Drug Metabolism and Pharmacokinetics 31 (2016) 385–388

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: http://www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Short communication

Involvement of organic cation transporter 3 (Oct3/Slc22a3) in the bioavailability and pharmacokinetics of antidiabetic metformin in mice

Yoshiyuki Shirasaka [a, b], Nora Lee [a], Weibin Zha [a], David Wagner [a], Joanne Wang [a, *]

[a] Department of Pharmaceutics, School of Pharmacy, University of Washington, H272 Health Sciences Building, Seattle, WA 98195-7610, USA
[b] Department of Biopharmaceutics, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form
6 April 2016
Accepted 21 April 2016
Available online 30 April 2016

Keywords:
Metformin
OCT3
Transporter
Intestinal absorption
Bioavailability
Mouse

ABSTRACT

Metformin is widely used for the treatment of type II diabetes mellitus. It was reported to be substrate of OCT3/Oct3, which is expressed in the brush boarder membrane of the enterocytes. However, the role of OCT3/Oct3 in the intestinal absorption process of metformin remains obscure. In the present study, we aimed to clarify the impact of Oct3 on the oral bioavailability and pharmacokinetics of metformin in mice, by means of *in vivo* pharmacokinetic study using wild-type ($Oct3^{+/+}$) and Oct3-knockout ($Oct3^{-/-}$) mice. When metformin (8.0 mg/kg) was intravenously administered to male $Oct3^{+/+}$ and $Oct3^{-/-}$ mice, $AUC_{0-\infty}$ of metformin was evaluated to be 659 ± 133 μg h/mL and 734 ± 213 μg h/mL, respectively. In the case of orally administered metformin (15 mg/kg), $AUC_{0-\infty}$ was 578 ± 158 μg h/mL and 449 ± 101 μg h/mL in $Oct3^{+/+}$ and $Oct3^{-/-}$ mice, respectively. Based on these pharmacokinetic parameters, absolute bioavailability (*F*) of metformin in $Oct3^{+/+}$ mice was evaluated as 46.8%, and it was significantly decreased to 32.6% in $Oct3^{-/-}$ mice. Taking into account the fact that metformin undergoes negligible metabolism, these results imply that intestinal absorption of metformin is mediated at least in part by Oct3 in mice.

1. Introduction

Metformin is widely used for the treatment of hyperglycemia in patients with type II diabetes mellitus. It undergoes neither hepatic metabolism nor biliary excretion and over 90% of the absorbed metformin is eliminated unchanged in the urine. Therefore, the fraction absorbed of metformin is considered to be very close to its bioavailability of 50–60% in human. This relatively high absorption rate of metformin, in spite of its high hydrophilicity (log *P* value of −1.43), is likely to be due to the involvement of transporter-mediated processes in intestinal absorption [1].

Metformin has been well characterized *in vitro* as a substrate of organic cation transporters (OCTs) in the SLC22A family. OCTs play an important role in cellular uptake and accumulation of metformin in various organs [2]. In the intestine, OCT3 and OCT1 have been reported to be localized in the apical and basolateral membrane of enterocytes, respectively [3–6]. However, Han et al. also reported that OCT1/Oct1 is apically expressed in Caco-2 cells and human and mouse intestines [7]. Therefore, the localization of OCT1 in the intestinal epithelium still remains inconclusive. On the other hand, OCT3 is expressed unequivocally in the apical membrane of enterocytes and mediates the uptake of organic cations from the lumen into the enterocytes [5,6]. These findings indicate the possibility that OCT/Oct transporters contribute to intestinal absorption of metformin. However, the roles of OCTs in intestinal transport and oral absorption of metformin *in vivo* remains to be elucidated.

In the present study, we focused on mouse Oct3 and aimed to evaluate the impact of OCT3/Oct3 transporter on intestinal absorption of metformin *in vivo*. Here, we show, by means of *in vivo* pharmacokinetic study using Oct3-knockout ($Oct3^{-/-}$) mice, that Oct3 contributes to the oral bioavailability and pharmacokinetics of metformin *in vivo*.

Abbreviations: AUC, area under the plasma concentration–time curve; CL, clearance; C_{max}, maximum concentration; OCT/Oct, organic cation transporter; SLC, solute carrier; t_{max}, time to maximum concentration.

* Corresponding author.
E-mail address: jowang@u.washington.edu (J. Wang).

http://dx.doi.org/10.1016/j.dmpk.2016.04.005
1347-4367/

2. Materials and methods

2.1. Materials

[^{14}C]Metformin hydrochloride was purchased from Moravek Biochemicals, Inc. (Brea, CA). 1,1-Dimethylbiguanide hydrochloride (metformin hydrochloride) was purchased from Sigma–Aldrich (St. Louis, MO). All other chemicals and general reagents were of analytical grade or better and were obtained from various commercial sources such as Invitrogen (Carlsbad, CA) or Applied Biosystems (Foster City, CA).

2.2. Animals

The Oct3 (Slc22a3) null mice of the FVB inbred strain were originally developed by Dr. Denise Barlow [8] and maintained by Dr. Alfred Schinkel (Netherlands Cancer Institute). After rederivation at Charles River Laboratories (16), breeding pairs of wild-type (Oct3$^{+/+}$) and Oct3-knockout (Oct3$^{-/-}$) mice were kindly provided to us by Dr. John Markowitz (University of Florida) with approval from Dr. Schinkel. These mice were housed in the specific pathogen-free facility at the University of Washington (Seattle, WA). Male Oct3$^{+/+}$ and Oct3$^{-/-}$ mice (10–12 weeks old) were housed five per cage with free access to commercial chow and tap water, and were maintained on a 12-h light/dark cycles in an air-controlled room. All animal experimentation was carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council. The animal protocol for this study was approved by the Institutional Animal Care and Use Committee at the University of Washington.

2.3. In vivo pharmacokinetic study

Mice were randomly divided into two groups: an intravenous metformin group (8 mg/kg; 4 mL/kg metformin dissolved in saline) and an oral metformin group (15 mg/kg; 10 mL/kg metformin dissolved in saline). For pharmacokinetic study of intravenous metformin, male Oct3$^{+/+}$ and Oct3$^{-/-}$ mice (10–12 weeks old) were fasted for ~10 h. Under anesthesia (2–5% isoflurane), mice were administered 8 mg/kg metformin containing 0.1 mCi/kg [^{14}C] metformin by retro-orbital injection. At various time points (0–480 min), mice (n = 6–9 mice at each time point) were anesthetized, followed by the submandibular puncture (as submandibular bleeding method). Blood was collected using a heparinized micro blood collecting tube (Thermo Fisher Scientific Inc, Waltham, MA) and centrifuged at 5000 × g for 10 min at 4 °C. Plasma was collected and stored at −80 °C until further analysis.

The pharmacokinetic study of orally administered metformin in Oct3$^{+/+}$ and Oct3$^{-/-}$ mice has been previously described [2]. The plasma concentration data obtained from this study were used to obtain oral pharmacokinetic parameters in the present study. Mice were administered 15 mg/kg metformin containing 0.2 mCi/kg [^{14}C]metformin by oral gavage. Plasma metformin concentrations were determined by liquid scintillation counting. Plasma concentration of metformin was expressed as ng/mL.

2.4. Data analysis

Plasma concentration–time curves of metformin were plotted and analyzed. Because metformin concentrations in plasma were sampled in different animals at each time point (1–3 points sampling), the population pharmacokinetics and Bayesian estimation was used to calculate the mean and standard deviation of the area under the concentration–time curves (AUCs) and other pharmacokinetic parameters, using the numerical analysis program for pharmacokinetics NAPP (Version 2.3) [9]. The extent of absolute oral bioavailability (F) expressed as a percentage was directly calculated by dividing the oral AUC by the intravenous AUC normalized to dose:

$$F(\%) = \frac{AUC_{oral}}{AUC_{IV}} \times \frac{Dose_{IV}}{Dose_{oral}} \times 100 \quad (1)$$

where AUC_{IV} and AUC_{oral} are the area under the plasma concentration-versus-time curve after intravenous and oral administrations (ng·h/mL), respectively. $Dose_{oral}$ and $Dose_{IV}$ represent oral and intravenous dosage amounts, respectively.

3. Results and discussion

The impact of Oct3 on the oral bioavailability and pharmacokinetics of metformin in mice was assessed by means of *in vivo* pharmacokinetic study using Oct3$^{-/-}$ mice. When metformin (8.0 mg/kg) was intravenously administered to male Oct3$^{+/+}$ and Oct3$^{-/-}$ mice, $AUC_{0-\infty}$ of metformin was evaluated to be 659 ± 133 μg h/mL and 734 ± 213 μg h/mL, respectively (Fig. 1 and Table 1). After oral administration (15 mg/kg), the $AUC_{0-\infty}$ of metformin was 578 ± 158 μg h/mL and 449 ± 101 μg h/mL in Oct3$^{+/+}$ and Oct3$^{-/-}$ mice, respectively. Based on these pharmacokinetic parameters, absolute bioavailability (F) of metformin in Oct3$^{+/+}$ mice was estimated as 46.8%, and it was markedly decreased to 32.6% in Oct3$^{-/-}$ mice (Table 1). Taking into account the fact that metformin undergoes negligible metabolism (hepatic or non-hepatic), the result implies that intestinal absorption of metformin is mediated at least in part by Oct3 in mice.

The lower bioavailability of metformin in Oct3$^{-/-}$ mice is considered to be mainly due to a reduction of oral $AUC_{0-\infty}$ caused by a lack of Oct3-mediated absorption process of metformin (Table 1). In addition, it may also result from an increase in $AUC_{0-\infty}$ of metformin in Oct3$^{-/-}$ mice after intravenous administration (Table 1). The higher $AUC_{0-\infty}$ of metformin in Oct3$^{-/-}$ mice may be presumably due to a reduced distribution of metformin to peripheral tissues in Oct3$^{-/-}$ mice. This consideration is supported by previous report demonstrating that overall metformin exposures in salivary gland, skeletal muscle and heart were significantly reduced in Oct3$^{-/-}$ mice compared to Oct3$^{+/+}$ mice [2]. However, V_d of metformin showed unexpected significant increase in Oct3$^{-/-}$ mice compared to Oct3$^{+/+}$ mice (1.74 ± 0.28 L/kg vs. 1.86 ± 0.12 L/kg), while V_d/F was significantly lower in Oct3$^{-/-}$ mice than in Oct3$^{+/+}$ mice (5.40 ± 1.38 L/kg vs. 4.30 ± 0.46 L/kg) (Table 1). The apparently inconsistent effects of Oct3 on the distribution volume of metformin between after intravenous (V_d) and oral (V_d/F) administrations may be explained by a complicated intestinal accumulation of metformin due to apical expression of Oct3 in enterocytes. Assuming that after intravenous administration intestinal Oct3 is mainly involved in efflux transport of metformin from enterocyte to intestinal lumen, absence of Oct3 may lead to higher cellular accumulation of metformin, resulting in an apparent enhancement of metformin distribution to intestine in Oct3$^{-/-}$ mice. In contrast, taking into consideration the finding that metformin absorption may be mediated by Oct3, absence of Oct3 may cause lower intracellular concentration of metformin in the intestine after oral administration, resulting in apparent reduction of metformin distribution to intestine in Oct3$^{-/-}$ mice. In order to prove the validity of these hypotheses, further studies with Oct3$^{-/-}$ mice are needed.

As shown in Table 1, CL_{tot} of metformin after intravenous administration is almost comparable between Oct3$^{+/+}$ and Oct3$^{-/-}$ mice. Therefore, it is reasonable to consider that Oct3 deletion does not affect metformin clearance. Although Oct3 is reportedly