Biochemical Pharmacology 84 (2012) 1088–1095

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Potential for food–drug interactions by dietary phenolic acids on human organic anion transporters 1 (SLC22A6), 3 (SLC22A8), and 4 (SLC22A11)

Li Wang, Douglas H. Sweet *

Department of Pharmaceutics, Virginia Commonwealth University, 410 N 12th Street, Richmond, VA 23298-0533, USA

ARTICLE INFO

Article history:
Received 11 June 2012
Accepted 25 July 2012
Available online 31 July 2012

Keywords:
Drug–drug interactions
Drug–food interaction
Pharmacokinetics
Renal transport
Solute carriers

ABSTRACT

Phenolic acids exert beneficial health effects such as anti-oxidant, anti-carcinogenic, and anti-inflammatory activities and show systemic exposure after consumption of common fruits, vegetables, and beverages. However, knowledge regarding which components convey therapeutic benefits and the mechanism(s) by which they cross cell membranes is extremely limited. Therefore, we determined the inhibitory effects of nine food-derived phenolic acids, *p*-coumaric acid, ferulic acid, gallic acid, gentisic acid, 4-hydroxybenzoic acid, protocatechuic acid, sinapinic acid, syringic acid, and vanillic acid, on human organic anion transporter 1 (hOAT1), hOAT3, and hOAT4. In the present study, inhibition of OAT-mediated transport of prototypical substrates (1 μM) by phenolic acids (100 μM) was examined in stably expressing cell lines. All compounds significantly inhibited hOAT3 transport, while just ferulic, gallic, protocatechuic, sinapinic, and vanillic acid significantly blocked hOAT1 activity. Only sinapinic acid inhibited hOAT4 (~35%). For compounds exhibiting inhibition > ~60%, known clinical plasma concentration levels and plasma protein binding in humans were examined to select compounds to evaluate further with dose–response curves (IC_{50} values) and drug–drug interaction (DDI) index determinations. IC_{50} values ranged from 1.24 to 18.08 μM for hOAT1 and from 7.35 to 87.36 μM for hOAT3. Maximum DDI indices for gallic and gentisic acid (≫0.1) indicated a very strong potential for DDIs on hOAT1 and/or hOAT3. This study indicates that gallic acid from foods or supplements, or gentisic acid from salicylate-based drug metabolism, may significantly alter the pharmacokinetics (efficacy and toxicity) of concomitant therapeutics that are hOAT1 and/or hOAT3 substrates.

1. Introduction

The term, phenolic acid, describes a large group of compounds that contain an aromatic ring bearing hydroxyl and carboxyl substituents [1]. In plants, phenolic acids are synthesized from phenylalanine or tyrosine via the shikimate pathway and they are widely distributed in fruits, vegetables, and beverages [2]. As phenolic acids have been reported to possess anti-oxidant, anti-carcinogenic, and anti-inflammatory activities, they are believed to reduce the risk of developing chronic diseases, e.g., diabetes, cardiovascular disorders, and certain cancers [1–3]. A number of phenolic acids showed marked systemic exposure in vivo after daily consumption of phenolic acid-rich food [3,4].

Additionally, in vivo metabolism generates phenolic acids. For example, gentisic acid was identified as one of the major metabolites of salicylate, and urinary elimination of gentisic acid accounted for 0.4–1.7% of the total administered dose of salicylate drugs [5,6]. Another study indicated that ferulic and isoferulic acids may be metabolites of caffeic acid [7]. Finally, anthocyanins (ACNs) were recently identified as an important dietary source of phenolic acids. ACNs are flavonoids which are widely distributed in flowers, berries, grapes, red wine, and blood orange juice, producing blue and red color [8]. Daily consumption of ACNs was estimated as 3–215 mg [8]. Yet, it was reported in clinical studies that the bioavailability of ACNs was poor [8]. However, in vitro studies demonstrated that phenolic acids represent major stable degradation products of ACNs, and this was confirmed in clinical studies [9–11]. Thus, phenolic acids are commonly found in the systemic circulation even in individuals not actively taking phenolic acid containing dietary supplements or alternative medicines.

Abbreviations: ACN, anthocyanin; BCRP, breast cancer resistance protein; C_{max}, maximum plasma concentration; CHO, Chinese hamster ovary; DDI, drug–drug interaction; ES, estrone sulfate; f_u, fraction unbound in plasma; FDA, Food and Drug Administration; HEK, human embryonic kidney 293; h, human; IC_{50}, median maximal inhibitory concentration; K_i, inhibitory constant; K_m, Michaelis constant; MDR1, multidrug resistance transporter 1; OAT, organic anion transporter; OATP1B1, organic anion transporting polypeptide 1B1; OCT2, organic cation transporter 2; PAH, *p*-aminohippuric acid; SLC, solute carrier.

* Corresponding author. Tel.: +1 804 828 3860; fax: +1 804 828 8359.
E-mail addresses: wangl4@vcu.edu (L. Wang), dsweet@vcu.edu (D.H. Sweet).

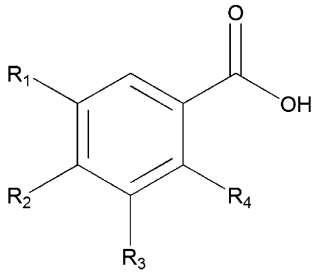
0006-2952/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcp.2012.07.027

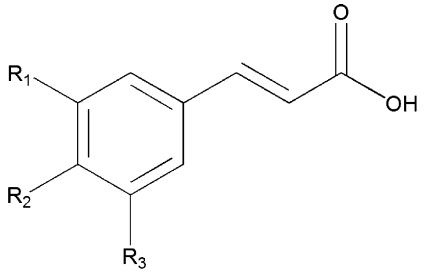
Several transporter families are responsible for the systemic disposition of organic acids. Among these, the Solute Carrier 22 (SLC22; organic cation/anion/zwitterion transporters) family is a key mediator in the distribution and renal tubular secretion of a multitude of endogenous and exogenous organic anions [12,13]. Many toxins, toxicants, and drugs (including antibiotics, antiviral and anticancer agents) are known organic anion transporter (OAT) substrates [12,13]. Further, some phenolic acids, e.g., tanshinol, rosmarinic acid, and salvianolic acid B, exhibit their highest tissue concentration in the kidney compared to other tissues, e.g., liver, intestine, and brain, after dosing [14,15]. In accordance, the kidney is known to express more OAT family members than any other tissue [12]. In particular, human organic anion transporter 1 (hOAT1; SLC22A6), hOAT2 (SLC22A7), and hOAT3 (SLC22A8), expressed in the basolateral membrane of renal proximal tubule cells, and hOAT4 (SLC22A11), hURAT1 (SLC22A12), and hOAT10 (SLC22A13) located in the apical membrane, are major constituents of the renal organic anion transport pathway.

OAT-mediated drug–drug interactions (DDIs), manifesting as altered renal clearance and longer terminal plasma half-life, have ben observed in a number of clinical and pre-clinical studies. For example, renal clearance of benzylpenicillin and ciprofloxacin was reduced when co-administered with probenecid, a known OAT inhibitor, in clinical practice [16,17]. In accord with these clinical findings, pharmacokinetic studies using organic anion transporter 3 knockout mice demonstrated significantly reduced renal elimination of benzylpenicillin, ciprofloxacin, and methotrexate [18,19]. In response to our increased knowledge of such clinically relevant drug–transporter interactions, and the impact they have on drug safety, the United States Food and Drug Administration (FDA) and the European Medicines Agency have issued guidance documents outlining conditions under which, prior to approval, any new drug entity should be investigated for potential DDIs on seven identified transporters; OAT1, OAT3, organic cation transporter 2 (OCT2), organic anion transporting polypeptide 1B1 (OATP1B1), OATP1B3, multidrug resistance transporter 1 (MDR1), and breast cancer resistance protein (BCRP) (http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf and http://www.ema.europa.eu/ema/index.jsp?curl=pages/includes/document/document_detail.jsp?webContentId=WC500090112&murl=menus/document_library/document_library.jsp&mid=WC0b01ac058009a3dc&jsenabled=true). Thus, the potential clinical significance of OAT1- and OAT3-mediated DDIs is already clearly recognized.

As most phenolic acids are small and mainly exist as anions at physiological pH, it is possible that they are OAT substrates or inhibitors. For example, some hydroxycinnamic acids, including caffeic acid, dihydrocaffeic acid, dihydroferulic acid, and ferulic acid were identified as hOAT1 and/or hOAT3 substrates [20]. This study further demonstrated that these compounds, as well as their glucuronide- and sulfate-conjugated metabolites, inhibited hOAT1 or hOAT3 transport activity [20]. Caffeic acid was identified as a competitive inhibitor for hOAT1 and hOAT3, with IC_{50} estimates of 16.6 μM and 5.4 μM, respectively [21]. As a consequence, hOAT1/hOAT3-mediated transport of antifolates and antivirals was inhibited by caffeic acid [21]. Finally, ellagic acid, which is a dietary polyphenol found in many fruits and vegetables, was demonstrated to be a potent inhibitor for hOAT1 (IC_{50} = 207 nM) and hOAT4 [22]. Therefore, it is necessary to explore the potential interaction of phenolic acids with OATs, in order to determine what transport processes influence the distribution and elimination (and hence efficacy and toxicity) of these compounds. This information is also vital to avoid potential drug/food–drug interactions.

In the present study, the inhibitory effects of nine dietary phenolic acids, *p*-coumaric acid, ferulic acid, gallic acid, gentisic acid, 4-hydroxybenzoic acid, protocatechuic acid, sinapinic acid, syringic acid, and vanillic acid, on the transport of *p*-aminohippuric acid (PAH) mediated by hOAT1 and the transport of estrone sulfate (ES) mediated by hOAT3 and hOAT4 were characterized. For potent inhibitors, dose–response studies were conducted in order to derive inhibitory constants (IC_{50} and K_i values) to aid evaluation of potential for clinical DDIs. Among these phenolic acids, gallic acid exhibited highest affinity for hOAT1 and hOAT3, with corresponding maximum DDI indices (unbound maximum plasma concentration/IC_{50}) of 5.52 and 0.76, respectively. Gentisic acid exhibited a marked DDI index of 7.25 for hOAT3 (assuming 90% binding to plasma protein). These findings suggested there is a strong potential for gallic acid- and gentisic acid-associated food/drug–drug interactions with co-administered clinical therapeutics that are OAT substrates, including altered drug pharmacokinetics, pharmacodynamics, and toxicity.





Compounds	R1	R2	R3	R4
Gallic acid	OH	OH	OH	H
Gentisic acid	OH	H	H	OH
4-Hydroxybenzoic acid	H	OH	H	H
Protocatechuic acid	OH	OH	H	H
Syringic acid	OCH_3	OH	OCH_3	H
Vanillic acid	OCH_3	OH	H	H

Compounds	R1	R2	R3
p-Coumaric acid	H	OH	H
Ferulic acid	OCH_3	OH	H
Sinapinic acid	OCH_3	OH	OCH_3

Fig. 1. Chemical structures of the nine dietary phenolic acids investigated in this study.