Involvement of Carnitine/Organic Cation Transporter OCTN1/SLC22A4 in Gastrointestinal Absorption of Metformin

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Received 23 December 2012; revised 16 April 2013; accepted 18 April 2013

Published online 10 May 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jps.23595

ABSTRACT: Metformin is a widely used oral anti-diabetic, but the molecular mechanism(s) of its gastrointestinal membrane permeation remains unclear. Here, we examined the role of carnitine/organic cation transporter OCTN1/SLC22A4, which is localized on apical membranes of small intestine in mice and humans, in metformin absorption. The maximum plasma concentration (C_{max}) after oral administration of metformin (50 mg/kg) in *octn1* gene knockout mice (*octn1* $^{-/-}$) was higher than that in wild-type mice, with only a minimal difference in terminal half-life, but C_{max} in *octn1*$^{-/-}$ mice given a higher dose (175 mg/kg) was lower than that in wild-type mice. Systemic elimination of metformin after intravenous administration was similar in the two strains, suggesting the possible involvement of OCTN1 in the gastrointestinal absorption. OCTN1-mediated uptake of metformin was observed in human embryonic kidney 293 cells transfected with mouse *OCTN1* gene, but much lower than the uptake of the typical substrate [^{3}H]ergothioneine (ERGO). In particular, the distribution volume for OCTN1-mediated uptake increased markedly and then tended to decrease as the metformin concentration was increased. Efflux of metformin preloaded in intestinal epithelial cell line Caco-2 was inhibited by ERGO. Overall, the present findings suggest that OCTN1 transports metformin and may be involved in its oral absorption in small intestine. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3407–3417, 2013

Keywords: oral absorption; organic cation transporter; pharmacokinetics; ADME; drug transport; renal excretion

INTRODUCTION

Metformin is an antihyperglycemic agent used as first-line therapy for type 2 diabetes mellitus. The main effect of metformin is to reduce hepatic glucose production through inhibition of the mitochondrial respiratory chain complex I, but metformin also exhibits various other pharmacological activities, including reduction of fatty liver, decrease of vascular complications, and decrease in the incidence of cancer and cancer-related mortality.[1,2] On the contrary, metformin also causes the fatal adverse effect of lactic acidosis in rare cases. Thus, an overall understanding of the pharmacokinetics of metformin is needed to better understand the pharmacology and toxicology associated with the clinical use of this drug.

After oral absorption, metformin is distributed to liver and kidney, and is mainly excreted into urine. Systemic elimination via various xenobiotics transporters has been reported in experimental animals and humans. Those transporters include organic cation transporter (OCT) 1, OCT2, multidrug and toxin extrusion (MATE) 1, and MATE-2K.[3–7] In

Abbreviations used: ERGO, ergothioneine; HEK, human embryonic kidney; IS, internal standard; MATE, multidrug and toxin extrusion; OCT, organic cation transporter; OCTN, carnitine/organic cation transporter; PMAT, plasma membrane monoamine transporter; TEA, tetraethylammonium.

Additional Supporting Information may be found in the online version of this article. Supporting Information

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Journal of Pharmaceutical Sciences, Vol. 102, 3407–3417 (2013)

liver and kidney, OCTs are localized on basolateral membranes, whereas MATEs are localized on apical membranes. Metformin is a substrate of these transporters. Thus, the mechanism(s) underlying the uptake and excretion of this drug in the clearance organs has been quite well investigated.

On the contrary, only limited information is available on the mechanism of permeation of metformin across the apical membranes in small intestine. Metformin is classified as a Class III compound in the biopharmaceutics drug disposition classification system originally proposed by Shugarts and Benet.[8] For such compounds, absorptive transporters could have a prominent effect on the pharmacokinetics following oral dosing.[8] Song et al.[5] examined gastrointestinal absorption of metformin in a single-pass rat intestinal perfusion model and proposed the involvement of passive diffusion and an active carrier-mediated saturable mechanism in membrane permeation in duodenum. Indeed, uptake of metformin by proton-stimulated OCT (plasma membrane monoamine transporter, PMAT) was subsequently reported in a gene transfectant system.[9] PMAT is localized on apical membranes of small intestine in humans,[9] and a cluster of intronic single nucleotide polymorphisms in the *PMAT* gene could be associated with decreased metformin absorption.[10] On the contrary, apical membrane transporters for metformin, MATE1, and MATE2-K are also expressed in human small intestine, although at much lower levels than in other expressing organs.[11] These findings, however, do not exclude the possible involvement of another transporter in gastrointestinal absorption of metformin.

Carnitine/organic cation transporter (OCTN) 1 accepts various types of organic cations and zwitterionic compounds as substrates.[12–14] We have recently reported localization of this transporter in apical membranes of small intestine in mice and humans.[15] In addition, *octn1* gene knockout mice (*octn1*$^{-/-}$) were recently constructed, and involvement of this transporter in gastrointestinal absorption of a naturally occurring antioxidant, ergothioneine (ERGO), has been demonstrated, at least in rodents.[15,16] These *octn1*$^{-/-}$ mice could be a useful tool to examine the roles of OCTN1 in the body. Although there has been no report that metformin is a substrate of OCTN1, OCTs and OCTN have approximately 30% structural homology and belong to the same solute carrier 22A family, so there could be an overlap of substrate specificity. Therefore, in the present study, we examined the involvement of OCTN1 in the absorption and disposition of metformin, using *octn1*$^{-/-}$ mice and cell lines stably transfected with *OCTN1* gene.

MATERIALS AND METHODS

Reagents and Animals

Metformin was obtained from LKT Laboratories Inc. (St. Paul, Minnesota). Furosemide and D(−)-mannitol were purchased from Wako Pure Chemical Industries (Osaka, Japan). [^{3}H]ERGO (1 Ci/mmol) was obtained from Moravek Biochemicals Inc. (Brea, California). All other reagents were commercial products of reagent grade. The *octn1*$^{-/-}$ mice were generated according to the previous report.[16] Animals were maintained, and experiments were performed according to the Guideline for the Care and Use of Laboratory Animals in Kanazawa University.

Oral Administration of Metformin

Male mice (7–9 weeks old) were fasted overnight with free access to water, and anesthetized with ether. Metformin (50 or 175 mg/kg) dissolved in saline was orally administered by gavage. Blood samples (20 μL) were then collected at designated time intervals from the tail vein and centrifuged to obtain plasma, which was stored at −30°C until determination of metformin. Pharmacokinetic parameters after oral administration were estimated by means of moment analysis using the WinNonLin software package (Professional version 5.2; Pharsight, Mountain View, California).

Intravenous Infusion of Metformin

After overnight fasting, mice were anesthetized by intraperitoneal injection of pentobarbital. Metformin (6 mg/kg) was injected as a loading dose, followed by infusion via the jugular vein of metformin [120 μg/(min·kg) dissolved in saline containing 3.0% (w/v) mannitol]. Blood samples were collected at designated time intervals from the jugular vein on the other side. Urine samples were collected by washing the bladder with saline through polyethylene tubing (SP31; Natsume, Tokyo, Japan). In a different group of mice, metformin was intravenously administered at the same dose, and blood and bile samples were collected from the jugular vein and gallbladder through polyethylene tubing (SP10, Natsume). At 2 h after the start of infusion, the mice were decapitated, and tissues were excised. The small intestine was divided into three parts. All samples were stored at −30°C until metformin determination.

Total clearance (CL_{tot}), renal clearance (CL_{renal}), biliary clearance with respect to plasma concentration ($CL_{bile,plasma}$), and biliary clearance with respect to liver concentration ($CL_{bile,liver}$) were calculated as follows:

$$CL_{tot} = \frac{I}{C_{ss,plasma}} \quad (1)$$