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Interactions of green tea catechins with organic anion transporting polypeptides

Megan Roth, Barbara N. Timmermann and Bruno Hagenbuch

Department of Pharmacology, Toxicology and Therapeutics, The University of Kansas Medical Center, Kansas City, Kansas (M.R., B.H.); Department of Medicinal Chemistry, The University of Kansas, Lawrence, Kansas (B.N.T.); The University of Kansas Cancer Center, Kansas City, Kansas (B.H.)

Running Title: Effect of green tea catechins on OATP function

Address of correspondence:

Bruno Hagenbuch

Department of Pharmacology, Toxicology and Therapeutics

The University of Kansas Medical Center

3901 Rainbow Blvd.

Kansas City, KS 66160

Phone: +1-913-588-0028

Fax: +1-913-588-7501

E-Mail: bhagenbuch@kumc.edu

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Abbreviations: DHEAS, dehydroepiandrosterone sulfate; DMSO, dimethyl sulfoxide; EC, (-)-epicatechin; ECG, (-)-epicatechin gallate; EGC, (-)-epigallocatechin gallate; EGCG, (-)-epigallocatechin gallate; OATP, organic anion transporting polypeptide.

Abstract:

Organic anion transporting polypeptides (OATPs) are multispecific transporters that mediate the uptake of numerous drugs and xenobiotics into cells. Here, we examined the effect of green tea (*Camellia sinensis)* catechins on the function of the four OATPs expressed in human enterocytes and hepatocytes. Uptake of the model substrate estrone-3-sulfate by cells expressing OATP1A2, OATP1B1, OATP1B3 or OATP2B1 was measured in the absence and presence of the four most abundant flavanols found in green tea. Uptake by OATP1A2, OATP1B1, and OATP2B1 was inhibited by epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) in a concentration dependent way. In contrast, OATP1B3-mediated uptake of estrone-3-sulfate was strongly stimulated by EGCG at low substrate concentrations. The effect of EGCG on OATP1B3 was also studied with additional substrates: uptake of estradiol-17β-glucuronide was unchanged while uptake of Fluo-3 was noncompetitively inhibited. Both ECG and EGCG were found to be substrates of OATP1A2 (K_m values of 10.4 μM and 18.8 μM, respectively) and OATP1B3 (34.1 μM and 13.2 μM, respectively), but not of OATP1B1 or OATP2B1. These results indicate that two of the major flavanols found in green tea have a substantial effect upon the function of OATPs expressed in enterocytes and hepatocytes and can potentially alter the pharmacokinetics of drugs and other OATP substrates. In addition, the diverse effects of EGCG on the transport of other OATP1B3 substrates suggest that different transport/binding sites are involved.

Introduction:

Adverse drug-drug interactions are a common result of comorbidity and polypharmacy, and pose a significant health threat. Furthermore, dietary supplements are increasingly popular and some of their ingredients have the potential for additional drug interactions. These adverse drug interactions may be caused by alterations in efflux (Durr et al., 2000) and uptake transporters (Fattinger et al., 2000), such as the organic anion transporting polypeptides (OATPs). OATPs are multispecific transporters that mediate the cellular uptake of a wide range of amphipathic compounds, including numerous drugs (Hagenbuch and Gui, 2008). Four well-characterized OATPs are expressed in the small intestine and the liver, where the likelihood of drug-drug or drug-food interactions is the greatest: OATP1A2, OATP1B1, OATP1B3 and OATP2B1.
Both OATP1A2 and OATP2B1 are expressed at the apical membrane of enterocytes (Kobayashi et al., 2003; Glaeser et al., 2007) where they can contribute to the absorption of drugs like statins, sartans, fexofenadine, talinolol and methotrexate (Shimizu et al., 2005; Badagnani et al., 2006; Ho et al., 2006; Kitamura et al., 2008; Shirasaka et al., 2010). In the liver, OATP1B1, OATP1B3, and OATP2B1 are expressed at the basolateral membrane of hepatocytes (Abe et al., 1999; König et al., 2000; Kullak-Ublick et al., 2001). Here, these proteins are involved in the removal of drugs from the bloodstream into hepatocytes. With their broader substrate specificity, OATP1B1 and OATP1B3 are thought to play a more important role in hepatocellular drug uptake than OATP2B1 (Smith et al., 2005; Hagenbuch and Gui, 2008; Kindla et al., 2009).

The importance of OATPs to drug disposition has been highlighted by pharmacokinetic studies that correlated changes in the bioavailability of drugs with polymorphisms of OATPs (Kalliokoski and Niemi, 2009). Thus, inhibition or stimulation of OATP function by food or dietary supplements can alter the pharmacokinetics of OATP substrates and potentially lead to adverse effects. Recent studies have indicated that flavonoids found in fruit juices, in green tea, and in many dietary supplements can alter the function of OATP1A2, OATP1B1 and OATP2B1 (Wang et al., 2005; Fuchikami et al., 2006; Bailey et al., 2007). Such interactions can affect the bioavailability of drugs like fexofenadine and celiprolol, known OATP substrates (Greenblatt, 2009).

Green tea is a commonly consumed beverage, and has received much attention for its reputed health benefits. Several epidemiological studies have shown a reduced risk of gastrointestinal cancers among those who regularly consume green tea (reviewed in Liu et al., 2008). Compared to other tea preparations, green tea is characterized by very high concentrations of catechins, which make up 30-40% of its dry weight. The catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG). EGCG, the most predominant catechin in green tea, has been highly studied for its *in vitro* effects. Due to the many apparent health benefits of green tea and EGCG, green tea beverages and extract supplements are widely used, creating an increased risk of adverse interactions. EGC and EGCG have been shown to inhibit OATP1B1-mediated uptake of dehydroepiandrosterone sulfate (DHEAS) (Wang et al., 2005) while all four catechins inhibited estrone-3-sulfate uptake mediated by OATP2B1 (Fuchikami et al., 2006). However, the effect of catechins on the function of OATP1A2 and OATP1B3 has not been reported and it is not known

whether any of these four catechins are transported by any of the OATPs. Therefore in the present study we asked the question whether all four major catechins inhibit OATP-mediated uptake and whether they are transported by OATPs.

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Materials and Methods:

Materials. [^{3}H]Estrone-3-sulfate (54.26 Ci/mmol) and [^{3}H]estradiol-17β-glucuronide (41.8 Ci/mmol) were purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). [^{3}H]Epigallocatechin gallate (10 Ci/mmol) was purchased from American Radiolabled Chemicals, Inc. (St. Louis. MO). Unlabeled estrone-3-sulfate, estradiol-17β-glucuronide, (-)-epicatechin, (-)-epigallocatechin, (-)-epicatechin gallate and (-)-epigallocatechin gallate were purchased from Sigma-Aldrich (St. Louis, MO). Fluo-3, pentapotassium salt was purchased from Invitrogen (Carlsbad, CA). Green tea (*Camellia sinensis*) biomass was provided by the Royal Estates Tea Company, a Division of Thomas J. Lipton, Co. (Englewood Cliffs, NJ). A sample of green tea biomass was extracted exhaustively with 10 mL H_2O (70 °C, 10 min). The extract was concentrated *in vacuo* and dried overnight at 30 °C in a vacuum oven. Dulbecco's Modified Eagle's Medium was purchased from Caisson Laboratories (North Logan, UT), and Eagle's Minimum Essential Medium was purchased from ATCC (Manassas, VA). Fetal bovine serum was obtained from HyClone (Logan, Utah). All other materials were purchased from Sigma-Aldrich (St. Louis, MO) or Invitrogen (Carlsbad, CA).

OATP Expression. CHO cells stably transfected with human OATP1B1, OATP1B3 and OATP2B1 were generated in our lab previously, and were cultured as described (Gui et al., 2008; Pacyniak et al., 2010). OATP1A2 was transiently expressed in HeLa or HEK293 cells. HeLa cells were grown in Eagle's Minimum Essential Medium supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. HEK-293 cells were grown in Dulbecco's Modified Eagle's Medium containing 4.5 g/L D-glucose, 2 mM L-glutamine, 25 mM Hepes buffer, and 110 mg/L sodium puruvate,

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supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. All cells were maintained in a humidified environment at 37 °C and 5% CO_2.

Transport Assays. Cells expressing OATP1B1 or OATP1B3 were seeded on 24-well plates and induced in the absence of geneticin in media containing 5 mM sodium butyrate 24 hours prior to uptake experiments. CHO cells stably expressing OATP2B1 were seeded on 24-well plates 48 hours prior to uptake experiments; OATP2B1 expression in this cell line did not require sodium butyrate induction. OATP1A2 was transiently expressed in HeLa or HEK-293 cells. HeLa cells were seeded on 12-well plates and transfected using the vaccinia virus T7 system, essentially as previously described (Lee et al., 2005). Between 16 and 20 hours prior to uptake experiments, cells were infected with vaccinia virus in serum-free OptiMEM medium and incubated for one hour at 37°C. After washing, cells were transfected with pcDNA5/FRT containing the open reading frame of a His-tagged OATP1A2 or with the empty vector using Lipofectamine 2000, as per manufacturer's instructions. HEK-293 cells were seeded on 24-well plates pretreated with poly-D-lysine, and were transfected with pExpress-1 (Express Genomics, Inc., Frederick, MD) containing OATP1A2 or with the empty vector approximately 48 hours prior to uptake experiments.

Uptake experiments were performed essentially as previously described for CHO (Gui et al., 2008), and HEK293 cells (Weaver and Hagenbuch, 2010). HeLa uptake buffer contained 100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM Hepes, and was adjusted to pH 7.5 with Trizma base. Catechins were dissolved in DMSO, and stock solutions were stored at -20 °C. A final DMSO concentration of 1% was maintained in all experiments. After brief washing, cells were incubated at 37 °C with

uptake buffer containing substrate and inhibitors. Uptake was terminated by removing the uptake solution and washing four times with ice-cold uptake buffer. To measure uptake of radiolabeled substrates, cells were lysed with 1% Triton X-100 in PBS, and the radioactivity was quantified with liquid scintillation counting. To measure uptake of Fluo-3, cells were lysed with 1% Triton X-100 in PBS containing 1 mM $CaCl_2$, and fluorescence was quantified on a Bio-Tek Synergy HT microplate reader (Winooski, VT) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. Unlabeled catechins were detected using a Quattro Premier high performance liquid chromatography tandem mass spectrometer (Waters Corp., Milford, MA) in electrospray negative ion mode using a Phenomenex C18 column (50 x 2.1mm, 5 μM) at 40 °C. Mobile phase consisted of 60/40 acetonitrile and 1% acetic acid, and was eluted isocratically with a flow fate of 0.3 mL per minute. Cells were lysed in mobile phase containing 1 μM ethyl gallate, and lysate was centrifuged at 20,000xg for 20 minutes to remove protein prior to injection. The transitions monitored were 441.15 > 169.1 for ECG, 457.05 > 169.1 for EGCG, and 197.17 > 124.2 for ethyl gallate (internal standard). QuanLynx software (Waters Corp.) was utilized to quantify mass spectrometry data. Protein concentrations were determined with a BCA assay kit (Thermo Fisher Scientific, Rockford, IL) and uptake was corrected for protein. Net OATP-mediated uptake was defined as the uptake by OATP-expressing cells minus the uptake by the appropriate control cell line (wild-type CHO cells for OATP1B1 and OATP1B3, CHO cells stably expressing empty vector for OATP2B1, and cells transiently expressing the empty vector for OATP1A2.)

Calculations and Statistics. All calculations were performed using Prism 5 (GraphPad Software Inc., La Jolla, CA). Determination of IC_{50} values and kinetic parameters were performed within the initial linear period of uptake after correcting for protein and subtracting uptake by the control cell line. Statistical analysis was performed with Two Way ANOVA followed by the Bonferroni post-test.

Results

Characterization of OATP1A2 and OATP2B1 expression systems. OATP2B1 was expressed at high levels on the plasma membrane of the stably transfected OATP2B1-expressing CHO cells as confirmed using an anti-His antibody (data not shown). In order to minimally characterize OATP2B1 and the transiently expressed OATP1A2 at a functional level, we used the model substrate estrone-3-sulfate. Uptake of 100 µM estrone-3-sulfate was linear for at least 30 seconds for both OATP1A2 and OATP2B1 (data not shown); therefore, subsequent experiments were performed at 30 seconds for OATP1A2 and 20 seconds for OATP2B1. In both systems, transport of estrone-3-sulfate was saturable, with apparent K_m and V_{max} values of 16.1 ± 0.2 µM and 640 ± 150 pmol/mg*min for OATP1A2 and 14.8 ± 4.0 µM and 2.54 ± 0.57 nmol/mg*min for OATP2B1, respectively.

Effect of green tea extract and catechins on OATP-mediated uptake of estrone-3-sulfate. To determine the effects of green tea components on OATP function, we measured OATP-mediated uptake of 0.1 µM estrone-3-sulfate in the presence of 0.03 µg/mL green tea extract or 100 µM green tea catechin under initial linear rate conditions. Epicatechin (EC) and epigallocatechin (EGC) did not significantly affect estrone-3-sulfate uptake by any of the four cell lines (Figure 1A-D). Epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) inhibited uptake of estrone-3-sulfate by OATP1A2, OATP1B1 and OATP2B1. Interestingly, OATP1B3-mediated uptake of estrone-3-sulfate was unaffected by ECG but strongly stimulated by EGCG (Figure 1C). To further characterize the effect of the gallated catechins on OATP-mediated uptake of estrone-3-sulfate, we determined uptake of 0.1 µM estrone-3-sulfate in the presence of

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increasing concentrations of ECG or EGCG. As shown in Figure 2, both ECG and EGCG exhibited a concentration dependent inhibition of estrone-3-sulfate uptake mediated by OATP1A2, OATP1B1 and OATP2B1 (Figure 2A, B, D). Uptake by OATP1A2 and OATP2B1 was more strongly inhibited by ECG (IC_{50} values of 10.2 μM and 35.9 μM, respectively) than by EGCG (54.8 μM and 101 μM, respectively), while uptake by OATP1B1 was more strongly inhibited by EGCG than by ECG (IC_{50} values of 7.8 μM and 58.6 μM, respectively). EGCG stimulated estrone-3-sulfate uptake by OATP1B3 five-fold at concentrations of 30-300 μM; the stimulatory effect remained at 1 mM EGCG, however it was greatly reduced (Figure 2C).

Substrate dependent effect of ECG and EGCG on OATP1B3-mediated uptake.

Based on previous evidence for substrate dependent stimulation of OATP1B3 (Gui et al., 2008), we measured the effects of increasing concentrations of ECG and EGCG on the uptake of two additional model substrates of OATP1B3, estradiol-17β-glucuronide (0.1 μM) and Fluo-3 (1 μM), as well as estrone-3-sulfate (0.1 μM). As can be seen in Figure 3A, ECG inhibited uptake of estradiol-17β-glucuronide with an IC_{50} value of 120 μM, slightly inhibited estrone-3-sulfate uptake at concentrations higher than 100 μM, and strongly inhibited uptake of Fluo-3 (IC_{50} = 7.2 μM). EGCG had no effect on the uptake of estradiol-17β-glucuronide, however it strongly inhibited uptake of Fluo-3 with an IC_{50} value of 8.4 μM while stimulating estrone-3-sulfate uptake with an EC_{50} value of 10.5 μM (Figure 3B).

To investigate the mechanism of this substrate dependency, we determined the effect that ECG and EGCG had on the kinetic parameters of each affected OATP1B3 substrate (Figure 4). Both ECG and EGCG non-competitively inhibited OATP1B3-

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mediated uptake of Fluo-3, reducing the V_{max} from 9.8 ± 0.6 pmol/mg*min to 4.3 ± 0.8 and 5.6 ± 0.6, respectively, while having no effect on the K_m (2.5 ± 0.8 μM, 2.1 ± 1.8 μM and 2.7 ± 1.4 μM) (Figure 4A and 4B). ECG also demonstrated non-competitive inhibition of estradiol-17β-glucuronide uptake, decreasing the V_{max} from 240 ± 40 pmol/mg*min to 110 ± 40 pmol/mg*min, while not affecting K_m (19 ± 3 μM to 17 ± 5 μM) (Figure 4C). Surprisingly, EGCG also significantly decreased the maximal rate of estrone-3-sulfate transport, reducing the V_{max} from 2.1 ± 0.1 nmol/mg*min to 0.36 ± 0.03 nmol/mg*min (Figure 4D). However, the K_m was also strongly decreased, from 95 ± 9 μM to 12 ± 5 μM. This five- to ten-fold increase in affinity results in the stimulation of estrone-3-sulfate transport observed at the low (0.1 μM) concentrations used in the initial inhibition experiments.

OATP-mediated uptake of ECG and EGCG. Given that inhibitors of transport are sometimes also substrates, we tested whether either ECG or EGCG was transported by these four OATPs. We measured accumulation of 100 μM ECG or EGCG in the OATP-expressing or control cells after 10 minutes incubation. As summarized in Figure 5, both OATP1A2 and OATP1B3 transported ECG and EGCG. Although ECG and EGCG were clear inhibitors of OATP1B1 and OATP2B1, we did not detect significant uptake by either OATP. Uptake of both catechins by OATP1A2 and OATP1B3 increased with time and was linear for at least 2-5 minutes (data not shown). Uptake was saturable, with apparent K_m values between 10 and 34 μM (Figure 6). OATP1A2 transports EGCG with a maximal rate of transport (V_{max}) almost twice that of ECG (100 and 60 pmol/mg*min, respectively), while OATP1B3 has a V_{max} about six times higher for ECG than for EGCG (2.2 and 0.340 nmol/mg*min, respectively).

Discussion:

The present study addressed the question of whether the four major green tea catechins affect the activity of all OATPs expressed in the small intestine and liver. Additionally, we investigated whether the catechins that do alter OATP activity are transported by OATPs. Our results demonstrate that while EC and EGC have minimal effect on OATPs, ECG and EGCG significantly alter the function of all four OATPs investigated. We found that the effects of ECG and EGCG on OATP1B3-mediated transport were substrate dependent, and could cause non-competitive inhibition or stimulation of activity. In addition, we showed that both ECG and EGCG are substrates of OATP1A2 and OATP1B3 but are not transported by OATP1B1 or OATP2B1, despite their strong inhibition of estrone-3-sulfate transport by these two proteins.

Epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) significantly inhibited the uptake of estrone-3-sulfate by all four OATPs at a concentration of 100 μM (Figure 2). The USDA Database for the Flavonoid Content of Selected Foods reported average concentrations in brewed green tea to be 19.73 mg/100 mL (450 μM) ECG and 77.81 mg/ 100 mL (430 μM) EGCG, with the maximal concentrations of each catechin in the low millimolar range (Agricultural Research Service, 2007). These compounds inhibited estrone-3-sulfate uptake by OATP1A2 and OATP2B1, expressed at the lumen of enterocytes, with IC_{50} values ranging from 10 to 100 μM (Figure 2). Assuming a gastric fluid volume of 100 to 500 mL, drinking a cup or two of tea on an empty stomach would result in intestinal concentrations of ECG and EGCG within the range that alters OATP transport. The physiological relevance of altered OATP1B1 and OATP1B3 transport is more ambiguous, as the bioavailability of catechins is low. A single dose

study in healthy volunteers found that consumption of 1600 mg of EGCG resulted in mean peak plasma concentrations (C_{max}) of 7.4 µM, with values ranging from 5.8 µM to 11.3 µM (Ullmann et al., 2003). The IC_{50} and EC_{50} values of EGCG on OATP1B3-mediated uptake of Fluo-3 and estrone-3-sulfate (8.4 µM and 10.5 µM, respectively) and on OATP1B1-mediated transport of estrone-3-sulfate (IC_{50} = 7.8 µM), are well within this range, indicating the physiological relevance of these interactions for those who take high-dose supplements. The same authors found that daily consumption of 800 mg EGCG resulted in an average C_{max} of 5.3 µM after 10 days (Ullmann et al., 2004). In addition, the bioavailability of EGCG was shown to increase with increasing doses, indicating that a saturable presystemic elimination process is involved in the low systemic bioavailability (Chow et al., 2001). If this presystemic elimination occurs via the liver, the OATP1B1- and OATP1B3-expressing hepatocytes may be exposed to these EGCG concentrations at more moderate doses as well.

We identified both ECG and EGCG as novel substrates for OATP1A2 and OATP1B3 (Figures 5 and 6). It is important to note that although these two catechins were inhibitors of OATP1B1 and OATP2B1 (Figures 1 and 2), we did not see any uptake by either of these OATPs (Figure 5). This result corroborates the finding that many inhibitors of transporters are not substrates of those transporters. It has been shown that ECG and EGCG are taken up into Caco-2 cells, and that uptake of ECG was saturable and stimulated by low pH (Vaidyanathan and Walle, 2003). The authors suggested that this transport was mediated by the monocarboxylate transporter MCT1 (Vaidyanathan and Walle, 2003). However, direct transport of ECG or EGCG by MCT1 to our knowledge has not been reported and so far no uptake transporter has been

identified for green tea catechins. Given that OATP1A2 is expressed in enterocytes, our results suggest that OATP1A2 could be involved in the absorption of ECG and EGCG from the gut. Furthermore, given that OATP1A2 can transport numerous drugs including fexofenadine (Cvetkovic et al., 1999), several antibiotics like levofloxacin (Maeda et al., 2007), methotrexate (Badagnani et al., 2006), statins (Fujino et al., 2005; Ho et al., 2006), and talinolol (Shirasaka et al., 2010), there is the potential for food-drug interactions like the ones described for fruit juices (Dresser et al., 2002; Lilja et al., 2004; Greenblatt, 2009) in patients that complement their prescription drugs with over the counter green tea supplements. A similar danger may exist for OATP1B3. It is unknown whether the low systemic bioavailability of ECG and EGCG is due to efflux from enterocytes or to a high first-pass effect. Efficient uptake via OATP1B3 into hepatocytes, however, could contribute to the low bioavailability of these compounds.

This study clearly demonstrates that OATP-mediated transport may be affected in different ways by the same compound, depending on the substrate being transported. We found that ECG inhibited OATP1B3-mediated uptake of estrone-3-sulfate, estradiol-17β-glucuornide, and Fluo-3, but to very different extents (Figure 3A). Inhibition of estrone-3-sulfate transport was too weak to further characterize; however, estradiol-17β-glucuornide and Fluo-3 were both inhibited in a non-competitive manner. EGCG, which differs from ECG by a single hydroxyl group, stimulated OATP1B3 activity with respect to estrone-3-sulfate transport, inhibited transport of Fluo-3, and had no effect on the transport of estradiol-17β-glucuronide (Figure 3B). Uptake of Fluo-3 was non-competitively inhibited, as was uptake of estrone-3-sulfate at high substrate concentrations. The stimulation of estrone-3-sulfate at low substrate concentrations

was found to be caused by increased substrate affinity. In addition, although 100 μM ECG did not inhibit OATP1B3-mediated estrone-3-sulfate uptake, and although EGCG did not affect OATP1B3-mediated estradiol-17β-glucuronide uptake, both catechins are substrates of OATP1B3 (Figures 1, 3, and 5). Together, these results suggest the presence of multiple substrate binding sites or translocation pathways on OATP1B3.

These results emphasize that, at least in the case of OATP1B1- and OATP1B3-mediated transport, it is crucial to test more than one substrate when screening for potential inhibitors. The International Transporter Consortium recently suggested that possible OATP inhibition by new molecular entities should be tested using a prototypical substrate such as estradiol-17β-glucuronide (Giacomini et al., 2010). However, we showed that OATP1B3-mediated uptake of estradiol-17β-glucuronide was not affected by EGCG while uptake of Fluo-3 was inhibited and uptake of estrone-3-sulfate was stimulated (Figure 3). Similarly, while a previous study found that EGC inhibited OATP1B1-mediated uptake of dehydroepiandrosterone sulfate (DHEAS) (Wang et al., 2005), we did not see significant inhibition of OATP1B1-mediated uptake of estrone-3-sulfate in the presence of EGC. Thus, we propose that for OATP1B1 and OATP1B3, the effect of potential inhibitors should always be tested by using several substrates instead of a single prototypical substrate. Similar substrate dependent effects have previously been observed for rat Oatp1a4 (Sugiyama et al., 2002), for human OATP1B1 (Noe et al., 2007), and for human OATP1B3 (Gui et al., 2008). In our previous study (Gui et al., 2008), the non-OATP substrate clotrimazole stimulated OATP1B3-mediated estradiol-17β-glucuronide uptake, did not affect uptake of estrone-3-sulfate and inhibited uptake of Fluo-3. Together these data clearly demonstrate that stimulation as well as

inhibition is substrate dependent, and indicate the presence of multiple substrate binding sites.

A previous study showed inhibition of OATP2B1-mediated uptake of estrone-3-sulfate by all four catechins (Fuchikami et al., 2006). However, we found that only ECG and EGCG significantly inhibit OATP2B1-mediated estrone-3-sulfate transport. These differences could be explained by the 10-fold higher substrate concentration used in the current study and highlights the difficulty in predicting *in vivo* effects based on *in vitro* data. However, in both studies the effects of EC and EGC on OATP2B1-mediated transport were much weaker than the effects of ECG and EGCG, suggesting that ECG and EGCG are the green tea catechins most likely to alter OATP-mediated drug uptake.

In conclusion, we have demonstrated that the green tea compounds ECG and EGCG are substrates for OATP1A2 and OATP1B3 suggesting that these two transporters could be involved in the disposition of these two catechins. We also demonstrated that compounds like ECG and EGCG can affect OATPs in a substrate dependent manner. This highlights the importance of using multiple and clinically relevant substrates when screening for potential drug-drug interactions with this family of transporters. Because of increasing use of green tea catechins, particularly EGCG, in dietary supplements and because ECG and EGCG can significantly alter the function of OATPs involved in drug disposition, the results of this study suggest that there is a significant possibility of adverse drug-catechin interactions.

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Authorship Contributions:

Participated in research design: Roth, Timmermann, Hagenbuch

Conducted experiments: Roth

Performed data analysis: Roth, Hagenbuch

Wrote or contributed to the writing of the manuscript: Roth, Timmermann, Hagenbuch

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Footnotes

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b) Part of this work was previously presented in poster form: Roth, M., Timmermann, B. and Hagenbuch, B. (2009) Interaction of Green Tea Catechins with Organic Anion Transporting Polypeptides. FASEB J. 23:748.4

c) Bruno Hagenbuch

Department of Pharmacology, Toxicology and Therapeutics

The University of Kansas Medical Center

3901 Rainbow Blvd.

Kansas City, KS 66160, USA

Phone: +1-913-588-0028

Fax: +1-913-588-7501

E-Mail: bhagenbuch@kumc.edu

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Figure legends:

Figure 1: Effect of green tea extract and catechins on OATP-mediated estrone-3-sulfate uptake. Cells were coincubated with 0.1µM [^{3}H]estrone-3-sulfate (E3S) and 0.03 µg/mL green tea extract, 100 µM of epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), or the vehicle control (1% DMSO) at 37 ºC for 20 seconds (OATP1B1, OATP1B3, OATP2B1) or 30 seconds (OATP1A2). After correcting for protein, uptake into empty vector (OATP1A2, OATP2B1) or wildtype control cells (OATP1B1, OATP1B3) was subtracted to determine OATP-mediated uptake. Values are expressed as a percentage of vehicle control; each value is the mean ± SEM of three independent experiments. Asterisks represent statistically significant differences from DMSO control (** $p < 0.005$, *** $p < 0.001$).

Figure 2: Concentration dependent effects of green tea catechins on OATP-mediated estrone-3-sulfate uptake. Cells were coincubated with 0.1 µM [^{3}H]estrone-3-sulfate (E3S) and increasing concentrations of epicatechin gallate (ECG) or epigallocatechin gallate (EGCG) at 37 ºC as described in the legend to Figure 1. Values are expressed as a percentage of vehicle control; each value represents the mean ± SEM of three independent experiments.

Figure 3: Substrate-dependent effects of epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) on OATP1B3-mediated transport. OATP1B3-expressing and wildtype CHO cells were coincubated with 0.1 µM [^{3}H]estrone-3-sulfate (E3S, circles), 0.1 µM [^{3}H]estradiol-17β-glucuronide (E17β, squares), or 1 µM Fluo-3

(triangles) and increasing concentrations of epicatechin gallate (ECG; **A**) or epigallocatechin gallate (EGCG; **B**) at 37 ºC as described in the legend to Figure 1. Values are expressed as a percentage of vehicle control; each value represents the mean ± SEM of three independent experiments.

Figure 4: Effect of epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) on the kinetics of OATP1B3-mediated transport. OATP1B3-expressing and wildtype CHO cells were coincubated with the stated concentrations of ECG or EGCG (squares), or the vehicle control (1% DMSO, circles), and increasing concentrations of Fluo-3 (**A**, **B**), estradiol-17β-glucuronide (E17β, **C**), or estrone-3-sulfate (E3S, **D**). **A**-**C** are representative graphs from at least three independent experiments, each value shown in **D** represents the mean ± SEM of at least three independent experiments.

Figure 5: Uptake of green tea catechins by OATPs. Cells were incubated with 100 µM epicatechin gallate (ECG) or epigallocatechin gallate (EGCG) at 37 ºC for ten minutes. Uptake by each OATP-expressing cell line was divided by the uptake by its appropriate control cell, and is expressed as fold uptake over control. Each value represents the mean ± SD of at least two experiments performed in triplicate. Asterisks represent statistically significant uptake compared to control cell lines (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$).

Figure 6: Kinetics of epicatechin gallate and epigallocatechin gallate uptake mediated by OATP1A2 or OATP1B3. Uptake of increasing concentrations of

epicatechin gallate (ECG; **A**, **B**) or [^{3}H]-epigallocatechin gallate (EGCG; **C**, **D**) was measured at 37º C under initial linear rate conditions. After subtracting the values obtained with control cells, net OATP1A2- (**A**, **C**) or OATP1B3- (**B**, **D**) mediated uptake was fitted to the Michaelis-Menten equation to determine K_m and V_{max} values. (**A**) and (**B**) plot mean data points from at least three independent experiments; (**C**) and (**D**) are representative graphs with mean ± SEM of three independently determined K_m values.

Figure 1

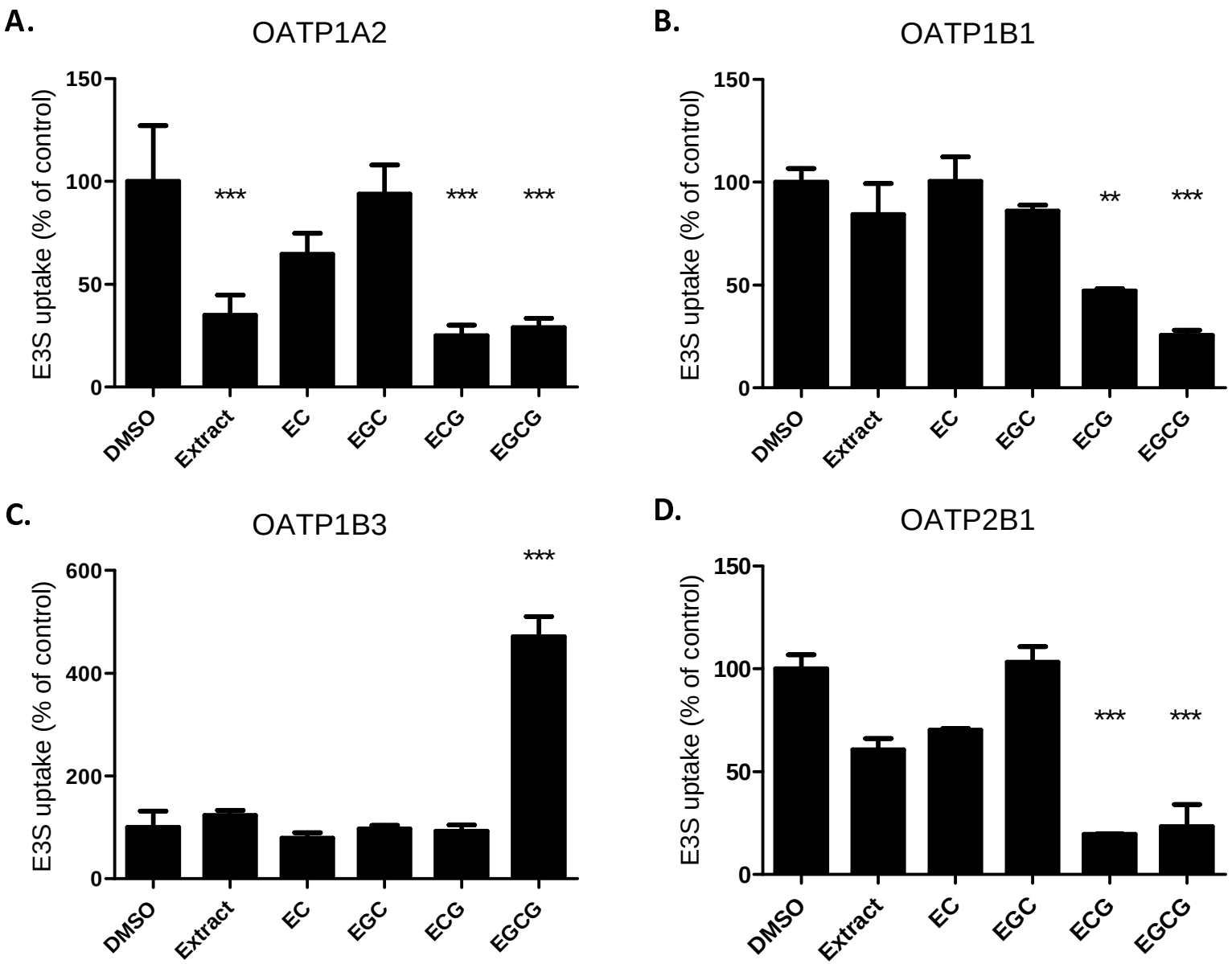

Figure 2

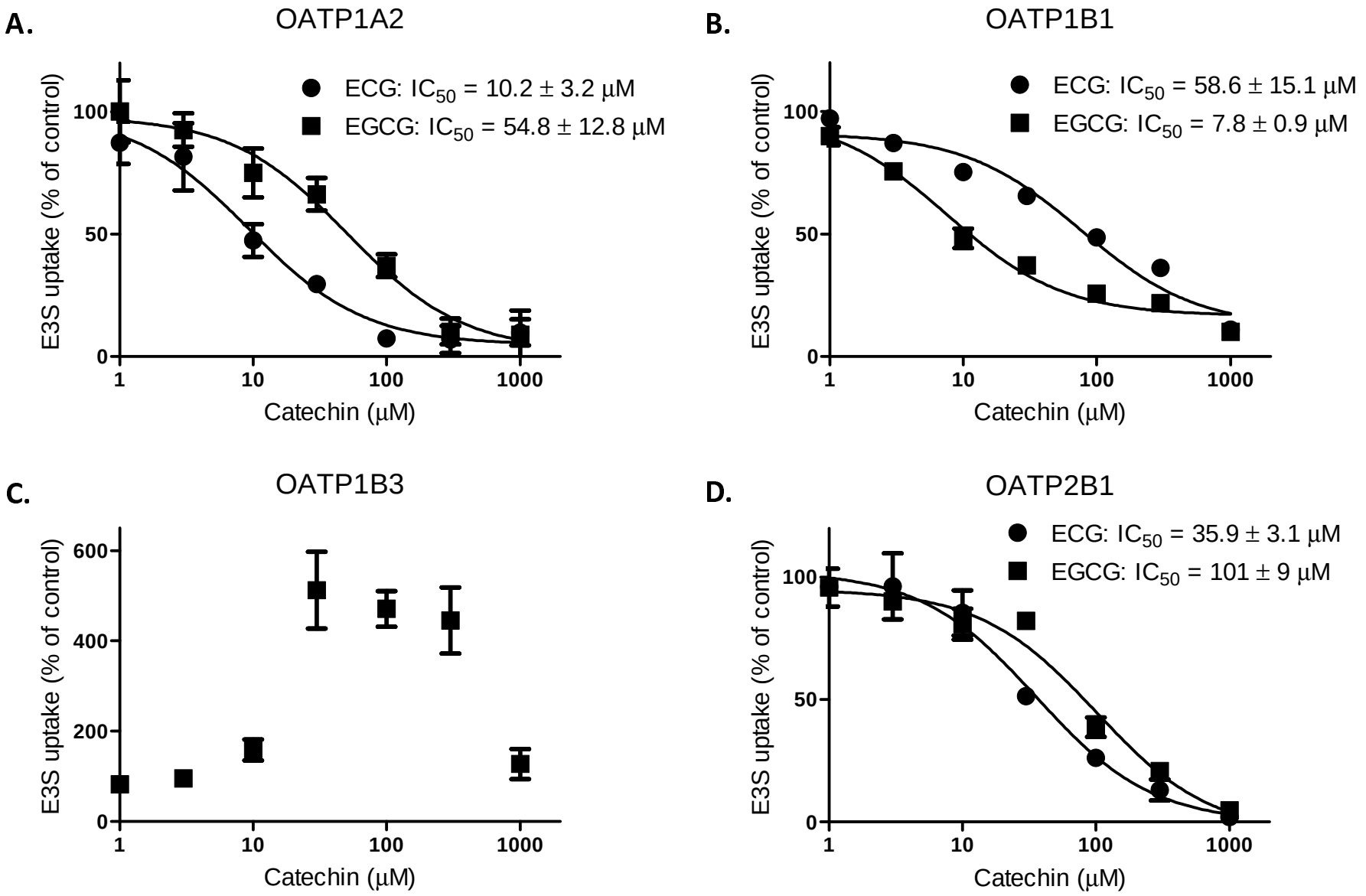

Figure 3

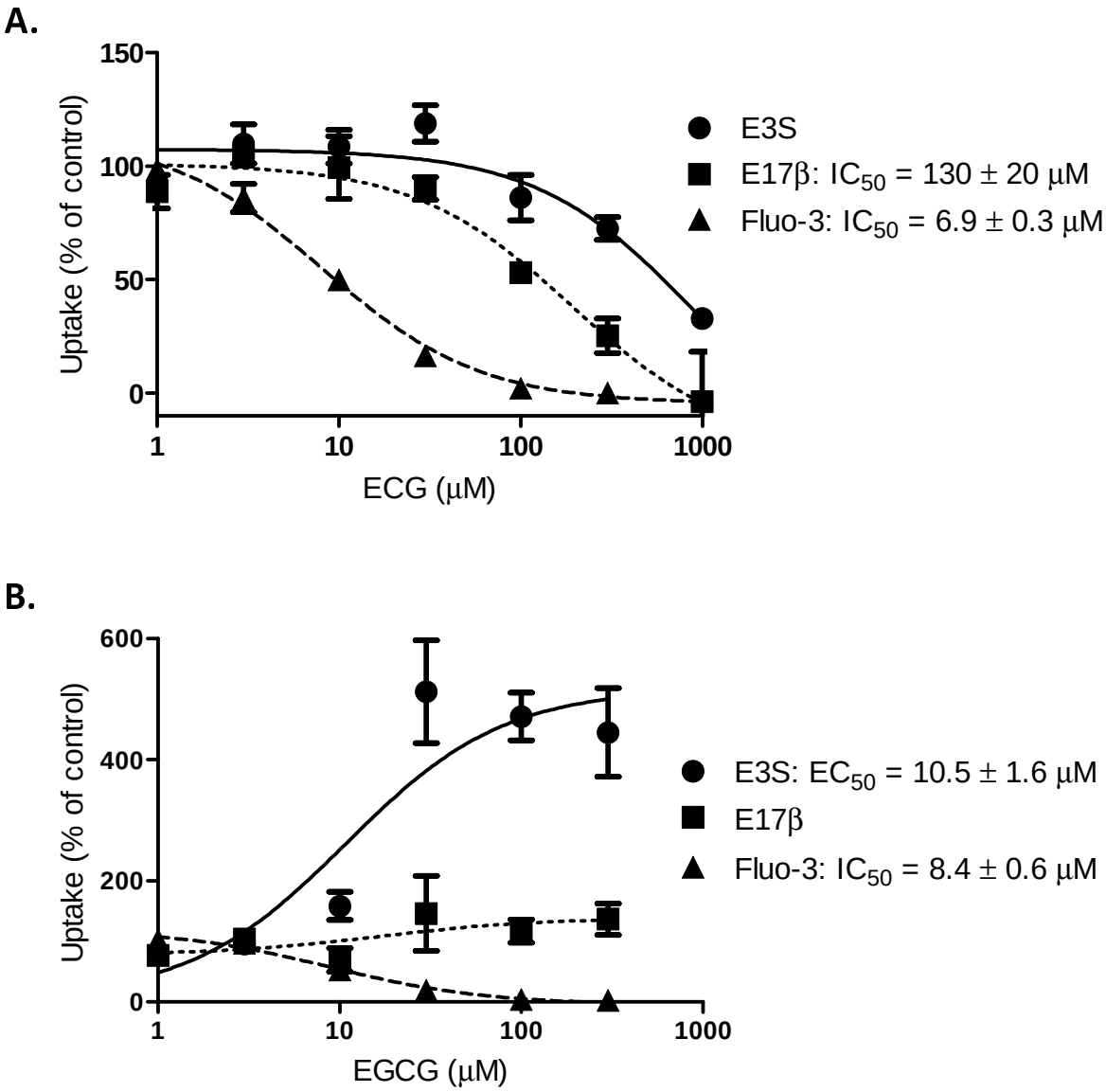

Figure 4

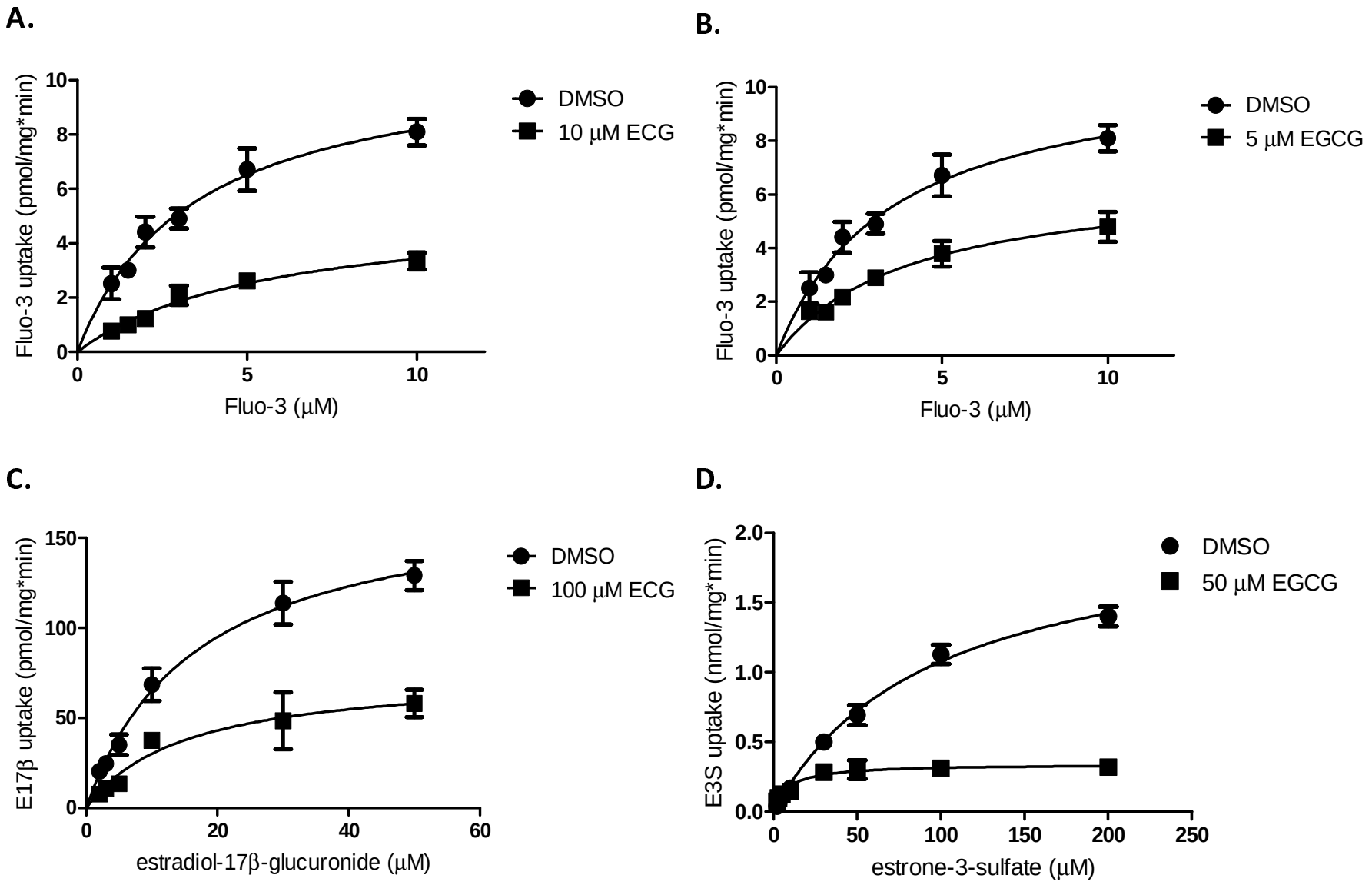

Figure 5

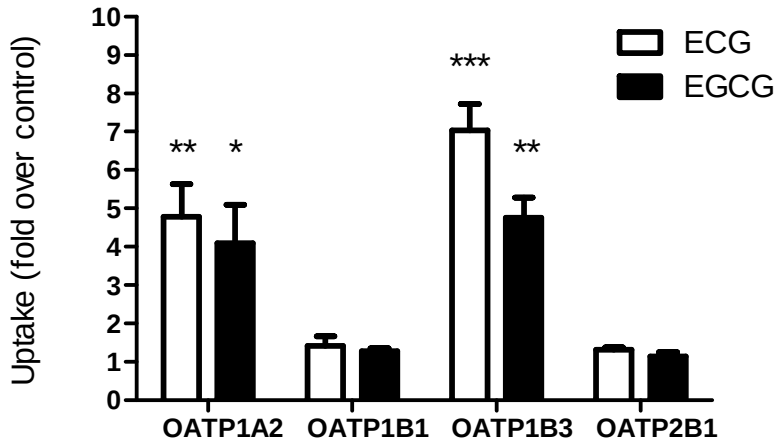

Figure 6