Goldenseal-Mediated Inhibition of Intestinal Uptake Transporters Decreases Metformin Systemic Exposure in Mice

Victoria O. Oyanna,† Kenisha Y. Garcia-Torres,† Baron J. Bechtold, Katherine D. Lynch, M. Ridge Call, Miklós Horváth, Preston K. Manwill, Tyler N. Graf, Nadja B. Cech, Nicholas H. Oberlies, Mary F. Paine, and John D. Clarke


Received April 11, 2023; accepted July 24, 2023

ABSTRACT

Goldenseal is a perennial plant native to eastern North America. A recent clinical study reported goldenseal decreased metformin Cmax and area under the blood concentration versus time curve (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 human embryonic kidney 293 cells overexpressing each transporter. The goldenseal extract, normalized to berberine content, was the strongest inhibitor of each transporter (IC50: 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 Cmax 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) (Weber et al., 2003; Mandal et al., 2020). 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 (Villinski et al., 2003; Abidi et al., 2006; Pirillo & Catapano, 2015). 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 (Wu et al., 2005; Hung et al., 1997; Asher et al., 2017).

ABBREVIATIONS: AUC, area under the blood concentration versus time curve; Cmax, maximum blood concentration; HEK, human embryonic kidney; IC50, half-maximal inhibitory concentration; Igut, 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.
Metformin is the first-line medication for the management of type 2 diabetes, which affects approximately 33 million people in the US (Centers for Disease Control and Prevention, 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 Cmax 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-hydastine, and a goldenseal extract normalized to berberine. The extract was the most potent (IC50 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 mono-amine 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 293 cells overexpressing each transporter. Second, the effects of orally administered goldenseal extract, berberine, (→)-β-hydastine, 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# 1157-70-4) was purchased from Enzo Life Sciences (Farmingdale, NY). 1,1-Dimethyl-4-phenylpyridinium iodide (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 (Mornmouth, NJ). (→)-β-Hydastine (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). Hydastinine chloride monohydrate (CAS# 65945-18-8), [3H]-1-methyl-4-phenylpyridinium iodide (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. Human embryonic kidney 293-LV cells overexpressing human OCT3, PMAT, or THTR2 were used to assess the inhibitory effect of each test article. The probe substrate was [3H]-1-methyl-4-phenylpyridinium iodide 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 quindine (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-hydastine, and hydastinine were tested at 10 and 100 μM. If >40% inhibition was observed at the higher concentration, then the 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 (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 and 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.

![Oral metformin/oral inhibitor](image1)

**Fig. 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-hydastine (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).
The groups and test articles for the oral metformin/oral inhibitor study are provided (Fig. 1A). Test articles were dissolved in 45% polyethylene glycol 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-hydastine were calculated to match their μg/ml content in the goldenseal extract (142 and 91 μg/ml, respectively). Blood (−25 μl) was collected through the tail vein into heparinized capillary tubes at 0.25, 0.5, 1, 2, 4, and 12 hours after administration of the test article(s). 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 × 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 liquid chromatography with tandem mass spectrometry analysis. Metformin concentrations were quantified using a QTRAP 6500 ultra-high-performance liquid chromatography with tandem mass spectrometry system (AB Sciex, Framingham, MA). Metformin was separated from matrix components using an ACQUITY UPLC BEH HILIC column (2.1 × 100 mm) at 30°C using the same mobile phases, flow rate, and gradient program from a published method (Schfer-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 > 201.139, 1,1-dimethyl-d6-biguanide HCl 136.174 > 118.130, and time to Cmax (tmax) were obtained directly from the concentration-time profiles.

Pharmacokinetic Analysis. The pharmacokinetics of metformin were determined via non-compartmental analysis methods using Phoenix WinNonlin (v8.3; Certara, Princeton, NJ). AUC was calculated as the ratio of the AUC of metformin in the presence to absence of inhibitor. AUC ratio (AUCR) for metformin was calculated as AUC_1 / AUC_0 × 100. The AUC ratio (AUCR) for metformin was calculated as AUC_1 / AUC_0 × 100. The pharmacokinetics of metformin were determined in Caco-2 cell monolayers (Han 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; Shirsaka et al., 2022). Han et al. and Shirsaka et al. reported different percent contributions for PMAT (20% versus 7%), SERT (20% versus 3%), 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 Km values for metformin toward SERT varied markedly (0.5–4 mM) (Han et al., 2015; Shirsaka 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

### Results

**Transport Inhibition Ssays.** 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-hydastine and hydrastidine inhibited all three transporters by <40%. The goldenseal extract was a more potent inhibitor than berberine of all three transporters; the IC₅₀ values for berberine alone were 2.8- to 7.4-fold higher than those for the extract (Fig. 2).

<table>
<thead>
<tr>
<th><strong>Concentration</strong> (μM)</th>
<th>OCT3 (%)</th>
<th>PMAT (%)</th>
<th>THTR2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goldenseal extract*</td>
<td>1.75</td>
<td>26 (14)</td>
<td>28 (3.8)</td>
</tr>
<tr>
<td>17.5</td>
<td>4.5 (2)</td>
<td>15 (4.5)</td>
<td>43 (12)</td>
</tr>
<tr>
<td>Berberine</td>
<td>10</td>
<td>55 (3.9)</td>
<td>8 (12)</td>
</tr>
<tr>
<td>100</td>
<td>95 (0.36)</td>
<td>71 (6.6)</td>
<td>43 (12)</td>
</tr>
<tr>
<td>(−)-β-hydastine</td>
<td>10</td>
<td>3 (9.5)</td>
<td>9 (7.3)</td>
</tr>
<tr>
<td>100</td>
<td>24 (6.0)</td>
<td>37 (7.3)</td>
<td>-4 (5.6)</td>
</tr>
<tr>
<td>Hydrastidine</td>
<td>10</td>
<td>5 (6.5)</td>
<td>11 (6.6)</td>
</tr>
<tr>
<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 (S.D.) of transporter activity by test article relative to vehicle (n=3).

**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-hydastine, and imatinib (Fig. 3). Relative to vehicle, goldenseal extract and imatinib decreased geometric mean metformin Cₘₐₓ by 31% and 25%, respectively, whereas berberine and hydrastine had no effect (Table 2). Goldenseal extract decreased geometric mean metformin AUC₀₋₁₂ by 30%, whereas imatinib, berberine, and (−)-β-hydastine had no effect. All test articles had no effects on geometric mean metformin AUC₀₋₁₂, t₁/₂, and median tₘₐₓ. Intravenously administered metformin was quantifiable in blood at all time points in the absence and presence of orally administered goldenseal extract and imatinib (Fig. 4). Neither the extract nor imatinib affected geometric mean metformin AUC₀₋₁₂ and t₁/₂ (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; Shirsaka et al., 2022). Han et al. and Shirsaka et al. reported different percent contributions for PMAT (20% versus 7%), SERT (20% versus 3%), 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 Km values for metformin toward SERT varied markedly (0.5–4 mM) (Han et al., 2015; Shirsaka 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 IC₅₀ values when tested alone compared with the goldenseal extract (Fig. 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 berberine inhibited metformin systemic exposure, but with higher IC₅₀ values when tested alone compared with the goldenseal extract (Nguyen et al., 2021), suggesting that other unidentified goldenseal constituents contribute to inhibition of these transporters.

The difference in the effects of goldenseal on metformin AUC₅₀ 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 and 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; Liang et al., 2015). OCT1 is localized on the basolateral membrane of hepatocytes and contributes to clearance from the blood.

**Fig. 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 S.D.s, respectively, in the absence or presence of test articles (n = 8 per group).

**TABLE 2**

<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>Cmax (μM)</td>
<td>1.6 (21)</td>
<td>1.1* (18)</td>
<td>1.4 (28)</td>
<td>1.4 (23)</td>
<td>1.2* (14)</td>
</tr>
<tr>
<td>AUC0-4h (μM·h)</td>
<td>4.7 (24)</td>
<td>3.3* (17)</td>
<td>4.1 (26)</td>
<td>4.4 (21)</td>
<td>3.7 (12)</td>
</tr>
<tr>
<td>AUCR0-4h</td>
<td>NA</td>
<td>0.69 (0.59–0.83)</td>
<td>0.87 (0.67–1.15)</td>
<td>0.93 (0.82–1.07)</td>
<td>0.79 (0.65–0.96)</td>
</tr>
<tr>
<td>AUC0-12h (μM·h)</td>
<td>7.6 (22)</td>
<td>6.3 (12)</td>
<td>6.9 (25)</td>
<td>7.2 (20)</td>
<td>6.7 (13)</td>
</tr>
<tr>
<td>AUCR0-12h</td>
<td>NA</td>
<td>0.83 (0.74–0.95)</td>
<td>0.92 (0.71–1.19)</td>
<td>0.95 (0.82–1.11)</td>
<td>0.89 (0.74–1.07)</td>
</tr>
<tr>
<td>AUCinf (μM·h)</td>
<td>7.0 (12)</td>
<td>6.6 (12)</td>
<td>7.2 (25)</td>
<td>7.3 (16)</td>
<td>6.9 (14)</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>2.0 (10)</td>
<td>2.4 (28)</td>
<td>2.0 (19)</td>
<td>2.0 (22)</td>
<td>2.0 (9)</td>
</tr>
<tr>
<td>tmax (h)a</td>
<td>1.5 (1 – 2)</td>
<td>2.0</td>
<td>2.0 (1–2)</td>
<td>2.0 (1–4)</td>
<td>2.0 (1–4)</td>
</tr>
</tbody>
</table>

Values are geometric means (CV%) unless indicated otherwise. *Geometric means (90% confidence intervals). aMedians (ranges). AUC0-4h, area under the blood concentration-time curve from 0 to 4 h; AUC0-12h, AUC from 0 to 12 h; AUCinf, AUC from time of dosing extrapolated to infinity based on the last observed concentration; AUCR, ratio of AUC0-12h in presence of test article to vehicle, where AUC0-12h is AUC from the time of dosing to time of the last observation; CV, coefficient of variation; NA, not applicable; t1/2, elimination half-life; tmax, time to reach Cmax. *P < 0.05 compared with vehicle by one-way ANOVA with Dunnett’s post-test. Sample sizes for all values were n=8, except AUCinf and t1/2 sample sizes which were n=6 for vehicle and goldenseal and n=7 for hydrastine and imatinib.
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 S.D.s, respectively, in the absence or presence of test articles (n = 7 per group).

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

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

### Authorship Contributions

**Participated in research design:** Oyanna, Garcia-Torres, Bechtold, Lynch, Call, Horváth, Manwill, Graf, Cech, Oberlies, Paine, Clarke.

**Conducted experiments:** Oyanna, Garcia-Torres, Bechtold, Lynch, Call, Horváth, Clarke.

**Contributed new reagents or analytic tools:** Manwill, Graf, Cech, Oberlies.

**Performed data analysis:** Oyanna, Garcia-Torres, Horváth, Clarke.

**Wrote or contributed to the writing of the manuscript:** Oyanna, Bechtold, Lynch, Call, Horváth, Manwill, Cech, Oberlies, Paine, Clarke.

### References


Address correspondence to: Dr. John D. Clarke, 412 E. Spokane Falls Blvd, Washington State University, College of Pharmacy and Pharmaceutical Sciences, Spokane, WA 99202. E-mail: j.clarke@wsu.edu