Full paper

The effects of rabeprazole on metformin pharmacokinetics and pharmacodynamics in Chinese healthy volunteers

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ABSTRACT

The aim was to investigate the role of rabeprazole on the pharmacokinetics (PK) and pharmacodynamics (PD) of metformin. The in vitro inhibition assays on metformin transport were carried out and showed that the half maximal inhibitory concentration (IC50) of rabeprazole on OCT2-mediated metformin transport was 26.0 μM, whereas the IC50 on MATE1-mediated metformin transport inhibition was 4.6 μM. Fifteen healthy Chinese male volunteers were enrolled and given two different doses of metformin plus the co-administration of placebo or rabeprazole. Plasma concentrations of metformin were measured up to 12 h after the second dose. The glucose-lowering effects and the variation of insulin concentrations during the oral glucose tolerance test (OGTT). The AUC0-12 of metformin plus placebo was 28,276 ± 5187 ng/ml h, which was significantly higher than AUC0-12 of metformin plus placebo (24,691 ± 3129 ng/ml h). Thus, rabeprazole can modestly influence the PK of metformin, suggesting the precaution of using the two drugs together. In OGTTs, rabeprazole decreased the values of AUC insulin and the maximum insulin concentration. Although rabeprazole showed inhibition effect on OCT2-mediated metformin transport, the glucose-lowering effect of metformin remained the same regardless of its PK changes. Further studies are needed to warrant the effect of rabeprazole on metformin. © 2016 Japanese Pharmacological Society. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Metformin is an insulin-sensitizing agent, widely used in the treatment of type 2 diabetes mellitus (DM), as well as in non-alcoholic fatty liver disease (NAFLD) (1–4). Metformin down-regulates blood glucose through increasing the insulin sensitivity in muscle, improving the efficiency of the lactate oxidative and enhancing the proportion of glucose oxidation (5). Additionally, it can decrease hepatic glucose output and gluconeogenesis (6). It was even reported that metformin could be used to treat polycystic ovary syndrome (PCOS) via the regulation of pituitary gonadotropin secreting cells (7) and protect against carbon tetrachloride induced hepatotoxicity (8). During the last decade, increasing evidence have suggested that metformin plays an positive role in cancer treatment and prevention (9,10).

Metformin is a substrate of organic cation transporters (OCTs) and multidrug and toxin extrusion 1 (MATE1) transporter, which localized at liver and gastrointestinal or renal epithelium responsible for drug absorption and excretion (11,12). Metformin does not undergo metabolism in the liver or other organs and is excreted unchanged through kidney tubules (13). Renal dysfunction or drug–drug interactions (DDIs) through the inhibition of OCTs and MATE1 transporters could cause the accumulation of metformin and increase the risk of lactic acidosis (14). Based on computational analysis and high-throughput assay, Y. Kido et al. (15) had discovered a number of drugs, including rabeprazole that have a large inhibition potency on OCT2. Moreover, Nies et al. (16) found that rabeprazole might influence the uptake of metformin by inhibiting OCT1, OCT2, and OCT3 in a concentration-dependent manner, using in vitro transporting assay (IC50 = 3.0, 5.7, and 3.0 μM, respectively).
Clinically, about 15%–30% patients experienced gastrointestinal side effects during metformin therapy (17). Rabeprazole was prescribed to treat gastrointestinal diseases, particularly gastroesophageal reflux. Therefore, co-administration of rabeprazole and metformin is frequently occurred for diabetic patients. However, there was no data about the PK and PD variation in Chinese after co-administration of these two drugs. Here, we recruited 15 Chinese healthy volunteers to illustrate the possible influence of PK and PD of metformin after co-administration of rabeprazole, which would be indicative for doctors to prescribe these two drugs properly.

2. Materials and methods

2.1. Chemicals and reagents

The Flp-In transfection system, Dulbecco’s modified Eagle’s medium (DMEM), Lipofectamine 2000, hygromycin, Opti-MEM reduced serum medium, TRizol, and fetal bovine serum were purchased from Invitrogen. The pcDNA5-hOCT2 and -hMATE1 plasmids were already constructed in the laboratory of Yan Shun from University of Maryland. All HEK-293 Flp-In cells stably expressing these transporters were established by selection against hygromycin (75 µg/ml) according to the Flp-In transfection system instruction (Invitrogen). [14C]-metformin was purchased from Moravek Biochemicals Inc. (Brea, CA). Rabeprazole and unlabeled metformin were obtained from Sigma Chemical Co. LLC. (St. Louis, MO).

2.2. In vitro inhibition of metformin transport by rabeprazole

HEK-293 cells stably overexpressing transporters and mock HEK-293 cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, and 75 µg/ml hygromycin, and were maintained in 25-cm² plastic flasks at 37 °C in a humidified atmosphere with 5% CO₂. Twenty-four hours before the uptake experiments, cells were digested with trypsin containing 0.25% EDTA and counted. 2 × 10⁵ Cells were plated in each well. The protocol of uptake experiment was according to the study of Qing Li (18) and briefly indicated as following. During the experiment of OCT2 inhibition, the cells were washed once with pre-warmed KRH buffer and then incubated with KRH buffer containing different concentrations of rabeprazole with 10 µM [14C]-metformin plus 40 µM unlabeled metformin for 10 min. The uptake was halted by removing the KRH buffer and washing the cells with ice-cold KRH buffer for 3 times. For the inhibition of MATE1 transport, the cells were firstly washed with pre-warmed K⁺ based buffer and then incubated in K⁺ based buffer containing 30 mM NH₄Cl for 10 min at 37 °C, and thereafter incubated in the uptake buffer (NH₄Cl-free) for another 5 min. The culture medium was then changed to the uptake buffer containing a series of concentrations of inhibitors with 10 µM [14C]-metformin plus 40 µM unlabeled metformin for 10 min and the assays were stopped by addition of ice-cold substrate-free uptake buffer, and the cells were washed 3 times. At last, 300 µL 0.5 N sodium hydroxide (NaOH) were added in each well, and thereafter the plate was shaked for 30 min and added 0.5 N hydrochloric acid (HCL) to neutralize the buffer. 300 µL cell lysate was transferred to scintillation tube containing 3 mL Biodegradable Counting Cocktail buffer (Fisher Scientific Inc., Pittsburgh, PA). Radioactivity was counted by a multi-purpose scintillation counter (Beckman LS6500 Counter, Brea, CA). Protein concentrations were measured using a BCA protein assay kit (Bio-Rad Co. Hercules, CA), which was used for normalizing radioactivity values.

2.3. Subjects

Our study protocol was approved by the Ethics Committee Institute of Clinical Pharmacology, Central South University (Project No: CTXY-140012). Fifteen healthy Chinese males were enrolled in the study, and all of them provided informed written consent before enrollment. The baseline characteristics of subjects were: age, 25.3 ± 6.5 years; height, 172.2 ± 4.5 cm; and weight, 76.9 ± 9.7 kg (mean ± SD). Subjects with anemia, abnormal of hepatic enzyme level, serum creatinine level>1.50 mg/dl, history of drug abuse, or presentation of any one of the criteria for metabolic syndrome were excluded from our study. Also excluded were those consuming more than two drinks a week, smoking more than ten cigarettes a day, or taking any medication in the past 3 months.

2.4. Clinical study design

This was a randomized, two-crossover study with a 14-day wash out period. In each phase, fifteen volunteers randomly received placebo or rabeprazole (20 mg, 10 mg per tablet) orally from day-1 to day-7 at 8:00 AM to attain the steady state concentration (Misato Plant of Eisai Co., Ltd, Xiangya Hospital of Central South University, ChangSha, China) and two doses of metformin (250 mg per tablet; Neptunus Pharmaceutical Co., Ltd, Changsha, China). The first dose of metformin (1000 mg) was taken orally on day-6 at 20:00 PM, and the second dose (750 mg) metformin together with rabeprazole or placebo was given on day-7 at 8:00 AM with a 12 h interval (18,19). In each period, the meal was supplied by the Clinical Trials Center at Hunan Cancer Hospital. Two hours after the last dose of metformin, oral glucose tolerance test (OGTT) was performed following the injection of 75 g glucose.

2.5. Sample collection

After drug administration, blood samples (5 ml per time point) for measuring plasma metformin concentrations were collected immediately before and 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10.0, 12.0 h. Blood samples for determining plasma glucose and insulin concentration were collected immediately before and 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 3.0 h after glucose ingestion. After collection, the blood samples were centrifuged in 3000 rpm for 10 min in 4 °C, and all the plasma samples were separated and frozen at –80 °C pending assaying.

2.6. Metformin plasma concentration analysis

Metformin assaying was performed on a Shimadzu LC-2010C HPLC system (Kyoto, Japan) with autosampler and ultraviolet detector. Simple protein precipitation was carried out by adding 400 µl acetonitrile with coumarin as internal standard into 200 µl plasma. The mixture was vortexed for 10 min and centrifuged at 16,000 g for 10 min. An aliquot of the supernatant was transferred to a vial, and 20 µl of this sample solution was injected into the HPLC-UV system column (4.60 mm x 200 mm, i.d., 5 µm, Hypersil BDS C₁₈ column) with UV detection at 232 nm. The mobile phase was composed of 34% acetonitrile and 66% aqueous phase with the flow rate at 1.00 ml/min. Maximum metformin concentration (Cmax) and the time of maximum concentration (Tmax) were determined, and the area under the metformin plasma concentration curve (AUC) was calculated by the linear trapezoidal rule. The elimination rate constant (Ke) 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 (t1/2) was calculated from the equation t₁/₂ = ln(2)/Ke.
2.7. Statistics analysis

Paired Sample T test was used to analyze the PK and PD data of metformin with placebo or rabeprazole. SPSS v.17.00 (IBM Corp., Armonk, NY, USA) software was used here. And all data were mean values ± standard deviation (SD), p < 0.05. Apparent IC50 values for inhibition of metformin uptake by rabeprazole were calculated for each individual experiment by fitting the values to the Hill equation using GraphPad Prism 5 (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

3.1. In vitro inhibition of OCT2- or MATE1-mediated metformin transport

Recently, Nies et al. (16) found that rabeprazole is not the substrate of OCT2 but the inhibitor of OCT2. It was reported that rabeprazole can inhibit OCT2-mediated metformin transport with IC50 at 5.7 μM. However, the data from our study showed that the IC50 value of rabeprazole on OCT2-mediated metformin transport inhibition was 26.0 μM, whereas the IC50 value on MATE1-mediated metformin transport inhibition was 4.6 μM (Fig. 1).

3.2. Effects of rabeprazole on the plasma concentration of metformin

The plasma concentrations of metformin co-administered with placebo or rabeprazole were shown in Fig. 1, and Table 1 showed that AUC0–12 of metformin co-administered with rabeprazole increased by 14.5% (24,690 ± 3129 vs 28,276 ± 5,186, p = 0.001) compared with placebo (see Fig. 2). The difference in AUC0–6 and AUC0–12 also had statistical significance (AUC0–6 increased by 15.9%, p = 0.001; AUC0–12 increased by 14.1%, p = 0.005). The Cmax value of metformin plus rabeprazole also increased by 18.6%, whereas the Tmax and T1/2 value were not significantly altered.

3.3. Effects of rabeprazole on the glucose-lowering effect of metformin

The plasma glucose concentrations after metformin administration were determined and showed in Fig. 3. The maximum glucose level (Gmax) and the area under the plasma glucose concentration–time curve (AUCglucose) of metformin plus placebo or rabeprazole group were characterized using the trapezoidal rule. However, no significant differences were found in Gmax or AUCglucose between placebo and rabeprazole group (Table 2).

3.4. The effect of rabeprazole on metformin in lowering insulin concentration

Insulin concentration in peripheral blood was detected and showed in Fig. 4. The maximum insulin concentration (INmax) and the area under the serum insulin concentration–time curve (AUCinsulin) were also characterized. Compared with the group of metformin plus placebo, rabeprazole can significantly decrease the value of both INmax and AUCinsulin by 20.4% (Table 2).

4. Discussion

In the past decade, the understanding of the role of drug transporters on PK gives us more insights on the causes of drug–drug interactions. Besides, pharmacogenomics study of drug transporter elucidate that gene mutations also play a role in drug PK and PD. The longer-term use of metformin will cause several adverse reactions. In another aspect, the co-administration of drugs, that inhibit its intestinal absorption or the uptake of metformin will cause several adverse reactions. Moreover, clinical studies in healthy population showed that co-administration of OCTs inhibitors can impact PK and PD of metformin (23,24).

Fig. 1. Inhibition of metformin uptake by rabeprazole. A. HEK-293 Flp-In cells stably expressing hOCT2. B. HEK-293 Flp-In cells stably expressing hMATE1. The cells were incubated with 10 μM [14C]-metformin for 10 min at 37 °C in the presence of various concentrations of metformin. Each point represents the mean ± SD of three monolayers from a typical experiment in several separate experiments.

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Metformin + placebo</th>
<th>Metformin + rabeprazole</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0–12 (ng/ml h)</td>
<td>24,691 ± 3129</td>
<td>28,276 ± 5,186</td>
<td>0.001</td>
</tr>
<tr>
<td>AUC0–6 (ng/ml h)</td>
<td>17,855 ± 2445</td>
<td>20,690 ± 3692</td>
<td>0.001</td>
</tr>
<tr>
<td>AUC0–12 (ng/ml h)</td>
<td>6801 ± 1399</td>
<td>7762 ± 1727</td>
<td>0.005</td>
</tr>
<tr>
<td>Cmax (ng/ml)</td>
<td>4220 ± 766</td>
<td>5003 ± 1023</td>
<td>0.001</td>
</tr>
<tr>
<td>Tmax(h)</td>
<td>1.23 ± 0.45</td>
<td>1.50 ± 0.68</td>
<td>0.164</td>
</tr>
<tr>
<td>t1/2(h)</td>
<td>4.56 ± 0.84</td>
<td>4.46 ± 0.94</td>
<td>0.635</td>
</tr>
</tbody>
</table>

The data shown for each parameter are mean ± SD: AUC12–30 area under the plasma concentration–time curve from time point a to time point b.
Impede the metformin transporting in intestine via the inhibition of OCT3 and PMAT were localized on the apical membrane of basal and apical layer of renal tubules, respectively, responsible for the excretion of metformin from plasma to urine. The change of alkaline environment indicates clinically significant role of OCTs (16). In our study, it was found that rabeprazole can strongly inhibit the transporting ability of OCTs (16). OCT2 and MATE1 are abundantly expressed on the basal membrane of heptocytes and may contribute to the translocation of metformin into heptocytes. It is possible that rabeprazole can reduce the accumulation of metformin in liver and influence the effects of its glucose-lowering.

In our study, although the PK of metformin was changed by rabeprazole, there was no alteration in the glucose-lowering effects of metformin between the groups co-administrated with placebo and rabeprazole. The similar results in OGTTs were also found in healthy subjects co-administrated with lansoprazole (24). In human body, especially the healthy individuals, the control of blood glucose concentration is an extremely complex process on which the influence of metformin is slender. However, it was interesting to find in our study that the plasma insulin concentrations during OGTTs were significantly lower in subjects treated with rabeprazole than placebo. To our knowledge, metformin alone can enhance the sensitivity of insulin and thereafter the insulin lowering effect of insulin, insulin levels, thereafter, decreased through the feedback regulation when rabeprazole was co-administrated. Also, this is a conjecture that rabeprazole may reduce the secretion of insulin, which need to be proved by the investigation of rabeprazole and metformin on plasma insulin in both healthy and diabetic populations.

### Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Metformin + placebo</th>
<th>Metformin + rabeprazole</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUCgly(0-3) (mmol/L h)</td>
<td>16.86 ± 2.08</td>
<td>17.19 ± 2.83</td>
<td>0.506</td>
</tr>
<tr>
<td>Cmaxgly (mmol/L)</td>
<td>7.21 ± 1.13</td>
<td>7.26 ± 1.01</td>
<td>0.824</td>
</tr>
<tr>
<td>AUCinsulin(0-3) (μIU/mL h)</td>
<td>148.2 ± 99.5</td>
<td>117.9 ± 68.4</td>
<td>0.013</td>
</tr>
<tr>
<td>Cmaxinsulin (μIU/mL)</td>
<td>86.8 ± 44.5</td>
<td>69.1 ± 32.9</td>
<td>0.002</td>
</tr>
</tbody>
</table>

The data shown for each parameter are mean ± SD.
There are also some limitations in our study. Firstly our study was only performed in healthy Chinese volunteers, whereas Samuel et al. (26) certified that the metformin PK-PD relationship had altered a lot in patients with type 2 DM by comparing with healthy subjects. Therefore, the study should be repeated in patients with type 2 DM. Furthermore, the urine samples from subjects after drug dosing were not collected. The urine clearance of metformin in placebo and rabeprazole co-administrated groups cannot be compared. As both metformin and rabeprazole are orally administered, it is necessary to further investigate whether rabeprazole can inhibit the PMAT-mediated metformin transport.

In summary, the combination of rabeprazole changed the pharmacokinetic feature of metformin, probably because of its inhibition on the renal metformin excretion. Although there was no obvious difference on the change of blood glucose levels between metformin plus placebo and rabeprazole during the OGTT tests, the levels of insulin was much lower in subjects treated with metformin plus rabeprazole. However, more investigations are needed to reveal the underlying mechanism of rabeprazole on insulin excretion.

Conflicts of interest

The authors declared there were no interest conflicts.

Acknowledgments

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