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Verapamil decreases the glucose-lowering effect of metformin in healthy volunteers

Sung Kweon Cho,[1,2] Choon Ok Kim,[3] Eun Seok Park[4] & Jae-Yong Chung[5]

[1]Department of Pharmacology, Yonsei University College of Medicine, Seoul, [2]Brain Korea 21 Project for Medical Science, Yonsei University, Seoul, [3]Department of Clinical Pharmacology, Yonsei University Severance Hospital, Seoul, [4]Department of Chemical Engineering, Graduate School of Engineering Yonsei University, Seoul and [5]Department of Clinical Pharmacology and Therapeutics, Seoul National University College of Medicine and Bundang Hospital, Seongnam, Korea

Correspondence
Professor Jae-Yong Chung MD PhD, Seoul National University Bundang Hospital, 173-82 Gumi-ro, Bundang-gu, Seongnam, Kyounggi 463-707, Korea.
Tel.: +82 31 787 3955
Fax: +82 31 787 4045
E-mail: jychung@snubh.org

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Organic cation transporters (OCTs) are known to play an important role in the cellular transport of metformin.
- An *in vitro* study reported that metformin uptake into hepatocytes through OCT1 was inhibited by verapamil.
- To date, there have been no clinical pharmacokinetic or pharmacodynamic drug interaction studies of metformin and verapamil.

WHAT THIS STUDY ADDS

- This study reports a significant pharmacodynamic drug interaction between metformin and verapamil.
- Verapamil remarkably decreases the glucose-lowering effect of metformin, without altering its pharmacokinetics.
- This is likely mediated by competitive inhibition of OCT1.
- Our study suggests that transporter-mediated drug interactions at the site of action are independent of plasma exposure.

AIM

The organic cation transporter 1 (OCT1) plays a key role in the cellular transport of metformin and its subsequent glucose-lowering effect. A recent non-clinical study reported that metformin uptake into hepatocytes is regulated via OCT1, and that uptake was strongly inhibited by verapamil. Therefore, we investigated the effects of verapamil co-administration on the pharmacokinetics and pharmacodynamics of metformin in humans.

METHODS

We evaluated the pharmacokinetics and the anti-hyperglycaemic effects of metformin using an oral glucose tolerance test (OGTT) in 12 healthy participants, before (day 1) and after metformin treatment (day 2), and again on days 15 and 16 after co-administration with verapamil.

RESULTS

Verapamil inhibited the ability of metformin to reduce maximum blood glucose concentrations (ΔG_{max}) by 62.5% ($P = 0.008$) and decreased the area under the glucose concentration–time curve (ΔAUC_{gluc}) by 238% ($P = 0.015$). However, verapamil did not significantly alter the C_{max} and the AUC of metformin, nor its renal clearance.

CONCLUSIONS

Our results suggest that verapamil remarkably decreases the glucose-lowering effect of metformin, possibly by acting as a competitive inhibitor of OCT1.

Introduction

Metformin, the first line oral antidiabetic drug, is believed to be the most frequently prescribed drug for the treatment of type 2 diabetes. The primary mechanism of metformin action is to inhibit glucagon-dependent increases in cAMP and glucose output in hepatocytes [1]. It has also been proposed that metformin enhances glucose uptake in peripheral tissues, thereby decreasing glucose absorption from the gastrointestinal tract [2]. More than 50% of orally administered metformin is absorbed into the blood and eliminated unmetabolized in urine [3]. Its renal clearance is greater than that of creatinine, indicating that tubular secretion is its major route of elimination [3]. However, the clinical efficacy of metformin is variable, and some patients do not show any significant antihyperglycaemic effects [4].

Metformin transport into the liver, kidneys and peripheral tissues is mediated by organic cation transporters (OCTs). OCT1 is primarily expressed in hepatocyte sinusoidal membranes [5], whereas OCT2 is primarily localized in the basolateral membrane of kidney tubules [6]. Metformin uptake by OCTs into the liver and kidney plays an important role in its pharmacokinetics and pharmacodynamics [7, 8].

Verapamil is an L-type calcium channel inhibitor used in the treatment of severe supraventricular tachycardia and hypertension [9]. Verapamil is also a potent inhibitor of P-glycoprotein (P-gp) function [10]. Interestingly, a recent non-clinical study reported that metformin uptake into hepatocytes through OCT1 was strongly inhibited by verapamil [11, 12]. Moreover, the IC_{50} value for verapamil acting on OCT1 was well below the estimated maximal portal vein concentration of verapamil in the liver directly after intestinal uptake ($C_{max,portal}$) [12]. These results suggest that there is a high risk of interaction between metformin and verapamil in the liver, and that co-administration of verapamil with metformin may result in a reduced hepatic uptake of metformin in humans.

In the present study, we hypothesized that the plasma concentration of metformin and its ability to decrease glucose concentrations is affected by verapamil, likely through its ability to inhibit OCT1 competitively. We therefore investigated the effect of verapamil on metformin pharmacokinetics (PK) and pharmacodynamics (PD) using the oral glucose tolerance test (OGTT) in 12 healthy subjects.

Methods

Subjects

Twelve healthy male subjects (age 27 ± 5 years; height 174.5 ± 6.0 cm; weight 70.5 ± 3.9 kg; fasting glucose 87 ± 3 mg dl^{-1}) participated in this study. Exclusion criteria were anaemia (haemoglobin < 12 g dl^{-1}), history of drug abuse, symptomatic coronary heart disease, significant elevation of hepatic enzyme levels (aspartate aminotransferase [AST] or alanine aminotransferase [ALT] > 60 IU l^{-1}), serum creatinine > 1.5 mg dl^{-1} or presentation of any criteria of metabolic syndrome. Subjects who were consuming more than two alcoholic drinks (at one time) twice a week, smoking more than 10 cigarettes per day, or taking any medication were also excluded.

Clinical study procedures

The study protocol was reviewed and approved by the Institutional Review Board of Severance Hospital in the Yonsei University Health System, Seoul, Korea (4–2010-0417, Clinicaltrial.gov: NCT01274130). All procedures were carried out in accordance with the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use-Good Clinical Practice (ICH-GCP) guidelines. Written informed consent for participation was obtained from all subjects before enrolment in the study. The participants were asked to maintain normal physical activity at least 5 days before the study began. Dieticians instructed the subjects regarding the meal plan, which was designed to maintain a carbohydrate intake of 200 to 250 g day^{-1} and instructed them to use a food diary to record food intake for 3 days before admission. The last meal before admission was eaten in the Clinical Trials Centre at Severance Hospital. After an overnight fast, a 3 h oral glucose tolerance test (OGTT) (75 g glucose) was performed at 10.00 h (day 1). The participants received a 1000 mg oral dose of metformin (Diabex Tab; Daewoong Pharmaceutical Co., Seoul, Korea) 10 h later. After fasting overnight, a 750 mg dose of metformin was administered at 08.00 h on day 2, followed by a second OGTT 2 h later. Blood and urine samples were collected to determine the pharmacokinetics of metformin. Carbohydrate-controlled meals were provided 5 h after the second metformin dose on day 2. Subjects were discharged on the morning of day 3. After 11 days without treatment, the subjects began the verapamil regimen (Isoptin SR Cap.; Ilsung Pharmaceutical Co., Seoul, Korea) (180 mg day^{-1}) for 3 days (day 14). The subjects were admitted to the Clinical Trials Centre the following day (day 15) for 2 days, where they were given verapamil, co-administered with metformin. Participants were instructed to restart the carbohydrate diet and maintain a food diary on day 13. The second OGTT tests, metformin and verapamil administration, and blood and urine collection were carried out according to the same protocol used from day 1 to day 3.

Blood and urine collection

For OGTT analysis, blood samples were collected before the ingestion of glucose and at 15, 30, 45, 60, 90, 120, 150 and 180 min after ingestion. To determine metformin concentrations in the plasma, blood samples were collected

before the second dose of metformin and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, 12 and 24 h after. After the second dose of metformin, participants were asked to drink 240 ml water every 4 h in order to maintain urine flow. The first portion of urine was voided and the subsequent samples were collected during the following time intervals: 0–4, 4–8, 8–12 and 12–24 h after the second dose of metformin. The volume and pH of urine were recorded before analysis. In order to calculate the creatinine clearance (CL_{cr}), the serum creatinine concentration was determined from a blood sample (3 ml) drawn before each of the admission times.

Metformin concentration analysis

Metformin concentrations in plasma and urine were determined using the highly specific and sensitive method of liquid chromatography-tandem mass spectrometry (API 3200; Applied Biosystems Sciex, Ontario, Canada). Samples were prepared for analysis by mixing an aliquot of the plasma or urine specimen with acetonitrile in the presence or absence of the internal standard formoterol. The mixture was vortexed for 5 min, and then centrifuged for 5 min at 10 000 rev min^{-1}. An aliquot of the supernatant was transferred to an autosampler vial and 1 µl was injected onto the column at 10°C. The mobile phase consisted of 75% acetonitrile, 25% double-distilled water and a 5 mM ammonium formate aqueous solution. The limit of quantification was 10 ng ml^{-1} in plasma and 0.5 µg ml^{-1} in urine. The intraday and interday coefficients of variation were <10%.

Glucose concentration analysis using an oral glucose tolerance test

Participants were on a carbohydrate-controlled diet (200–250 g day^{-1}) for 3 days prior to admission. Before conducting the first OGTT (10.00 h on day 1), all subjects had fasted for more than 14 h. Metformin lowers glucose production in patients with diabetes [13] and exerts the same effect in healthy subjects if their serum glucose concentrations are increased by glucose ingestion [7]. The OGTT was conducted four times: before and after metformin treatments, prior to the first verapamil administration (day 1 and day 2), and after verapamil administration (day 15 and day 16). The maximum glucose concentration (G_{max}) was determined and the area under the serum glucose concentration–time curve (AUC_{gluc}) was calculated using the trapezoidal rule. AUC_{gluc60} was defined as the area under the glucose curve from 0 to 60 min after glucose ingestion, which was the period during which plasma glucose concentration increased. The difference between G_{max} and AUC_{gluc} before and after metformin administration (ΔG_{max} and ΔAUC_{gluc}) is considered to be the glucose lowering action of metformin [14]. The effect of verapamil on the glucose-lowering action of metformin was calculated as the differences in ΔG_{max} and ΔAUC_{gluc} values (ΔG_{max}, ΔAUC_{gluc60}, and ΔAUC_{gluc}) before and after verapamil administration (on days 1 and 2 and again on days 15 and16) in each subject.

Pharmacokinetics

The pharmacokinetic parameters were calculated by non-compartmental analysis using Phoenix WinNonlin 6.1 (Pharsight Corporation, Mountain View, CA, USA). The maximum metformin concentration (C_{max}) and the time required to reach the maximum concentration (t_{max}) were determined. The area under the plasma metformin concentration–time curves (AUC_{met}) from 0 to 24 h was calculated using the linear trapezoidal rule. The elimination rate constant (k_e) was estimated from the slope of the best-fit line determined by linear regression analysis of the log-transformed concentration–time curve. The elimination half-life ($t_{1/2}$) was then calculated from the equation $t_{1/2} = \ln(2)/k_e$. The apparent volume of distribution, V_z/F, was calculated using the formula dose/λ_z*AUC(0,∞). The clearance of metformin (CL_R) was calculated as the total amount of metformin excreted in urine over 24 h divided by AUC_{met}. The clearance of creatinine (CL_{cr}) was calculated from the Cockcroft–Gault equation ([140–age] × (body weight, kg) × (0.85 if female)/(72 × serum creatinine). Metformin secretion clearance ($SrCL_R$) was calculated by subtracting CL_{cr} from CL_R.

Statistical analysis

Measurements from the same subjects before and after verapamil treatment were compared using the Wilcoxon signed-rank test. The data were analyzed using SPSS v.20.0 (IMB Corp., Armonk, NY, USA). Data were expressed as mean values ± standard deviation (SD). $P < 0.05$ was considered significant. The general linear model was used to evaluate the effects of metformin, verapamil and their interaction on the glucose parameters.

Results

Glucose-lowering effect of metformin

Before the first metformin dose, verapamil did not alter the baseline serum glucose concentration. In the absence of metformin administration, G_{max} and AUC_{gluc} were not significantly affected by verapamil treatment (147 ± 17 mg dl^{-1} and 141 ± 11 mg dl^{-1} before and after verapamil treatment, respectively, $P = 0.158$; 18 791 ± 1521 mg dl^{-1} min and 18 381 ± 1768 mg dl^{-1} min, $P = 0.347$). However, the glucose-lowering effect of metformin was considerably reduced by co-administration of verapamil (Figure 1). The ability of metformin to reduce ΔAUC_{gluc60} and AUC_{gluc} was compared with and without verapamil co-treatment (Table 1). Verapamil treatment decreased ΔG_{max} by 62.5% (16 mg dl^{-1} and 6 mg dl^{-1} with or without verapamil co-administration, $P = 0.010$) and

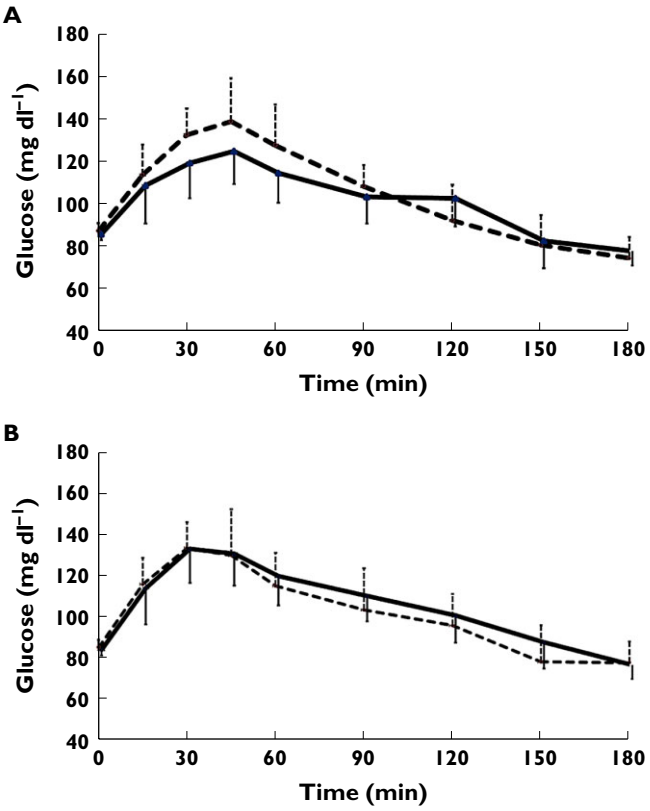

Figure 1

Serum glucose concentrations were determined by OGTT before and after metformin administration. (A) Serum glucose profile without verapamil administration (B) Serum glucose profile with verapamil administration. Data are expressed as mean ± SD ($n = 12$). (▬) before metformin; (▬) after metformin

the ΔAUC_{gluc60} by 101% (594 ± 500 mg dl^{-1} min and −6 ± 556 mg dl^{-1}·min, $P = 0.008$). Verapamil also decreased the ΔAUC_{gluc} by 238% (509 ± 1224 mg dl^{-1} min *vs.* −702 ± 1103 mg dl^{-1} min, $P = 0.015$). Based on the difference in the glucose lowering effect of the two groups, the power was calculated above 90% (93% and 97% for ΔG_{max} and ΔAUC_{gluc}, respectively). By general linear model analysis, the interacting effects of metformin and verapamil appeared to be significant or close to significance (P values for G_{max}, AUC_{gluc}, and AUC_{gluc60} were 0.06, 0.054, and 0.015, respectively), while the effects of verapamil were not significant, and those of metformin were significant for G_{max} ($P = 0.0003$) and AUC_{gluc60} ($P = 0.017$).

Metformin pharmacokinetics

The metformin plasma concentration profiles before and after verapamil treatment are shown in Figure 2. The AUC from the period 0–24 h (AUC_{met}) and the maximum metformin concentration (C_{max}) were consistent with previous studies [7, 14–16].

After verapamil treatment, the pharmacokinetic parameters of metformin were not significantly altered as shown in Table 2. Verapamil did not significantly alter the $t_{1/2}$ of metformin, and did not significantly decrease its renal clearance (CL_R), or its net tubular secretion ($SrCL_R$). Overall, the pharmacokinetics of metformin were not affected by verapamil co-administration.

Discussion

In the present study, we found that verapamil considerably inhibited the glucose-lowering effect of metformin. Verapamil may inhibit metformin action and/or increase glucose concentrations through its own pharmacological action. Verapamil could increase glucose concentrations at high doses by blocking pancreatic L-type calcium channels and by increasing insulin resistance at the cellular level [17]. However, when comparing the baseline glucose concentrations before and after verapamil treatment (G_{max} and AUC_{gluc} on day 1 *vs.* day 15 before metformin administration), we did not observe any verapamil-induced hyperglycaemia. These results suggest that verapamil inhibited the glucose-lowering effect of metformin. To our knowledge, this is the first study to report a substantial pharmacodynamic drug interaction between verapamil and metformin in humans.

Verapamil significantly inhibited the pharmacodynamic effects of metformin, but had no effect on the pharmacokinetics, which is consistent with our hypothesis that verapamil may act as a competitive inhibitor of OCT1-mediated metformin transport [11]. Five amino acids in the innermost cavity of OCT1 constitute the substrate binding cleft [18] and cysteine is critical for binding and subsequent transport [19]. We hypothesize that when these two organic cationic drugs are administered simultaneously, the binding of verapamil to OCT1 prevents metformin binding and transport.

The plasma concentration of metformin was not altered after verapamil treatment. The concentration of metformin in the liver is relatively lower than the concentration in blood [14], which is consistent with our results showing that the verapamil interaction with OCT1 in the liver does not affect the systemic exposure of metformin and its renal clearance. Wang *et al.* showed that the systemic exposure to metformin was not altered in an OCT1 knockout mouse, as compared with a WT mouse, even though the urinary excretion and the volume of distribution were significantly different [20]. A change in absorption or in the kinetics of elimination is unlikely, as there is no evidence that verapamil is a substrate for OCT2 or any other absorption transporter, such as the plasma monoamine transporter (PMAT) [21]. Although not significant in this study, the apparent volume of distribution (V_z/F) of metformin decreased after verapamil treatment (243.6 ± 76.2 l *vs.* 188.1 ± 74.2 l). We therefore postulate that verapamil may decrease the distribution of metformin or increase its bioavailability by limiting its distribution

Table 1

The glucose-lowering effect parameters of metformin with and without verapamil treatment in healthy participants (n = 12)

	Without verapamil	With verapamil	Difference mean (95% confidence interval)	P
ΔG_{max} (mg dl^{-1})	16 ± 13	6 ± 10	10 (3, 18)	0.010
ΔAUC_{gluc60} (mg dl^{-1} min)	594 ± 500	–6 ± 556	600 (248, 952)	0.008
ΔAUC_{gluc} (mg dl^{-1} min)	509 ± 1224	–702 ± 1103	1211 (399, 2024)	0.015
G_{max} (mg dl^{-1}) before metformin	147 ± 17	141 ± 11	6 (–3, 15)	0.158
AUC_{gluc} (mg dl^{-1} min) before metformin	18,791 ± 1521	18,381 ± 1768	410 (–621, 1439)	0.347

Data were evaluated using a Wilcoxon signed-rank test and expressed as mean ± SD. ΔG_{max}, difference in maximum glucose concentration before and after metformin treatment; ΔAUC_{gluc60}, difference in partial glucose AUC (0 to 60 min after ingestion, during which glucose concentration increases) before and after metformin treatment; ΔAUC_{gluc}, difference in total AUC_{gluc} before and after metformin treatment.

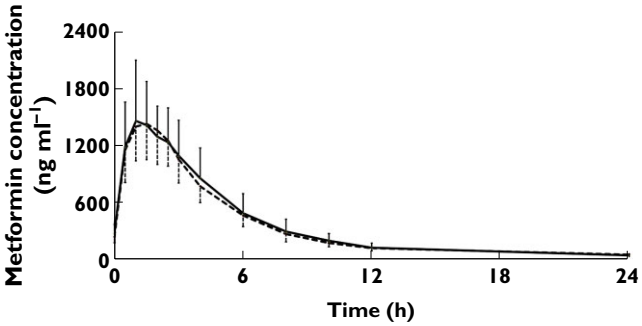

Figure 2

The plasma concentration–time curve of metformin on day 2 (without verapamil treatment) and day 15 (with verapamil treatment). Metformin concentrations were measured after the second dose of metformin. **--**, without verapamil; **—**, with verapamil

into central compartments, such as the liver, or by reducing its hepatic extraction. Both mechanisms could be the result from inhibition of OCT1-mediated hepatic transport. Other transporters such as OATP1A2 [22] or OCTN1 [23] may be involved, but the affinity of these transporters for metformin is relatively small and the IC_{50} of these transports for verapamil is relatively large compared with OCT1 [22]. Thus, the contribution of these transporters is not likely to be involved in verapamil-mediated inhibition of metformin activity. Our previous study showed the positive effect of rifampicin on OCT1 expression and metformin-mediated decreases in glucose [14]. Combined with our present study, these data suggest OCT1-mediated transport is critical for metformin action. Patients with diabetes may also take probiotics or other medications, such as antihypertensive drugs. These drugs may affect the clinical efficacy of metformin. Moreover, it has been shown that the metformin-mediated decrease in glucose is impaired in individuals expressing genetic variants of OCT1 [7]. Therefore, further study is needed to characterize the effect of transporters polymorphism (OCT1, OCT2, MATE1, and MATE2-K), as well as other co-administrated drugs, on metformin-mediated decreases in glucose [24–26].

There are some limitations in the present study. First, verapamil is a strong inhibitor of other transporters, including P-glycoprotein (P-gp) and the multidrug and toxin extrusion (MATE) transporter [10, 27, 28]. There is no evidence to suggest that metformin is a substrate for P-gp or that P-gp-inhibits the metformin-mediated decrease in glucose. Metformin is a substrate for the MATE transporter, and inhibition by verapamil may affect its glucose-lowering action [29, 30]. However, since MATE is expressed in the apical membrane of hepatocytes, the enhanced glucose lowering effect of metformin is likely due to inhibition of MATE. These data support our hypothesis that the uptake of metformin into hepatocytes could be decreased due to OCT1 inhibition and suggests that inhibition of transport to the bile duct does not occur. Further, as verapamil does not affect the pharmacokinetics of metformin, it is less likely that it interacts with other metformin transporters. A second limitation is that the results obtained from healthy volunteers may differ from those in the general population or diabetic patients. This prospective study was designed to minimize the effect of confounding factors and clearly shows that, although verapamil does not affect basal serum glucose concentrations, it considerably inhibits metformin action. Since metformin was recently proposed to be an inhibitor of glucagon-dependent glucose production [1], the effect of verapamil on metformin in diabetic patients may differ, based on the status of glucagon. However, the action of metformin is mediated after the uptake of metformin into intracellular components of the hepatocyte. Since the effect of verapamil on OCT1 takes place at the cellular membrane level, we assume that there will not be a difference in the drug–drug interactions between healthy people and diabetic patients, unless the hepatic expression level of OCT1 is different. A small scale study in diabetic patients is needed to evaluate this interaction mechanism. Finally, further investigation is needed to correlate our results with verapamil binding to OCT1 *in vivo* and its subsequent inhibition of OCT1-mediated metformin transport into

Table 2

Pharmacokinetic parameters of metformin in healthy participants (n = 12) with and without verapamil treatment

	Without verapamil	With verapamil	Difference mean (95% confidence interval)	P
AUC_{met} (ng ml^{-1} h)	8,221.6 ± 1,755.6	8,835.3 ± 2,432.0	–613.7 (–1990.3, 762.9)	0.530
$t_{1/2}$ (h)	7.66 ± 2.15	6.15 ± 2.14	1.51 (0.02, 3.00)	0.060
C_{max} (ng ml^{-1})	1,510.7 ± 365.9	1,640.7 ± 599.0	–130.1 (–467.1, 206.9)	0.754
t_{max} (h)	1.5 [1.0–2.5]	1.5 [0.5–3.0]	0 (–)	0.324
CL_R (ml min^{-1})	662.0 ± 139.7	649.6 ± 92.4	12.4 (–61.2, 86.0)	0.718
$SrCL_R$ (ml min^{-1})	562.8 ± 137.1	553.0 ± 91.1	9.8 (–65.8, 85.3)	0.781
CL_{cr} (ml min^{-1})	99.2 ± 14.2	96.5 ± 15.0	2.7 (–4.4, 9.7)	0.432
V_z/F (l)	243.6 ± 76.2	188.1 ± 74.2	55.5 (4.8, 106.2)	0.050

Data were evaluated using Wilcoxon signed rank test and expressed as mean ± SD. AUC_{met}, area under the plasma concentration–time curve from 0 to 24 h; $t_{1/2}$, elimination half-life; C_{max}, maximum plasma concentration; t_{max}, time of maximum plasma concentration; CL_R, renal clearance; $SrCL_R$, renal clearance by tubular secretion; CL_{cr}, creatinine clearance; V_z/F, apparent volume of distribution.

hepatocytes. Intra-hepatocyte molecular imaging or measuring portal vein concentrations will be considered in future studies.

In conclusion, we found that verapamil decreased the ability of metformin to lower glucose concentrations, without affecting its pharmacokinetics. Verapamil most likely acts as a competitive inhibitor of OCT1, preventing the transport of metformin into the liver. Verapamil–metformin interactions in patients with hypertension and type 2 diabetes may affect the efficacy and safety of the drugs. This drug–drug interaction may also alter metformin pharmacokinetics and pharmacodynamics in individuals expressing OCT1 genetic variants. A clinical study is necessary to assess OCT-based drug interactions and related genetic polymorphisms in patients with type 2 diabetes receiving metformin.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organizations for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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Author Contributions

Participated in research design: SKC, COK, J-YC.
Conducted clinical study: SKC, COK, J-YC.
Performed data analysis: SKC, J-YC.
Performed metformin analysis: ESP.

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