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Increased expression of renal drug transporters in a mouse model of familial Alzheimer's disease

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Abstract

Being a disease of the central nervous system (CNS), it is well established that the expression and function of drug transporters at the blood-brain barrier (BBB) are altered in Alzheimer's disease (AD). However, we have recently demonstrated in a mouse model of AD that the expression of key drug transporters and metabolizing enzymes was modified in peripheral organs, such as the small intestine and liver, suggesting that systemic drug absorption may be altered in AD. The purpose of this study was to determine whether the expression of drug transporters in the kidneys differed between 8–9 months old wild type (WT) and APP^{swe}/PSEN1^{dE9} (APP/PS1) transgenic mice, a mouse model of familial AD, using a quantitative targeted absolute proteomics (QTAP) approach. The protein expression of the drug transporters multidrug resistance-associated protein 2 (Mrp2), organic anion transporter 3 (Oat3), and organic cation transporter 2 (Oct2) was upregulated 1.6-, 1.3-, and 1.4-fold, respectively, in kidneys from APP/PS1 mice relative to WT mice. These results suggest that in addition to modified oral absorption of certain drugs, it is possible that the renal excretion of drugs that are Mrp2, Oat3, and/or Oct2 substrates could be modified in AD. These changes could affect the interpretation of studies conducted during drug development using this mouse model of AD, and potentially impact dosage regimens of such drugs used in this patient population.

Keywords

Alzheimer's disease; kidney; drug transporters; multidrug resistance-associated protein 2; organic cation transporter 2; organic anion transporter 3

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INTRODUCTION

Alzheimer's disease (AD), the leading cause of dementia, is a major health burden with one diagnosis every 65 seconds and a prediction of one diagnosis every 33 seconds by 2050¹. Individuals with AD are typically advanced in age, and often taking multiple medicines for their comorbidities, including diabetes, hypertension and pain, in addition to medicines that assist with cognition (e.g., acetylcholinesterase inhibitors). In fact, individuals with AD are prescribed up to 5–10 more repeat prescriptions than those without dementia, even after accounting for differences in age and sex². The dosing regimens for these medicines are generally based on those used in the aged population, without consideration for potential alterations to peripheral pharmacokinetics of drugs that may occur in AD.

The disposition of drugs is affected by multiple processes, however, transporters and metabolizing enzymes are critical determinants of drug absorption, distribution, metabolism and excretion (ADME)³. Indeed, the expression and function of transporters at the blood-brain barrier (BBB) such as P-glycoprotein (P-gp)⁴ and breast cancer resistance protein (BCRP)⁵, which normally restrict access of substrates into the brain, are affected in AD, and this is associated with altered CNS distribution of some drugs. In addition to altered expression of transporters at the BBB, we recently reported that the expression of peripheral drug transporters and drug metabolizing enzymes is altered in a mouse model of familial AD, suggesting that systemic pharmacokinetics of drugs also may be modified in AD⁶. In our previous study, we reported a 2.3-fold increase in the expression of multidrug resistance-associated protein 2 (Mrp2), a 1.9-fold reduction in the expression of monocarboxylate transporter 1 (Mct1), and a 3.6-fold increase in UDP-glucuronosyltransferase (Ugt) 2b5 expression in the small intestines of 8–9 month old APP^{swe}/PSEN1^{ΔE9} (APP/PS1) transgenic mice relative to wild-type (WT) mice⁶. While drug transporter expression was not modified in the livers of APP/PS1 mice, we detected a 1.3-fold increase in the hepatic expression of cytochrome P450 (Cyp) 51a1 and a 1.2-fold reduction in the expression of Cyp2c29, which was associated with hepatic lipidosis⁶. These findings suggest that the oral absorption of drugs that are substrates of MRP2 and MCT1 may be affected in individuals with AD. Indeed, there is evidence to suggest that systemic pharmacokinetics of certain drugs is modified in a mouse model of AD and in individuals with AD. For example, we previously reported that the oral absorption of a preclinical drug candidate PBT2 was significantly reduced in Tg2576 mice (another mouse model of familial AD) relative to WT mice⁷. Furthermore, the plasma concentrations of orally-administered rivastigmine were found to be 30–50% higher in individuals with AD relative to age-matched healthy volunteers⁸, confirming that systemic pharmacokinetics may be altered in AD.

Although the expression of drug transporters and metabolizing enzymes is modified in the small intestine and liver from a mouse model of AD, similar findings on the status of key determinants of renal excretion have yet to be reported. It is known that renal function may be impaired in AD with a decrease in the glomerular filtration rate⁹, which could significantly decrease the renal excretion of drugs. Furthermore, epidemiological studies have demonstrated that individuals with AD are prone to alterations in renal function¹⁰. It is conceivable that the expression of renal drug transporters is modified in AD based on our observations of altered transporter expression in other peripheral organs⁶ and the fact that

the expression of renal transporters can be affected by inflammatory mediators¹¹, which are increased in the plasma of individuals with AD^{12–14}. Such changes could impact the clearance of drugs and/or metabolites that are substrates for affected transporters, which may necessitate adjustments in dosing regimens of these drugs in AD.

The purpose of this study was to use a quantitative transporter absolute proteomics (QTAP) approach to quantify the expression of plasma membrane transporters and cytoplasmic enzymes from kidneys isolated from 8–9 month old WT and APP/PS1 mice. This sensitive and reproducible technique has been extensively used to measure peripheral organ transporter and metabolizing enzyme protein expression levels^{15,16}. This approach was used to generate the data demonstrating altered expression of proteins involved in drug transport and metabolism in the small intestine and liver of APP/PS1 mice⁶. Unravelling the expression profile of renal drug transporters and enzymes in an AD mouse model may inform critical clinical dosing regimens for the multiple medicines that are prescribed to individuals with AD.

MATERIALS AND METHODS

Materials.

Acetonitrile, ethylenediaminetetraacetic acid (EDTA), dithiothreitol (DTT), formalin, formic acid, phenylmethylsulfonyl fluoride (PMSF), phosphate-buffered saline (PBS), paraformaldehyde (PFA) and protease inhibitor cocktail were purchased from Sigma-Aldrich (St. Louis, MO). Stable-isotope labeled (internal standard) and unlabeled peptides used to quantify transporter and enzyme proteins were designed by the in-silico peptide selection criteria reported previously^{15,16} and synthesized by Thermo Fisher Scientific (Sedan Strasse, Germany) with >95% peptide purity.

Animals.

All animal studies were approved by the Monash Institute of Pharmaceutical Sciences Animal Ethics Committee (MIPS.2011.39) and undertaken in a manner consistent with the National Health and Medical Research Council guidelines for the care and use of animals for scientific purposes. The mice used in the study were either 8–9 month old female APP/PS1 (B6C3-Tg(APP^{swe},PSEN1^{dE9})85Dbo/Mmjax) or their littermate wild-type (WT) mice. These mice were obtained from Jackson Laboratories at 2–3 months of age and aged to 8–9 months of age at Monash University with free access to standard rodent chow and water.

Preparation of cytoplasmic and plasma membrane fractions from mouse kidney tissues for proteomic analysis.

Following removal from euthanized WT and APP/PS1 mice, kidneys were immediately snap frozen and subsequently stored at –80°C until required for sample preparation. The procedures to prepare cytoplasmic and plasma membrane fractions of kidney tissues were all undertaken at 4°C using procedures that have been published previously^{15,16} with minor modifications. In brief, isolated kidneys were cut into small pieces and homogenized using a Potter–Elvehjem homogenizer in pH 7.4 buffer prepared with 10 mM NaCl, 1.5 mM MgCl₂,

1 mM PMSF, a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO), and Tris-HCl. Kidney homogenates were centrifuged at 8,000 ×g for 10 min, after which the supernatants were collected and subsequently centrifuged at 100,000 ×g for 60 min at 4°C to obtain the cytoplasmic fraction in the supernatant. The remaining pellets were suspended in a solution containing 10 mM Tris-HCl (pH 7.4) and 250 mM sucrose that was layered on a 38% (w/v) sucrose solution. This suspension was centrifuged at 100,000 ×g for 40 min at 4°C, with the interfacial turbid layer containing the plasma membrane fraction isolated and stored at –80°C until required for proteomic analysis.

Quantitative transporter proteomic analysis (QTAP) of kidney cytoplasmic and plasma membrane fractions.

Prior to QTAP analysis, all kidney cytoplasmic and plasma membrane fractions were treated with trypsin and lysylendopeptidase as reported previously¹⁷. A cHiPLC nanoflex and nano-LC system (Eksigent Technologies, Dublin CA)-Triple TOF 5600 with parallel reaction monitoring (PRM) was used for simultaneous protein quantification of target molecules according to our previously published method⁶. In order to normalize drug metabolizing enzyme and transporter protein levels to total cellular protein, a Lowry protein assay (Bio-rad, Hercules, CA) was employed for quantification of total cellular protein. The expression of drug transporters or metabolizing enzymes (expressed as fmol/μg of total protein) in kidneys from WT mice was plotted against the expression in kidneys from APP/PS1 mice on a logarithmic scale.

Statistical analysis.

All data are expressed as mean ± SD. In order to determine any statistical differences between protein expression levels in kidneys from WT and APP/PS1 mice, a Student's *t*-test was performed using Prism software (GraphPad, La Jolla, CA), with a *p* value of 0.05 used as a cut-off for statistical significance.

RESULTS

APP/PS1 mice exhibit increased renal expression of drug transporters but not drug metabolizing enzymes.

The expression of both drug transporters and enzymes in plasma membrane and cytoplasmic fractions of kidneys from WT and APP/PS1 mice was screened using a QTAP approach. The expression of each of the transporters from plasma membranes and cytoplasmic fractions of kidney tissue is listed in Table 1. A graphical representation comparing the expression of both transporters and metabolizing enzymes in renal tissue between WT and APP/PS1 on a logarithmic scale is provided in Figure 1. The expression of Mrp2 was significantly (*p*=0.0001) increased by 1.6-fold in kidney plasma membranes from APP/PS1 mice relative to WT mice (Figure 1A, Table 1). Similarly, there was a 1.3-fold increase in the expression of Oat3 (*p*=0.01), and a 1.4-fold increase in the expression of Oct2 (*p*=0.005) in kidneys from APP/PS1 mice relative to those from WT mice. Finally, a modest increase in Asct2 (1.2-fold, *p*=0.01) was observed in kidneys obtained from APP/PS1 mice compared to WT mice. Unlike the changes in expression of various transporters, no significant difference in

the expression of relevant drug metabolizing enzymes was detected in kidneys from APP/PS1 and WT mice (Figure 1B, Table 1).

DISCUSSION

The expression of proteins involved in ADME (*i.e.*, drug transporters and metabolizing enzymes) is significantly modified in small intestinal and liver tissues derived from a mouse model of familial AD⁶. We have now extended these studies to assess the expression of renal drug transporters and selected drug metabolizing enzymes in this same mouse model (APP/PS1 mice) with the intent of identifying likely candidate drugs whose renal excretion may be affected in AD. This information is critical in drug development, where mouse models of AD are utilized, and may translate into a better understanding on how the multiple medicines prescribed to individuals with AD are handled in the body.

While there are reports of impaired renal function in AD such as decreased glomerular filtration rate⁹, which could significantly decrease the renal excretion of drugs, there are no reports of altered transporter expression or function in AD. This is likely because AD is mainly considered a disease of the brain. Scientists and clinicians have primarily focused on altered drug transporter expression at the BBB⁴⁵. However, given our observations of altered expression of drug transporters and enzymes in the small intestine and liver in AD⁶, and the growing realization that AD involves a systemic inflammatory component¹⁸, altered expression of renal drug transporters may occur in AD. In support of this, our data are the first to demonstrate that, in line with that previously reported in the small intestine from APP/PS1 mice, the renal expression of Mrp2 is significantly enhanced in APP/PS1 mice relative to WT mice (Table 1). Mrp2 is expressed at the apical (urine-facing) membrane of renal proximal tubular epithelial cells¹⁹, and therefore, increased urinary excretion of Mrp2 substrates would be expected in AD. Drugs that undergo renal excretion via this efflux transporter include etoposide, benzylpenicillin, saquinavir, ritonavir and glucuronidated and sulfated conjugates of numerous xenobiotics^{20,21}. The mechanism(s) responsible for the increased renal expression of Mrp2 in APP/PS1 mice is currently unknown. However, human renal proximal tubular epithelial cells express increased MRP2 following treatment with conjugated bile acids or human bile²². This increased MRP2 expression is likely a protective response aimed at increasing bilirubin clearance during obstructive jaundice, given that MRP2 is involved in the renal clearance of conjugated bilirubin²³. Increased plasma concentrations of cholic acid has been detected in APP/PS1 mice²⁴ and may contribute to the increased Mrp2 expression observed in the kidney. Whether this increase in renal Mrp2 in APP/PS1 mice is a protective response to increase bilirubin clearance (as is suggested in obstructive jaundice) is unknown. Further studies using human AD kidneys will be required to confirm if the increased Mrp2 expression in AD mouse is translated to the human setting.

In addition to the increased renal expression of Mrp2, we detected increased renal expression of the membrane transporters Oct2 and Oat3. Oct2 is expressed on the basolateral membrane of renal proximal tubular epithelial cells and is involved in the renal secretion of various cationic drugs such as metformin and pindolol²⁵, medicines that are likely to be taken by individuals with AD for diabetes and hypertension, respectively. The increased

expression of Oct2 suggests that the renal clearance of such compounds, and other substrates of this cationic transporter, would be increased in AD. A previous study has reported that the renal expression and function of Oct2 were significantly increased in rats with acute biliary obstruction²⁶, suggesting that the increase in renal Oct2 we observed in APP/PS1 mice may also result from increased plasma concentrations of bile acids, as described for Mrp2. Proteomic analysis also identified a significant increase in the expression of Oat3, a solute linked carrier protein expressed at the basolateral membrane of renal proximal tubular epithelial cells. Oat3 is involved in the renal excretion of various drugs including angiotensin 2 receptor blockers (valsartan, candesartan, losartan), diuretics (bumetanide, frusemide), beta lactam antibiotics (cefamandole) and some antiviral agents (zidovudine, valacyclovir)²⁷. Any increase in function of Oat3 is, therefore, likely to increase the renal clearance of such drugs, which might necessitate dosage adjustments to ensure therapeutic effectiveness. Interestingly, both the expression and function of Oat3 are increased in rodent models of acute biliary obstruction^{28,29}. Therefore, the increased Oat3 expression observed in APP/PS1 mice may also be due to increased circulating bile acid concentrations detected in these mice²⁴.

A slight increase in Asct2 expression in the kidney of APP/PS1 mice was noted, compared to WT mice, however, such a change is unlikely to impact drug clearance. Asct2 is a glutamine transporter expressed on the apical membrane (urine-facing) of renal proximal tubular epithelial cells, which is involved in the reuptake of glutamine from the urine³⁰. Asct2 also mediates the transport of D-serine³¹, the N-methyl-D-aspartate receptor co-agonist, which exhibit higher levels in the hippocampus and parietal cortex of individuals with AD relative to control subjects³². The increased Asct2 expression could contribute to the observations that individuals with AD have higher brain levels of glutamine³³ and D-serine³² (*i.e.*, through an increase in urinary reabsorption and plasma concentrations). However, the increased brain levels of glutamine and D-serine also may be due to reduced cerebral metabolism of glutamine³⁴ and elevated production of D-serine via serine racemase in brain³², respectively, as has been observed in APP/PS1 mice.

In summary, these studies have demonstrated that the renal expression of key drug transporters Mrp2, Oct2 and Oat3 is modestly, but significantly, increased in a mouse model of familial AD. These preclinical studies will lead to future studies assessing whether such changes lead to functional changes in renal excretion of drugs which are substrates of these transporters and whether similar alterations to transporter expression occur in human kidneys obtained from individuals with AD. Ultimately, these findings may guide the optimal design of dosage regimens in individuals with AD to ensure efficacy and minimize the toxicity of drugs that are substrates for these transporters.

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Figure 1.

Comparison of protein expression levels of (A) drug transporters in the plasma membrane and (B) drug metabolizing enzymes in the cytoplasmic fraction of kidney tissue isolated from 8–9 months old APP/PS1 and WT mice (n=3 mice per group). The top (green) and bottom (red) dashed lines represent a 2-fold upregulation or downregulation in protein expression, respectively, in APP/PS1 mice compared to WT mice. Data are presented as mean $\pm$ SD, with statistically significant upregulation in APP/PS1 mice denoted by a green data point, with $p < 0.05$ (Student's t-test; WT vs. APP/PS1 mice).

Table 1.

Protein expression of drug transporters and metabolizing enzymes in kidneys obtained from WT and APP/PS1 mice;

Protein	WT			APP/PS1			Ratio of APP/PS1:WT	<i>p</i> value
	Mean	±	S.D.	Mean	±	S.D.		
	(fmol/μg protein)			(fmol/μg protein)				
Plasma membrane								
<i>Abc transporters</i>								
Mdr1a	1.10	±	0.32	1.03	±	0.18	0.93	0.92
Mdr1b	0.83	±	0.10	0.99	±	0.04	1.19	0.12
Mrp1	0.53	±	0.13	0.67	±	0.10	1.28	0.32
Mrp2	2.77	±	0.46	4.39	±	0.17	1.58 *	0.0001
Mrp3	2.08	±	0.52	1.77	±	0.40	0.85	0.23
Mrp4	1.48	±	0.29	1.54	±	0.14	1.04	0.70
Mrp6	0.47	±	0.10	0.44	±	0.04	0.95	0.51
Bcrp	26.69	±	4.56	29.59	±	2.05	1.11	0.25
Abca3	0.69	±	0.24	0.79	±	0.07	1.15	0.89
<i>Slc transporters</i>								
Asct2	2.53	±	0.15	2.91	±	0.10	1.15 *	0.01
Sgt1	2.38	±	1.06	2.92	±	0.36	1.23	0.41
Sgt2	23.69	±	12.40	24.98	±	6.58	1.05	0.90
4F2hc	31.88	±	4.11	37.84	±	6.25	1.19	0.29
Mct1	10.69	±	1.57	14.50	±	2.72	1.36	0.12
Ata2	0.39	±	0.04	0.31	±	0.17	0.79	0.51
Glut1	3.78	±	0.52	4.36	±	0.19	1.15	0.24
Oat1	4.95	±	0.71	5.64	±	0.93	1.14	0.51
Oat2	0.94	±	0.29	0.92	±	0.15	0.98	0.78
Oat3	21.81	±	3.97	28.30	±	1.91	1.30 *	0.01
Oct2	9.67	±	0.82	13.46	±	0.75	1.39 *	0.005
Mate1	0.87	±	0.32	0.98	±	0.29	1.13	0.76
Ostb	0.53	±	0.06	0.46	±	0.18	0.86	0.42
Oatp1a6	1.14	±	0.16	1.00	±	0.33	0.88	0.71
Oatp3a1	0.74	±	0.12	0.82	±	0.17	1.11	0.76
Oatp4a1	0.48	±	0.10	0.52	±	0.16	1.10	0.72
Urat1	6.46	±	1.36	7.29	±	2.35	1.13	0.64
<i>Receptors</i>								
Tfr1	1.22	±	0.32	1.35	±	0.38	1.11	0.85
Insr	1.01	±	0.20	1.09	±	0.27	1.08	0.91
CD147	20.55	±	4.30	26.75	±	4.57	1.30	0.24
<i>Marker proteins</i>								

Protein	WT			APP/PS1			Ratio of APP/PS1:WT	<i>p</i> value
	Mean	±	S.D.	Mean	±	S.D.		
	(fmol/μg protein)			(fmol/μg protein)				
γ-Gtp	35.66	±	8.30	34.30	±	5.10	0.96	0.78
Na ⁺ /K ⁺ ATPase	295.52	±	67.94	341.73	±	63.30	1.16	0.66
Cytoplasmic fraction								
<i>Enzymes</i>								
Cyp2d22	1.39	±	0.15	1.35	±	0.36	0.97	0.85
Cyp2e1	0.56	±	0.15	0.55	±	0.10	0.99	0.98
Cyp51a1	5.31	±	1.04	5.20	±	1.50	0.98	0.91
Nadph	22.63	±	1.77	20.30	±	2.98	0.90	0.25
Ugt1a1	0.66	±	0.23	0.58	±	0.32	0.88	0.72
<i>Marker proteins</i>								
γ-Gtp	48.75	±	15.91	42.26	±	15.42	0.87	0.61
Na ⁺ /K ⁺ ATPase	196.53	±	36.85	203.93	±	15.38	1.04	0.76

*
p<0.05, WT vs. APP/PS1 mice (n=3 per group).