Title:

Goldenseal-mediated inhibition of intestinal uptake transporters decreases metformin systemic exposure in mice

Authors:

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Mechanistic study of the PK goldenseal-metformin interaction

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Abbreviations: AUC, area under the blood concentration versus time curve; Cmax, maximum blood concentration; HEK, human embryonic kidney; IC50, half-maximal inhibitory concentration; I gut, intestinal luminal inhibitor concentration; LC-MS/MS, liquid chromatography with tandem mass spectrometry; MPP+, 1-methyl-4-phenylpyridinium iodide; OCT, organic cation transporter; PEG, polyethylene glycol; PMAT, plasma monoamine transporter; SERT, serotonin transporter; THTR2, thiamine transporter 2; UHPLC, ultra-high performance liquid chromatography.
ABSTRACT
Goldenseal is a perennial plant native to eastern North America. A recent clinical study reported goldenseal decreased metformin $C_{\text{max}}$ and AUC by 27% and 23%, respectively, but half-life and renal clearance were unchanged. These observations suggested goldenseal altered processes involved in metformin absorption. The underlying mechanism(s) remain(s) unknown. One mechanism for the decreased metformin systemic exposure is inhibition by goldenseal of intestinal uptake transporters involved in metformin absorption. Goldenseal extract and three goldenseal alkaloids (berberine, (−)-β-hydrastine, hydrastinine) were tested as inhibitors of organic cation transporter (OCT) 3, plasma membrane monoamine transporter (PMAT), and thiamine transporter (THTR) 2 using HEK293 cells overexpressing each transporter. The goldenseal extract, normalized to berberine content, was the strongest inhibitor of each transporter ($IC_{50}$: 4.9, 13.1, and 5.8 μM for OCT3, PMAT, and THTR2, respectively). A pharmacokinetic study in mice compared the effects of berberine, (−)-β-hydrastine, goldenseal extract, and imatinib (OCT inhibitor) on orally administered metformin. Goldenseal extract and imatinib significantly decreased metformin $C_{\text{max}}$ by 31% and 25%, respectively, and had no effect on half-life. Berberine and (−)-β-hydrastine had no effect on metformin pharmacokinetics, indicating neither alkaloid alone precipitated the interaction in vivo. A follow-up murine study involving intravenous metformin and oral inhibitors examined the contributions of basolateral enteric/hepatic uptake transporters to the goldenseal-metformin interaction. Goldenseal extract and imatinib had no effect on metformin AUC and half-life, suggesting lack of inhibition of basolateral enteric/hepatic uptake transporters. Results may have implications for patients taking goldenseal with drugs that are substrates for OCT3 and THTR2.
SIGNIFICANCE STATEMENT

Goldenseal is used to self-treat respiratory infections and digestive disorders. We investigated potential mechanisms for the clinical pharmacokinetic interaction observed between goldenseal and metformin, specifically inhibition by goldenseal of intestinal uptake transporters (OCT3, PMAT, THTR2) involved in metformin absorption. Goldenseal extract inhibited all three transporters in vitro and decreased metformin systemic exposure in mice. These data may have broader implications for patients co-consuming goldenseal with other drugs that are substrates for these transporters.
Introduction

Goldenseal [*Hydrastis canadensis* L. (Ranunculaceae)] is an herbaceous perennial plant native to eastern North America (Pengelly et al., 2012). The roots and rhizomes of goldenseal have been used in Appalachian culture as a bitter tonic, eyewash, and topical agent (Pengelly et al., 2012). Goldenseal is widely available as a botanical dietary supplement that is used to self-treat digestive disorders and respiratory infections (Hussain, 2011). Constituents from goldenseal include alkaloids (e.g., berberine, canadine, (−)-β-hydrastine, hydrastinine), flavonoids (e.g., sideroxylin, 8-desmethyl-sideroxylin), and phenolic compounds (e.g., chlorogenic acid, neochlorogenic acid) (Mandal et al., 2020; Weber et al., 2003). Goldenseal and some of its constituents are reported to have anti-microbial, anti-inflammatory, hypolipidemic, and hypoglycemic properties in various *in vitro* and animal models (Abidi et al., 2006; Pirillo & Catapano, 2015; Villinski et al., 2003). However, their clinical efficacy remains unclear (Mandal et al., 2020). US sales of botanical dietary supplements from different retail channels increased by 9.7% from 2020 to 2021 (i.e., $11.3 to $12.4 billion) (Smith et al., 2022). Increasing sales of these products raise concerns that goldenseal may increase the risk for natural product-drug interactions and compromise the safety and efficacy of certain medications (Asher et al., 2017; Hung et al., 1997; Wu et al., 2005).

Metformin is the first-line medication for the management of type 2 diabetes, which afflicts approximately 33 million people in the US (National Diabetes Statistics Report, 2022) and 537 million people worldwide (Sun et al., 2022). A recent clinical study demonstrated that a well-characterized goldenseal product significantly decreased metformin area under the plasma concentration versus time curve (AUC) and maximum plasma concentration (C<sub>max</sub>) by 23% and 27%, respectively, but did not affect half-life or renal clearance (Nguyen et al., 2021). These pharmacokinetic data suggested altered intestinal absorption of metformin by goldenseal as a potential mechanism underlying this natural product-drug interaction.
Metformin is hydrophilic and depends on transporters for transcellular movement through enterocytes (Han et al., 2015; Liang and Giacomini, 2017). Inhibition of intestinal apical uptake transporters may explain the observed goldenseal-metformin interaction. Nguyen et al. demonstrated inhibition of the uptake transporter organic cation transporter (OCT) 1 by berberine, (–)-β-hydrastine, and a goldenseal extract normalized to berberine. The extract was the most potent (IC$_{50}$: 19, 6.6, and 2.6 μM, respectively) (Nguyen et al., 2021). However, localization of OCT1 in enterocytes is equivocal, and metformin is a substrate for other apically located intestinal uptake transporters, including OCT3, plasma monoamine transporter (PMAT), and thiamine transporter (THTR) 2 (Han et al., 2015; Liang and Giacomini, 2017). Whether goldenseal inhibits these transporters is not known.

The objective of this study was to address additional potential mechanisms underlying the pharmacokinetic goldenseal-metformin interaction. First, the aforementioned goldenseal extract and alkaloids were tested as inhibitors of OCT3, PMAT, and THTR2 activity in human embryonic kidney (HEK) 293 cells overexpressing each transporter. Second, the effects of orally administered goldenseal extract, berberine, (–)-β-hydrastine, and imatinib (OCT1 and OCT3 inhibitor) were evaluated on the oral pharmacokinetics of metformin in mice. Third, the effects of orally administered goldenseal extract were evaluated on the intravenous pharmacokinetics of metformin in mice to assess potential contributions of intestinal and hepatic basolateral transporters. Results suggest multiple transporters and (an) unidentified constituent(s) are involved in this natural product-drug interaction.
Materials and methods

Materials

Metformin hydrochloride salt (CAS# 1115-70-4) was purchased from Enzo Life Sciences (Farmingdale, NY). 1,1-Dimethyl-d₆-biguanide HCl (CAS# 1185166-01-1) was obtained from C.D.N. isotopes (Pointe-Claire, Quebec). The goldenseal extract was sourced and prepared from the same previously characterized commercial product used in the clinical study (Nguyen et al., 2021; Wallace et al., 2018). Berberine chloride hydrate (CAS# 68030-18-2) and imatinib (CAS# 152459-95-5) were purchased from MedChemExpress (Monmouth, NJ). (–)-β-Hydrastine (CAS# 118-08-1) was purchased from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Middle Franconia, Germany). Human embryonic kidney (HEK) 293-LV cells were provided by SOLVO Biotechnology (city, Hungary). Hydrastinine chloride monohydrate (CAS# 65945-18-8), [³H]1-methyl-4-phenylpyridinium iodide (MPP⁺) (CAS# 36913-39-0), [³H]thiamine (CAS# 67-03-8), pyrimethamine (CAS# 58-14-0), and quinidine (CAS# 56-54-2) were purchased from Sigma-Aldrich (St. Louis, MO). Fedratinib (CAS# 936091-26-8) was purchased from Selleckchem (Houston, Texas). All other chemicals and reagents were analytical grade.

In vitro transporter inhibition assays

HEK 293-LV cells overexpressing human OCT3, PMAT, or THTR2 were used to assess the inhibitory effect of each test article. The probe substrate was MPP⁺ for both OCT3 (0.02 µM) and PMAT (10 µM), for THTR2 was thiamine (0.03 µM). Reference inhibitors for OCT3, PMAT, and THTR2 were quinidine (1000 µM), pyrimethamine (100 µM), and fedratinib (30 µM), respectively. For the screening assays, goldenseal extract (normalized to berberine content) was tested at 1.75 and 17.5 µM. This concentration represents the amount of berberine in the extract, but does not include other constituents, which would also be present. Berberine, (–)-β-
hydrastine, and hydrastinine were tested at 10 and 100 µM. If ≥40% inhibition was observed at the higher concentration, then the half-maximum inhibitory concentration (IC₅₀) was determined. Each cell line was preincubated with transport buffer containing test article for 30 minutes at 37°C before the addition of transport buffer containing probe substrate and test article. Transport buffers used were Krebs-Henseleit (pH 7.4) for OCT3, Hank’s Balanced Salt Solution (pH 6.6) for PMAT, and Hank’s Balanced Salt Solution (pH 7.4) for THTR2. Transport was terminated after 3 minutes for OCT3 and THTR2 or 2 minutes for PMAT. Cells were rinsed twice with transport buffer and lysed with 0.1 M NaOH. The amount of probe substrate in cells was determined by liquid scintillation counting. Relative transporter-specific accumulation was calculated and expressed as a percent of control. Bottom constraints (0) were applied to all data sets, and a top constraint (100) was applied to the goldenseal extract data obtained for THTR2. Best-fit IC₅₀ values were determined by nonlinear least-squares regression using the standard and variable Hill slope equations and comparing the models via visual inspection of the observed versus predicted data and the Akaike Information Criterion in GraphPad Prism (v 9.3.1; San Diego, CA).

Mouse studies

The study protocol was approved and executed following the Institutional Animal Care and Use Committee guidelines at Washington State University. Handling, care, and maintenance of the animals occurred at the Program of Laboratory Animal Resources facility of Washington State University Health Sciences Spokane. Adult male FVB mice (25-30 g) were purchased from Jackson Laboratory (Bar Harbor, Maine). Mice were randomly placed in cages (two per cage) and maintained under a standard 12-hour light/dark cycle with free access to food and water. After acclimation, mice were randomly allocated to groups for oral metformin/oral inhibitor (n=8) or intravenous metformin/oral inhibitor (n=7) studies. All mice were fasted for four hours before and after gavage. The metformin dose was based on the single oral
dose (50 mg) used in the clinical study (Nguyen et al., 2021). The imatinib dose was based on two previous studies: the daily dose (600 mg) used for the treatment of Philadelphia chromosome-positive chronic myeloid leukemia and a previously reported clinical drug-drug interaction study (K. Giacomini, 2014). The doses were converted to mg/kg using a reference body weight of 60 kg (0.83 and 10 mg/kg for metformin and imatinib, respectively) (Nair & Jacob, 2016). The animal equivalent doses were then determined by allometric scaling using the mouse correction factor of 12.3, producing 10.2 mg/kg for metformin and 123 mg/kg for imatinib. The goldenseal extract dose was selected from a single oral dose of 2.7 g used in a clinical study by Gupta et al. and the total dose (3 g/day) used in the clinical study by Nguyen et al. (Gupta et al., 2009; Nguyen et al., 2021). The animal equivalent dose for goldenseal extract was 615 mg/kg. The final doses used in the mouse studies (10, 500, and 100 mg/kg for metformin, goldenseal extract, and imatinib, respectively) were selected based on allometric scaling and considering test article solubility in the vehicle.

The groups and test article doses for the oral metformin/oral inhibitor study are provided (Figure 1A). Test articles were dissolved in 45% PEG 400, 20% propylene glycol, and 35% molecular grade water (containing metformin) by heating at 37°C and homogenizing with a vortex mixer. The doses of berberine and (–)-β-hydrastine were calculated to match their µg/mg content in the goldenseal extract (142 and 91 µg/mg, respectively). Blood (~25 µL) was collected through the tail vein into heparinized capillary tubes at 0.25, 0.5, 1, 2, 4, 8, and 12 h after administration of the test article(s). Blood samples were stored at -80°C until analysis.

The groups and doses of the intravenous metformin/oral inhibitor study are provided (Figure 1B). Metformin was dissolved in sterile saline, whereas goldenseal extract and imatinib were prepared as described for the oral metformin/oral inhibitor study. Goldenseal extract, imatinib, or vehicle was administered by oral gavage, and metformin was administered 30 minutes later by retro-orbital injection. Blood (~25 µL) was collected through the tail vein into
heparinized capillary tubes at 0.083, 0.25, 0.5, 1, 2, 4, 8, and 12 h after injection of metformin. Blood samples were stored at -80°C until analysis.

**Quantitation of metformin in murine blood by liquid chromatography/tandem mass spectrometry**

Blood (2 µL) was added to 100 µL of internal standard (1,1-dimethyl-d₆-biguanide HCl, 2 ng/ml) prepared in 100% acetonitrile, mixed vigorously with a vortex mixer, and centrifuged at 15,000 x g for 10 minutes at 4°C. The supernatant (51 µL) was added to mobile phase B (49 µL), which consisted of 80 mM ammonium formate, formic acid and acetonitrile (100:0.5:900, v/v/v), and mixed with a vortex mixer. An aliquot (60 µL) was transferred to an autosampler vial for LC-MS/MS analysis. Metformin concentrations were quantified using a QTRAP 6500 UHPLC-MS/MS system (AB Sciex, Framingham, MA). Metformin was separated from matrix components using an ACQUITY UPLC® BEH HILIC column (2.1 x 100 mm) at 30°C using the same mobile phases, flow rate, and gradient program from a published method (Scherf-Clavel et al., 2019). The turbo electrospray source was operated in positive ionization mode. The following parameters in multiple reaction monitoring were used: metformin 130.174 → 60.055 m/z, 1,1-dimethyl-d₆-biguanide HCl 136.174 → 60.055 m/z, declustering potential 31 V, and collision energy 17 V. The calibration standards were linear from 0.039-20 ng/mL. Analyst® software (v1.7, AB Sciex) was used for data acquisition and quantification.

**Pharmacokinetic analysis**

The pharmacokinetics of metformin were determined via non-compartmental analysis methods using Phoenix WinNonlin (v8.3; Certara, Princeton, NJ). AUC for the oral metformin/oral inhibitor study and intravenous metformin/oral inhibitor study was determined using the linear up/log down trapezoidal method and log trapezoidal method, respectively. \( C_{\text{max}} \) and time to \( C_{\text{max}} \) (\( t_{\text{max}} \)) were obtained directly from the concentration-time profiles. The terminal
elimination rate constant ($\lambda_z$) was recovered via linear regression of at least the last three data points, and elimination half-life ($t_{1/2}$) was calculated as $\ln 2/\lambda_z$. The AUC ratio (AUCR) for metformin was calculated as the ratio of the AUC of metformin in the presence to absence of inhibitor. AUC from zero to infinity (AUC$_{inf}$) was calculated from time of dosing extrapolated to infinity based on the last observed concentration. Metformin oral bioavailability was calculated as the ratio AUC$_{inf, oral}$ to AUC$_{inf, iv}$ and correcting for dose.

**Statistical analysis**

Statistical analysis was conducted using GraphPad Prism (v 9.3.1; San Diego, CA). *In vitro* data are presented as means ± standard deviations (SDs) of three replicates. One-way analysis of variance with Dunnett’s post-hoc test was used to compare mean AUC, $C_{max}$, and $t_{1/2}$ between the presence and absence of inhibitor on log transformed data. A p-value <0.05 was considered statistically significant.
Results

Transport inhibition assays

The goldenseal extract and berberine inhibited OCT3, PMAT, and THTR2 in a concentration-dependent manner, with ≥40% inhibition observed at the higher concentration (Table 1). Nearly all concentrations of (–)-β-hydrastine and hydastinine inhibited all three transporters by <40%. The goldenseal extract was a more potent inhibitor than berberine of all three transporters; the IC\textsubscript{50} values for berberine alone were 2.8 to 7.4-fold higher than those for the extract (Figure 2).

Pharmacokinetics of metformin in mice

No overt effects were observed in the mice after administration of test articles in both the oral metformin/oral inhibitor and intravenous metformin/oral inhibitor studies. Orally administered metformin was quantifiable in blood samples at all time points in the absence and presence of orally administered goldenseal extract, berberine, (–)-β-hydrastine, and imatinib (Figure 3). Relative to vehicle, goldenseal extract and imatinib decreased geometric mean metformin C\textsubscript{max} by 31% and 25%, respectively, whereas berberine and hydrastine had no effect (Table 2). Goldenseal extract decreased geometric mean metformin AUC\textsubscript{0-4h} by 30%, whereas imatinib, berberine, and (–)-β-hydrastine had no effect. All test articles had no effects on geometric mean metformin AUC\textsubscript{0-12h}, t\textsubscript{1/2}, and median t\textsubscript{max}. Intravenously administered metformin was quantifiable in blood at all time points in the absence and presence of orally administered goldenseal extract and imatinib (Figure 4). Neither the extract nor imatinib affected metformin geometric mean AUC\textsubscript{0-12h} and t\textsubscript{1/2} (Table 3). Metformin oral bioavailability in the presence of vehicle, goldenseal extract, and imatinib was 62%, 46%, and 51%, respectively.
Discussion

This work investigated potential mechanisms underlying the observed clinical pharmacokinetic goldenseal-metformin interaction (Nguyen et al., 2021). Metformin is a substrate for multiple intestinal uptake transporters, including OCT1, OCT3, PMAT, THTR2, and serotonin transporter (SERT). As such, inhibition of one or more of these transporters could lead to a decrease in the extent of metformin absorption. Assessment of the percent contribution of each transporter to metformin uptake may provide insight into which transporter(s) may be responsible for the interaction. Two previous studies estimated the percent contribution of various transporters to metformin uptake in Caco-2 cell monolayers (Han et al., 2015; Shirasaka et al., 2022). Han et al. and Shirasaka et al. reported different percent contributions for PMAT (20% versus 10%), SERT (20% versus 2%), and passive diffusion (20% versus 55%). Each study also reported additional transporters that may contribute to metformin intestinal uptake. Han et al. included OCT1 (25%) and choline transporter (15%), whereas Shirasaka et al. included THTR2 (22%), OCT3 (10%), and other (unidentified) transporters (1%). These between-study variations make it difficult to determine the true contribution of each transporter to metformin intestinal uptake, but these studies demonstrated that multiple transporters are involved. Although choline transporter is expressed in Caco-2 cells, there is no evidence of mRNA or protein expression in human intestine (Han et al., 2015). Reported $K_m$ values for metformin towards SERT varied markedly (0.5-4 mM) (Han et al., 2015; Shirasaka et al., 2022), and SERT overexpressing cells were not available when the current work was initiated.

Collectively, to further elucidate the potential involvement of transporters in the observed pharmacokinetic goldenseal-metformin interaction, we investigated the in vitro inhibition of OCT3, PMAT, and THTR2 and conducted pharmacokinetic studies in mice.

Both the in vitro and in vivo data indicated that the goldenseal alkaloids hydrastinine and (–)-β-hydrastine did not contribute to the interaction because hydrastinine did not inhibit OCT1,
OCT3, PMAT, or THTR2 (Table 1), and (–)-β-hydrastine only inhibited OCT1 (Nguyen et al., 2021). In contrast, berberine inhibited all four transporters, but with higher IC\textsubscript{50} values when tested alone compared with the goldenseal extract (Figure 2) (Nguyen et al., 2021), suggesting that other unidentified goldenseal constituents contribute to inhibition of these transporters. Our oral metformin/oral inhibitor mouse study showed that the goldenseal extract decreased metformin systemic exposure, but berberine and (–)-β-hydrastine alone were insufficient to precipitate an interaction. Nguyen et al. estimated an I\textsubscript{gut} (intestinal luminal inhibitor concentration) for berberine of 354 µM in humans (Nguyen et al., 2021), which is much higher than the IC\textsubscript{50} values determined for the transporters tested in the current study. Because there is no clear guidance on how to estimate I\textsubscript{gut} in mice, it is difficult to determine whether berberine I\textsubscript{gut} was sufficient to inhibit the transporters. It is possible that other processes not dependent on berberine (i.e., passive diffusion) and/or unidentified goldenseal constituents contribute to this pharmacokinetic interaction.

Our oral metformin/oral inhibitor mouse study further investigated potential mechanisms underlying the pharmacokinetic interaction. Metformin pharmacokinetics in the presence of goldenseal extract were compared to imatinib, an OCT1 and OCT3 inhibitor (IC\textsubscript{50}, 1.4 and 4.4 µM, respectively) (Minematsu and Giacomini, 2011). Previous studies have reported 78% and 86% identity between human OCT1 and OCT3 with mouse Oct1 and Oct3 (Floerl et al., 2020; Wu et al., 2000). Both the goldenseal extract and imatinib decreased metformin C\textsubscript{max} (31% and 25%, respectively) by a comparable extent as that observed for goldenseal in the clinical study (27%) (Nguyen et al., 2021). The goldenseal extract and imatinib precipitated a non-significant decrease in metformin AUC\textsubscript{0-12h} (17% and 12%, respectively), while a slightly larger decrease in metformin oral bioavailability was observed (26% and 18%, respectively) due to a non-significant increase in AUC\textsubscript{0-12} after intravenous administration (see below for further discussion). The effects of the goldenseal extract and imatinib on metformin AUC\textsubscript{0-4h} were next compared because metformin is primarily absorbed from the small intestine (Song et al., 2006),
and small intestinal transit time in mice ranges from 3-6 h (Padmanabhan et al., 2013). The goldenseal extract and imatinib decreased metformin AUC0-4h by 30% and 21%, respectively (Table 2), which are comparable to the decrease in metformin AUCinf (23%) observed for goldenseal in the clinical study (Nguyen et al., 2021). The potential contribution of passive diffusion to metformin intestinal uptake (up to 50%) (Han et al., 2015; Shirasaka et al., 2022) may partially explain the modest decrease in metformin systemic exposure observed after goldenseal administration. The Cmax and AUC data, combined with no change in metformin half-life, further support that the interaction occurred primarily in the intestine in mice and humans. The oral metformin/oral inhibitor data also indicate imatinib inhibited mouse Oct1 and Oct3 and suggest goldenseal extract and imatinib act through similar mechanisms. Whether THTR2/Thtr2 contributes to the imatinib-metformin interaction is unclear because imatinib has not been tested as a THTR2/Thtr2 inhibitor.

The difference in the effects of goldenseal on metformin AUCinf and AUC0-12 between the clinical and mouse studies may reflect species differences in transporter inhibition and the percent contribution of each process to metformin uptake. For example, imatinib was reported to be a more potent inhibitor of mouse Oct1 than human OCT1 (IC50: 0.3 µM and 1.47 µM, respectively) but a more potent inhibitor of human OCT3 than mouse Oct3 (IC50: 4.36 µM and 10 µM, respectively) (Minematsu & Giacomini, 2011). Like imatinib, the goldenseal extract was a more potent inhibitor of human OCT1 (Nguyen et al., 2021) than OCT3 (current study) (IC50: 2.6 versus 4.9 µM). There may be species differences for other OCT/Oct inhibitors, including goldenseal constituents. Whether species differences exist for inhibitors of THTR2/Thtr2 and PMAT/Pmat are unknown based on lack of comparable inhibition data for both species. In addition, the percent contribution of each transporter to metformin uptake may differ between mice and humans. Collectively, species differences in metformin passive diffusion/active transport and/or goldenseal inhibitory potency for each transporter may determine the magnitude of the interaction.
Our intravenous metformin/oral inhibitor mouse study investigated the involvement of uptake transporters localized on the basolateral membrane of enterocytes and/or hepatocytes in the goldenseal-metformin interaction. Inhibition of enteric or hepatic basolateral uptake transporters would be expected to increase, whereas inhibition of enteric apical uptake transporters would be expected to decrease, systemic drug concentrations (Giacomini et al., 2010; Y. Liang et al., 2015). OCT1 is localized on the basolateral membrane of hepatocytes and contributes to clearance from the blood (K. M. Giacomini et al., 2010). OCT1 is also expressed in the intestine, but its membrane localization is equivocal (Müller et al., 2005; Han et al., 2013; Wenzel et al., 2021). Both the goldenseal extract and imatinib increased metformin AUC₀-12h by 22-27% (Figure 4). However, these values were not significantly different from control, suggesting that the inhibitory effect of the goldenseal extract may be attributed mainly to interactions with apical transporters in enterocytes. Although we did not quantify berberine and (−)-β-hydrastine systemic concentrations, previous data suggest that neither constituent is likely to achieve sufficient systemic concentrations to inhibit basolateral transporters at the doses administered in our study. For example, a single 2.7 g dose of goldenseal administered to humans produced a Cₘₐₓ of 1.75 and 470 nM for (−)-β-hydrastine and berberine, respectively (Gupta et al., 2015). These concentrations are substantially lower than the reported IC₅₀ values for berberine and (−)-β-hydrastine against OCT1 (19 and 6.6 µM, respectively) (Nguyen et al., 2021). We cannot rule out the possibility that other untested goldenseal constituents may inhibit basolateral transporters and contribute to this pharmacokinetic interaction.

As discussed above, one limitation of the present study is the well-known species differences in transporter expression and activity. Absolute expression of all transporters and their localization in mice is not well-defined. In addition, Pmat mRNA is not expressed in the small intestine of mice (Nakamichi et al., 2013), whereas PMAT mRNA has been detected in human intestinal tissue (Duan & and Wang, 2010; Han et al., 2015). However, the comparable
decrease in $C_{\text{max}}$ and unchanged half-life between the mouse and human studies suggest PMAT inhibition may not substantially contribute to the goldenseal-metformin interaction in humans.

In summary, the current work advances the mechanistic understanding of the pharmacokinetic interaction observed between the widely used natural product, goldenseal, and the most prescribed oral anti-diabetic drug, metformin, in human participants (Nguyen et al., 2021). Results from a comprehensive approach involving established human transporter-overexpressing cells and mouse pharmacokinetic studies supported that (1) the mechanism involves inhibition of intestinal uptake transporters by goldenseal, including OCT1, OCT3, and THTR2, and (2) either an unidentified constituent or multiple constituents in goldenseal is(are) required to precipitate the interaction. These results also may have implications for patients taking metformin with other OCT1/3 inhibitors, such as erlotinib and gefitinib, or taking goldenseal with other drugs that are substrates of OCT1, OCT3, and THTR2, such as acyclovir, atenolol, sumatriptan, and desipramine. Additional mechanistic in vitro and in vivo studies, including clinical evaluations, are needed to determine the clinical ramifications of this pharmacokinetic natural product-drug interaction.
Data Availability Statement

The authors declare that all the data supporting the findings of this study are contained within the paper.
Author Contributions

Participated in research design: VOO, KYGT, BJB, MFP, JDC

Conducted experiments: VOO, KYGT, BJB, KDL, MRC, MH, JDC

Contributed new reagents or analytic tools: PKM, TNG, NBC, NHO

Performed data analysis: VOO, KYGT, MH, JDC

Wrote or contributed to the writing of the manuscript: VOO, BJB, KDL, MRC, MH, PKM, NBC, NHO, MFP, JDC
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Footnotes

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Conflicts of Interest

None
Figure Legends

Figure 1. Experimental design for the mouse studies. (A) Oral metformin/oral inhibitor study (n=8 per group). Metformin (10 mg/kg) was administered by oral gavage alone or with goldenseal extract (500 mg/kg), berberine (71 mg/kg), (−)-β-hydrastine (46 mg/kg), or imatinib (100 mg/kg). (B) Intravenous metformin/oral inhibitor study (n=7 per group). Metformin (5 mg/kg) was administered intravenously 30 minutes after oral gavage of vehicle, goldenseal extract (500 mg/kg), or imatinib (100 mg/kg).

Figure 2. Inhibitory effects of goldenseal extract (gold squares) and berberine (blue triangles) on OCT3-, PMAT-, and THTR2-mediated uptake of probe substrates ([3H]-1-methyl-4-phenylpyridinium iodide [OCT3 and PMAT] and [3H]thiamine [THTR2]). Symbols and error bars represent means and SDs, respectively, of three replicates. Curves denote model-generated best fits to the data using the Hill slope equation.

Figure 3. Geometric mean metformin blood concentration versus time profiles for metformin after oral administration to mice (insets are semi-logarithmic plots). A single oral dose of metformin (10 mg/kg) was administered alone (black closed circles) or with a single oral dose of (A) goldenseal extract (500 mg/kg) (gold squares), (B) berberine (71 mg/kg) (blue triangles), (C) (−)-β-hydrastine (46 mg/kg) (red open circles), or (D) imatinib (100 mg/kg) (purple diamonds). Symbols and error bars denote geometric means and SDs, respectively, in the absence or presence of test articles (n=8 per group).

Figure 4. Geometric mean metformin blood concentration versus time profiles for metformin after intravenous administration to mice (insets are semi-logarithmic plots). A single intravenous dose of metformin (5 mg/kg) was administered alone (black closed circles) 30 minutes after a single oral dose of (A) goldenseal extract (gold squares) (500 mg/kg) or (B) imatinib (100 mg/kg) (purple diamonds). Symbols and error bars denote geometric means and SDs, respectively, in the absence or presence of test articles (n=7 per group).
### Table 1: Goldenseal extract and alkaloids tested as inhibitors of uptake transporters

<table>
<thead>
<tr>
<th></th>
<th>Concentration (µM)</th>
<th>OCT3 (%)</th>
<th>PMAT (%)</th>
<th>THTR2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Goldenseal</strong> extract*</td>
<td>1.75</td>
<td>26 (14)</td>
<td>28 (3.8)</td>
<td>8 (16)</td>
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<td></td>
<td>17.5</td>
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<td><strong>Berberine</strong></td>
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<td>8 (12)</td>
<td>-2 (5.6)</td>
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<td></td>
<td>100</td>
<td>95 (0.36)</td>
<td>71 (6.6)</td>
<td>43 (12)</td>
</tr>
<tr>
<td>(-)-β-hydrastine</td>
<td>10</td>
<td>3 (9.5)</td>
<td>-9 (7.3)</td>
<td>7 (22)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>24 (6.0)</td>
<td>37 (7.3)</td>
<td>-4 (5.6)</td>
</tr>
<tr>
<td><strong>Hydrastinine</strong></td>
<td>10</td>
<td>-5 (6.5)</td>
<td>11 (6.6)</td>
<td>2 (20)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>-3 (7.0)</td>
<td>43 (6.5)</td>
<td>19 (4.6)</td>
</tr>
</tbody>
</table>

*Normalized to berberine content.

Values represent arithmetic mean percent inhibition (SD) of transporter activity by test article relative to vehicle (n=3). OCT3, organic cation transporter 3; PMAT, plasma monoamine transporter; THTR2, thiamine transporter 2.
Table 2: Pharmacokinetics of oral metformin alone (presence of vehicle) or in combination with orally administered test articles in mice (n=8)

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Goldenseal extract</th>
<th>Berberine</th>
<th>(–)-β-hydrastine</th>
<th>Imatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>C&lt;sub&gt;max&lt;/sub&gt; (µM)</strong></td>
<td>1.6</td>
<td>1.1*</td>
<td>1.4</td>
<td>1.4</td>
<td>1.2*</td>
</tr>
<tr>
<td></td>
<td>(21)</td>
<td>(18)</td>
<td>(28)</td>
<td>(23)</td>
<td>(14)</td>
</tr>
<tr>
<td><strong>AUC&lt;sub&gt;0-4h&lt;/sub&gt;</strong></td>
<td>4.7</td>
<td>3.3*</td>
<td>4.1</td>
<td>4.4</td>
<td>3.7</td>
</tr>
<tr>
<td>(µM•h)</td>
<td>(24)</td>
<td>(17)</td>
<td>(26)</td>
<td>(21)</td>
<td>(12)</td>
</tr>
<tr>
<td><strong>AUCR&lt;sub&gt;0-4h&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</strong></td>
<td>NA</td>
<td>0.69</td>
<td>0.87</td>
<td>0.93</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>(0.59 - 0.83)</td>
<td>(0.67 - 1.15)</td>
<td>(0.82 - 1.07)</td>
<td>(0.65 - 0.96)</td>
<td></td>
</tr>
<tr>
<td><strong>AUC&lt;sub&gt;0-12h&lt;/sub&gt;</strong></td>
<td>7.6</td>
<td>6.3</td>
<td>6.9</td>
<td>7.2</td>
<td>6.7</td>
</tr>
<tr>
<td>(µM•h)</td>
<td>(22)</td>
<td>(12)</td>
<td>(25)</td>
<td>(20)</td>
<td>(13)</td>
</tr>
<tr>
<td><strong>AUCR&lt;sub&gt;0-12h&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</strong></td>
<td>NA</td>
<td>0.83</td>
<td>0.92</td>
<td>0.95</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>(0.74 - 0.95)</td>
<td>(0.71 - 1.19)</td>
<td>(0.82 - 1.11)</td>
<td>(0.74 - 1.07)</td>
<td></td>
</tr>
<tr>
<td><strong>AUC&lt;sub&gt;inf&lt;/sub&gt;</strong></td>
<td>7.0</td>
<td>6.6</td>
<td>7.2</td>
<td>7.3</td>
<td>6.9</td>
</tr>
<tr>
<td>(µM•h)</td>
<td>(12)</td>
<td>(12)</td>
<td>(25)</td>
<td>(16)</td>
<td>(14)</td>
</tr>
<tr>
<td><strong>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</strong></td>
<td>2.0</td>
<td>2.4</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(28)</td>
<td>(19)</td>
<td>(22)</td>
<td>(9)</td>
</tr>
<tr>
<td><strong>t&lt;sub&gt;max&lt;/sub&gt; (h)&lt;sup&gt;b&lt;/sup&gt;</strong></td>
<td>1.5</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>(1 - 2)</td>
<td>(1 - 2)</td>
<td>(1 - 4)</td>
<td>(1 - 4)</td>
<td></td>
</tr>
</tbody>
</table>

Values are geometric means (CV%) unless indicated otherwise. <sup>a</sup>Geometric means (90% confidence intervals). <sup>b</sup>Medians (ranges). CV, coefficient of variation; C<sub>max</sub>, maximum blood concentration; AUC<sub>0-4h</sub>, area under the blood concentration-time curve from 0 to 4 h; AUCR,
ratio of $\text{AUC}_{0-t}$ in presence of test article to vehicle, where $\text{AUC}_{0-t}$ is AUC from the time of dosing to time of the last observation; $\text{AUC}_{0-12h}$, AUC from 0 to 12 h; $t_{1/2}$, elimination half-life; $\text{AUC}_{\text{inf}}$, AUC from time of dosing extrapolated to infinity based on the last observed concentration; $t_{\text{max}}$, time to reach $C_{\text{max}}$; NA, not applicable. * $p < 0.05$ compared to vehicle by 1-way ANOVA with Dunnett’s post-test.
Table 3: Pharmacokinetics of intravenous metformin alone (presence of vehicle) or in the presence of orally administered goldenseal extract or imatinib in mice (n=7)

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Goldenseal extract</th>
<th>Imatinib</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC$_{0-12h}$ (µM•h)</td>
<td>5.5</td>
<td>7.0</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>(31)</td>
<td>(18)</td>
<td>(18)</td>
</tr>
<tr>
<td>AUCR$_{0-12h}^a$</td>
<td>NA</td>
<td>1.27</td>
<td>1.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1.10 – 1.48)</td>
<td>(0.97 – 1.50)</td>
</tr>
<tr>
<td>AUC$_{inf}$ (µM•h)</td>
<td>5.7</td>
<td>7.1</td>
<td>6.8</td>
</tr>
<tr>
<td></td>
<td>(30)</td>
<td>(19)</td>
<td>(18)</td>
</tr>
<tr>
<td>t$_{1/2}$ (h)</td>
<td>1.4</td>
<td>1.7</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>(34)</td>
<td>(18)</td>
<td>(39)</td>
</tr>
</tbody>
</table>

Values are geometric means (CV%) unless indicated otherwise. $^a$Geometric means (90% confidence intervals). CV, coefficient of variation; AUC$_{0-12h}$, area under the blood concentration-time curve from 0 to 12 h; AUCR, ratio of AUC$_{0-12h}$ in presence of test article to vehicle; AUC$_{inf}$, AUC from time of dosing extrapolated to infinity based on the last observed concentration; t$_{1/2}$, elimination half-life; NA, not applicable.
Figure 1
IC50 values represent model-generated best-fit estimates (95% confidence interval) of three replicates. IC50, half-maximum inhibitory concentration; OCT3, organic cation transporter 3; PMAT, plasma monoamine transporter; THTR2, thiamine transporter.

**Figure 2**
Figure 3
Figure 4