Interplay between Metformin and Serotonin Transport In the Gastrointestinal Tract: A Novel Mechanism for the Intestinal Absorption and Adverse Effects of Metformin

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Introduction

Metformin is a widely used drug for Type II diabetes mellitus. The mechanisms involved in its unexpectedly high oral bioavailability and intestinal adverse effects are unknown. Studies in our laboratory have shown that cation-selective transporters, including the serotonin reuptake transporter (SERT), mediate the apical uptake of metformin in Caco-2 cell monolayers, and that metformin is both a substrate and an inhibitor of SERT1-3. We provide evidence in this study that 1) SERT contributes to oral absorption of metformin, and 2) metformin inhibits SERT-mediated intestinal reuptake of serotonin resulting in increased intestinal motility and water retention in a mouse model.

Materials and Methods

Metformin Oral Absorption

All animal experiments were conducted according to approved protocols in UNC-Chapel Hill. For portal vein cannulation, C57BL/6 mice were fasted overnight and anesthetized with an intraperitoneal (IP) injection of urethane (1.2-1.5g/kg). A 2 cm abdominal midline incision was made and the intestines were pushed to the side in order to expose the portal vein. A silastic catheter was inserted into the portal vein and secured. [14C]metformin [1mM, 30μCi/mL, 1.3mg/kg] in the presence or absence of the SERT specific inhibitor paroxetine [1μM, 3μg/kg] was administered by oral gavage. Portal blood samples (20 μl each sample) were withdrawn through the portal cannula over four hours (at 5, 15, 30, 60, 120, and 240 min), and simultaneously, systemic blood samples were withdrawn through the tail vein of the same animal. To rule out any confounding effects of the anesthetics, similar studies were conducted in conscious mice. Doses of [14C]metformin [0.5mM, 15μCi/mL, 0.65mg/kg] in the presence and absence of paroxetine [1μM, 3μg/kg] in wild type C57BL/6 mice, and in the absence of paroxetine in mSERT−/− mice, were administered by oral gavage. Systemic blood samples (20 μl each sample) were withdrawn through the tail vein over six hours (at 5, 15, 30, 60, 120, 240 and 360 min). All blood samples were placed in heparinized capillary tubes and centrifuged at 9,000 g at 4°C for 10 min, and plasma samples were analyzed by liquid scintillation spectrometry.

Metformin Intestinal Adverse Effects

Intestinal Motility: Male BALB/c mice were fasted overnight prior to experimentation. Metformin and other gut motility modulating compounds (see below) were given to mice by oral gavage 30 min before oral administration of Alexa Fluor 680-conjugated Dextran (10kDa). Treatment groups included: saline control, metformin [15mg·kg−1, 50μL], paroxetine [1μM, 50μL], 5HT7 agonist cisapride [100μM, 50μL], 5HT1 antagonist GR113808 [25μM, 50μL], metformin plus GR113808 (dosed together, 30 min before dextran), paroxetine plus GR113808 (dosed together, 30 min before dextran), cisapride plus GR113808 [15min before cisapride oral dose, and dextran was administered 30 min after cisapride treatment]. At 5min, 15min, 30min, 60min and 60min post treatment with the fluorescent dye, animals were sacrificed using isoflurane followed by cervical dislocation. Stomach and intestines were harvested and fluorescent imaging was performed, to quantify the intestinal transit of fluorescent dextran. The distance from the intestinal wall to the anterior most region of the fluorescent signal in the intestine was designated as the intestinal transit of dextran. To represent this value as a percentage of the length of the small intestine (34 cm), the intestinal length was divided by 34. The motility of the small intestine was determined by dividing the percentage of dextran transit in the small intestine by the time required to travel that distance (i.e., percentage of small intestinal transit per minute). Data were analyzed by one-way ANOVA and Bonferroni post-hoc test. Data represent mean±S.D.; n=3, *p<0.05, are comparisons to the control.

Intestinal Water Content: Male C57BL/6 mice were fasted overnight prior to experimentation. Magnetic resonance imaging (MRI) was utilized for analysis of water content. Sixty minutes post treatment (same as above), the animals were placed in a temperature-controlled restraining chamber in the MRI instrument with isoflurane as inhalational anesthesia throughout imaging. The abdominal region of the animals was scanned (3 scans for each animal × 3 animals), and the large intestinal lumens at the junction of the transverse colon and the descending colon was selected for imaging. The proton content in the selected area was quantified and the spectra for water present in this area were generated. These spectra were analyzed by MatLab software (MathWorks Inc., Natick, MA, USA) to generate a water peak. The area under the curve (AUC) of this water peak was used as an indicator of water content in the selected area of the large intestine. The specific area selected for imaging was based on its biological significance for all animals to achieve the highest accuracy. Dose of metformin was given by one-way ANOVA and Bonferroni post-hoc test. Data represent mean±S.D.; n=5, *p<0.05, **p<0.01, ***p<0.001 are comparisons to the control.

Results

Paroxetine decreases metformin systemic AUC0–150 min by 9% (p<0.05) in conscious wild-type mice and by 6% (p<0.05) in mSERT−/− mice compared to wild-type mice, confirming the role of mSERT in intestinal oral absorption.

Conclusions and Discussion

1. Previous studies established that metformin is a substrate of SERT, and SERT mediates ~20% of the AP uptake of metformin in Caco-2 cells1. Current study shows that mSERT plays a minor role (~10%) in metformin oral absorption in mice, using in vivo inhibition and transporter knockout strategies.

2. Previous studies had showed that metformin is also an inhibitor of SERT (IC50 of ~50mM) at a concentration that is slightly higher than that achieved in the intestinal lumen, but close to that observed in the enterocytes3. Current study provides in vivo evidence that the inhibition of SERT by metformin leads to the decrease of reuptake of serotonin and increase of serotonin concentration in the intestine, that could result in the enhancement of intestinal motility and water retention.

3. This is the first study that demonstrates that the interaction between SERT and metformin in the GI tract leads to GI distress such as diarrhea. This finding has major scientific and medical significance, because metformin-mediated intestinal adverse effects have affected and continue to affect millions of patients on metformin therapy, leading to non-compliance.

4. Importantly, this is the first direct evidence that establishes a link between an exogenous activator of SERT, and an endogenous monoamine, serotonin. Endogenous monoamines are generally important molecules, such as hormones and neurotransmitters, and their homeostasis is critically important to biological functions. The interactions between these endogenous cations and exogenous drugs could lead to profound effects, and could be extrapolated to many other cationic drugs.

References

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Acknowledgment

Figure 1: Portal and Systemic Plasma Concentrations Over Time (Left) and AUC0−150 min (Right) of Metformin in Anesthetized Mice after Oral Administration

Figure 2: Systemic Plasma Concentrations Over Time (Left) and AUC0−150 min (Right) of Metformin in Conscious Mice after Oral Administration

Figure 3: Intestinal Transit of Fluorescent Dextran in Control (saline-treated) and Metformin-Treated Mice

Figure 4: Small Intestinal Transit of Fluorescent Dextran (Left) and Small Intestinal Motility (Right) after Treatments in Mice

Figure 5: Large Intestinal Water Content after Treatments in Mice