THE DIETARY POLYPHENOL ELLAGIC ACID IS A POTENT INHIBITOR OF hOAT1

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ABSTRACT:

Ellagic acid (EA), a polyphenol present in berries, has been demonstrated to be preventive of esophageal and colon cancer in animals. Here, we have studied the ability of organic anion transporters (OATs) and organic anion-transporting polypeptides (OATPs) to transport EA. The accumulation of radiolabeled [14C]EA, [3H]p-aminohippuric acid (PAH), [14C]glutarate, [3H]estrone sulfate, [3H]ochratoxin A, and [3H]taurocholic acid ± inhibitor(s) was tested in OAT- and OATP-expressing oocytes. Oocytes expressing human (h)OAT1, rat (r)Oat1, and hOAT4 accumulated 6.5-, 7.1-, and 8.9-fold more EA, respectively, than did water-injected oocytes. This accumulation was prevented by the prototype OAT inhibitors bromosulfophthalein and probenecid. rOatp1, mouse (m)Oat2, hOAT3, and mOat5 showed no EA transport. The uptake of the prototype OAT substrate PAH in hOAT1-expressing oocytes was dose dependently and potently inhibited by EA with an IC50 of 207 nM. In conclusion, we have demonstrated that the OAT family members hOAT1, rOat1, and hOAT4 mediate transport of EA, with a very high affinity for hOAT1.

Organic anion transporters (OATs) play a critical role in the distribution and elimination of a diverse array of exogenous and endogenous compounds. The substrates of OATs are small organic anions at physiologic pH and include a multitude of clinically used therapeutics such as angiotensin-converting enzyme inhibitors, β-lactam antibiotics, and nonsteroidal anti-inflammatory drugs. Certain drugs that are eliminated from the body mainly by the kidneys through the OATs can compete with other OAT substrates for transport. This competition for transport can cause retention of certain drugs, leading to longer plasma half-lives (Burckhardt and Burckhardt, 2003). Historically, the interaction was utilized to maintain penicillin plasma levels with the use of probenecid.

Plant polyphenols are the focus of much research for the ability to affect adverse human biological disease states. One of these polyphenols, ellagic acid (EA), found naturally in our diet (Lei et al., 2001), has been demonstrated to be a cancer-preventive agent for esophageal cancer in animal models (Stoner and Gupta, 2001). EA is a small organic anion at physiologic pH (Priyadarssini et al., 2002), and its accumulation in human intestinal Caco-2 cells was demonstrated to maintain penicillin plasma levels with the use of probenecid.

Xenopus laevis Oocyte Uptake Assay. X. laevis oocytes were obtained as described previously (Youngblood and Sweet, 2004). Substrate uptake assays were performed 3 days after injection with 20 ng of capped cRNA (hOAT1, rOat1, mOat2, hOAT3, mOAT4, mOat5, or rOatp1), as previously described (Cihlar et al., 1999). Oocytes were randomly divided into experimental groups of 10 and incubated for 60 min (Sweet et al., 1997) in 1 ml of oocyte Ringer medium containing 20 mM [14C]EA in all types of oocytes, 10 μM [3H]p-aminohippurate (PAH) in hOAT1 and rOat1 oocytes (Pritchard and Miller, 1993), 1 μM [3H]estrone sulfate in hOAT3 oocytes (Cha et al., 2001), 36.4 μM [14C]glutarate in mOat2 oocytes (Kobayashi et al., 2002), 1 μM [3H]ochratoxin A and 1 μM [3H]estrone sulfate in hOAT4 oocytes (Cha et al., 2000), 1 μM [3H]ochratoxin A in mOat5 oocytes (Youngblood and Sweet, 2004), and 300 nM [3H]taurocholate in rOatp1 oocytes (Li et al., 1998). The uptake inhibitor used was 1 mM probenecid (Cihlar et al., 1999) or 500 μM bromosulfophthalein (BSP) (A. C Whitley, D. H. Sweet, and T. Walle, manuscript submitted for publication). Other inhibitors used were 0.05 to 35 μM EA, 5 to 200 μM indoxyl sulfate, or 50 to 900 μM sodium salicylate (Khamdang et al., 2002; Enomoto et al., 2003). Oocytes were rapidly rinsed four times with ice-cold oocyte Ringer medium, and after digestion with sodium hydroxide, individual oocyte radioactivity was measured by liquid scintillation spectrometry with external quench correction. Each experiment was repeated in three different animals. Water-injected oocytes were included as negative controls in each experiment.

Statistics. Data are expressed as means ± S.E. Statistical differences were determined using analysis of variance followed by Dunnett’s multiple comparison test. Differences were considered significant when P ≤ 0.05.
Results

Oocyte Accumulation. Experiments in oocytes were conducted to identify specific transporters that recognize EA as a substrate. The uptake of radiolabeled positive markers for each transporter was used to confirm protein expression at the membrane. Probenecid and BSP were used as inhibitors of positive control uptake and exhibited significant inhibition in cRNA-injected oocytes with little or no effect in water-injected oocytes (data not shown). Accumulation of 20 μM $[^{14}C]$EA was measured in water-injected oocytes and oocytes expressing hOAT1, rOat1, mOat2, hOAT3, hOAT4, mOat5, or rOatp1. As seen in Fig. 1, $[^{14}C]$EA was significantly accumulated in oocytes expressing hOAT1 (6.4 ± 1.4-fold), rOat1 (7.0 ± 1.4-fold), and

**Fig. 1.** Cellular accumulation of EA in OAT-expressing oocytes. Oocytes were incubated for 60 min with 20 μM $[^{14}C]$EA in the presence or absence of 1 mM probenecid or 500 μM BSP. *, $P < 0.01$, $n = 19$ except for BSP treatment, where $n = 6$.

**Fig. 2.** Cellular accumulation of PAH in oocytes expressing hOAT1 (A) and rOat1 (B), and of OA in hOAT4-expressing oocytes (C). Oocytes were incubated for 60 min with 10 μM $[^{3}H]$PAH or 1 μM $[^{3}H]$OA in the presence or absence of 0.05 to 35 μM EA or 1 mM probenecid. The values are expressed as percentage of control (without inhibitor). *, $P < 0.05$, **, $P < 0.01$, compared to control ($n = 9$).
Studies in Caco-2 cells demonstrated that cellular accumulation of EA was dose dependently and potently inhibited by BSP (Whitley et al., manuscript submitted for publication). This finding suggested that members of the OAT and/or OATP families of transporters may mediate the cellular entry of EA. Therefore, to directly test the ability of OAT and OATP family members to transport EA, EA uptake was measured in oocytes expressing individual members of both families. hOAT4 and the orthologs hOAT1 and rOat1 were identified as transporters of EA. The increase in accumulation of EA when these transporters were expressed was completely inhibited by the commonly used OAT inhibitors, probenecid and BSP. Other members of the OAT family, as well as rOatp1, did not transport EA. Upon further investigation of hOAT1 and rOat1, EA displayed a potent ability to inhibit transport of PAH, a prototypical substrate (Pritchard and Miller, 1993).

Although EA per se has been reported to have very low oral bioavailability (Teel and Martin, 1988), significant plasma concentrations may be achieved from natural precursor sources, e.g., ellagitannins, as recently reported (Seeram et al., 2004). The very low IC50 of 207 nM for the EA inhibition of hOAT1 transport indicates an extraordinary affinity of this transporter for EA. This very high affinity could lead to EA-drug interactions, since hOAT1 is a key component in the renal secretion of a wide variety of therapeutics and endogenous substrates such as β-lactam antibiotics, angiotensin-converting enzyme inhibitors, nonsteroidal anti-inflammatory drugs, antiviral drugs, prostaglandins, and diuretics. It is interesting to compare the OAT1 IC50 for EA and the published OAT1 Km values of acyclovir (342 μM) and AZT (45.9 μM) (Burckhardt and Burckhardt, 2003). Therefore, based on the localization of OAT1, an EA-drug interaction could potentially be utilized to attain higher plasma and/or cerebrospinal fluid levels of either acyclovir or AZT by decreasing their renal elimination and/or cerebrospinal fluid elimination by the choroid plexus, respectively. This high affinity interaction may also lead to its development as a useful diagnostic in OAT1 transport research, i.e., its use as a “chemical knockout” of OAT1.

In conclusion, X. laevis oocytes expressing hOAT1, rOat1, and hOAT4 were all demonstrated to mediate efficient inhibitor-sensitive transport of EA. Finally, we showed the interaction between hOAT1 and rOat1 with EA to be one of high affinity. This study demonstrates the potential interaction of EA with therapeutics and/or endogenous substrates through OATs expressed in the kidney and/or blood-brain barrier.

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