used.

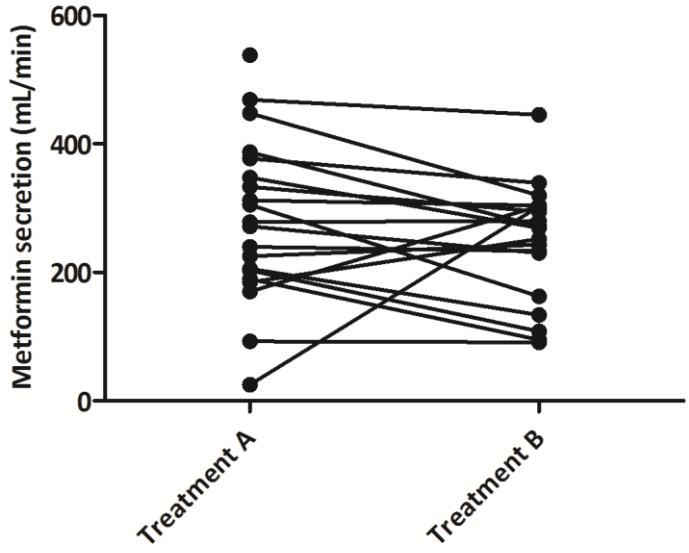


Figure 2 The 12-hour metformin secretion during treatment A and B shown per patient.

The urine of one subject was discarded during the trial; therefore, the metformin secretion of 19 subjects is shown in the Figure.

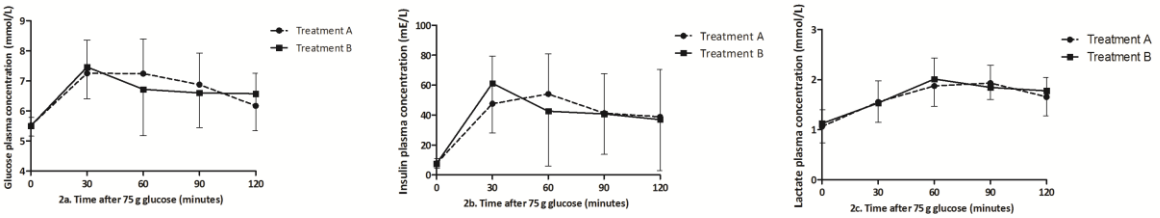


Figure 3 Results of the pharmacodynamic analysis. Glucose (3a), insulin (3b) and lactate (3c) area under the concentration-time curves from 0 to 2 hours are shown. Presented values are geometric means with geometric coefficients of variation (n = 19).

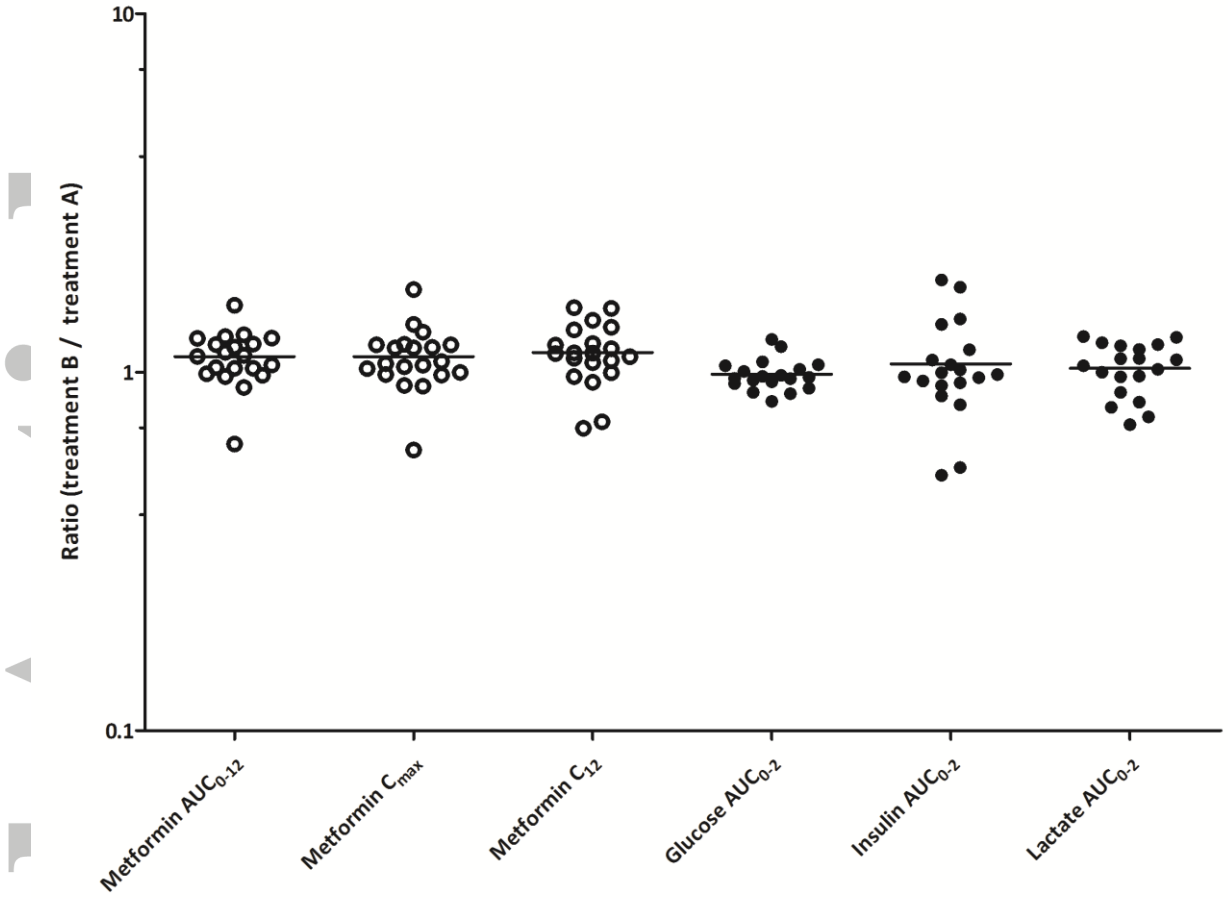


Figure 4 Ratios for treatment B/treatment A shown per subject for metformin area under the concentration-time curves from 0 to 12 hours, metformin maximum plasma concentration, metformin 12-hour plasma concentration, glucose area under the time curves from 0 to 2 hours ($AUC_{0–2}$), insulin $AUC_{0–2}$ and lactate $AUC_{0–2}$.

One subject did not tolerate the oral glucose tolerance test during treatment A; therefore, the ratio of 19 subjects is shown for glucose, insulin and lactate. $AUC_{0–12}$, area under the time curve from 0 to 12 hours; C_{max}, maximum plasma concentration; C_{12}, 12-hour plasma concentration; $AUC_{0–2}$, area under the time curve from 0 to 2 hours**.**

Table 1: Steady-state pharmacokinetic parameters of metformin (n=19) and daclatasvir (n = 20). Geometric means are shown with geometric coefficient of variation. Geometric mean ratios of treatment B (with daclatasvir) versus treatment A (without daclatasvir).

Metformin	**Treatment A (n = 19[a])**	**Treatment B (n = 20)**	**GMR, % (90% CI)**
AUC_{0-12} (h·mg/L)	12.41 (22)	13.54 (25)	109 (102–116)
C_{max} (mg/L)	2.06 (23)	2.23 (23)	108 (101–116)
C_{12} (mg/L)	0.34 (32)	0.38 (29)	112 (103–122)
T_{max} (h)[b]	1.9 (1–2.5)	1.9 (1–3.0)	–
$T_{1/2}$ (h)[c]	4.77 (19)	4.86 (22)	–
Daclatasvir	**Treatment B**	**Reference[d]**	
AUC_{0-24} (h·mg/L)	18.38 (44)	12.7 (41); 13.8 (26)	
C_{max} (mg/L)	1.85 (40)	1.34 (38); 1.41 (28)	
C_{24} (mg/L)	0.30 (63)	0.225 (54); 0.225 (36)	
T_{max} (h)[a]	1 (1–2.5)	2.0 (1.0; 6.0)	
$T_{1/2}$ (h)[b]	11.23 (23)	–	

[a] For treatment a 19 subjects are used for the pharmacokinetic analysis as 1 subject vomited during treatment.

[b] Values presented are medians (range)

[c] The apparent $T_{1/2}$ is calculated

[d] The reference values from Gandhi et al. are presented, showing two studies [24]

AUC_{0-12}, area under the time curve from 0 to 12 hours; AUC_{0-24}, area under the time curve from 0 to 24 hours; C_{12}, 12-hour plasma concentration; C_{24}, 24-hour plasma concentration; 90% CI, 90% confidence interval; C_{max}, maximum plasma concentration; GM, geometric mean; GMR, geometric mean ratio; $T_{1/2}$, elimination half-life; T_{max}, time to reach C_{max}

Table 2: Adverse events (AEs) reported during the trial, per treatment. Only AEs that were reported ≥5.0 % are shown.

	Treatment A (Total number AEs: 53)			Treatment B (Total number AEs: 76)		
	Subjects, n	**AEs, n**	**AEs, %**	**Subjects, n**	**AEs, n**	**AEs, %**
Diarrhoea	10	11	21	12	15	20
Fatigue	5	5	9	4	4	5
Stomach ache/cramps	5	5	9	6	10	13
Nausea	4	4	8	5	7	9
Sore throat	3	3	6	–	–	–
Vomiting	2	3	6	2	2	3
Common cold	3	3	6	–	–	–
Headache	2	2	4	7	8	11

Treatment A: 100 mg metformin twice daily.

Treatment B: 1000 mg metformin twice daily and 60 mg daclatasvir once daily.

AEs: adverse events

Toxicity grades were judged by the trial physician and graded using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (’DAIDS AE GradingTable’); version 1.0, December 2004, clarification August 2009 [23].