博 士 論 文

Metabolomic Study of Food Derived Compounds Interacting with ABC Drug Transporters

ABC 薬物トランスポーターと相互作用する食物由来化合物を 対象とした メタボロミクス研究

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Table of Contents

Abbreviations - 3 -

Abstract - 4 -

General Introduction - 5 -

I. Untargeted metabolomic analysis to identify endogenous BCRP substrates

Introduction - 7 -

Results - 9 -

Discussion - 29 -

Conclusion - 32 -

II. Effect of P-gp inhibitors on disposition of food derived steroidal alkaloids

Introduction - 33 -

Results - 35 -

Discussion - 43 -

Conclusion - 44 -

General Conclusion - 45 -

Materials and Methods - 46 -

Supplementary Figures - 55 -

Supplementary Tables - 57 -

References - 58 –

Acknowledgements - 64 -

Abbreviations

AIF	all ion fragmentation
AUC	area under the plasma concentration–time curve
BCRP	breast cancer resistance protein
BS	biochanin A sulfate
C_{max}	maximum plasma concentration
DDI	drug-drug interaction
DS	daidzein sulfate
ES	equol sulfate
ESI	electrospray ionization
GS	genistein sulfate
iPS	induced pluripotent stem
LC-MS/MS	liquid chromatography with tandem mass spectrometry
LC-TOFMS	liquid chromatography time-of-flight mass spectrometry
LY	lucifer yellow
NMQ	N-methyl-quinidine
P-gp	P-glycoprotein
PLS-DA	projection to latent structures-discriminant analysis
SULT	sulfotransferase
TKO	triples knock out (*Abcb1a/1b/Abcg2*$^{-/-}$)

ABSTRACT

Breast cancer resistance protein (BCRP) and P-glycoprotein (P-gp) belong to ABC drug transporters. They are localized on the apical side of plasma membranes in various organs and extrude their substrates from the cells. Potential inhibition of these transporters is a crucial issue during drug development, and utilization of its physiological substrates as biomarkers would be advantageous to evaluate the inhibition potential. The purpose of this study was to identify such BCRP/P-gp substrates in the body using metabolomics approaches. In BCRP study, mice were fed with a roasted soybean flour-containing diet which may highly include BCRP substrates isoflavones. Lapatinib was orally administered as a BCRP inhibitor, and plasma samples were collected. Ion peaks in the plasma samples were comprehensively measured by LC-TOFMS using all ion fragmentation mode and quantified by LC-MS/MS. Another screening with a similar sequence of analysis was also performed in plasma samples of *Bcrp*$^{-/-}$ and wild-type mice. Untargeted metabolomics with neutral loss search of sulfate moiety has revealed increase in plasma peaks of daidzein sulfate (DS) and genistein sulfate (GS) by lapatinib administration and *Bcrp*$^{-/-}$ mice. Oral administration of lapatinib significantly increased $AUC_{0\text{-}7h}$ for DS, GS, and equal sulfate (ES) by 3.56-, 5.60-, and 1.57-fold, respectively. Another BCRP inhibitor febuxostat also increased $AUC_{0\text{-}7h}$ of DS, GS, and ES. To demonstrate the effect of BCRP inhibitors on the intestinal disposition of the isoflavone sulfates, their appearance in the basal side of human iPS cell-derived small intestinal epithelial-like cells (F-hiSIEC) was examined to mimic physiological intestinal transport. After addition of daidzein, genistein, and equol to the medium of apical side, the appearance of DS, GS, and ES in the basal compartment significantly increased in the presence of lapatinib and febuxostat, suggesting that these BCRP inhibitors may inhibit intestinal BCRP and thereby increase appearance of the isoflavone sulfates. Both inhibitors reduced ATP-dependent uptake of DS and ES in BCRP-expressing membrane vesicles, indicating inhibition of BCRP-mediated DS and ES transport. In P-gp study, effect of P-gp inhibitors on the disposition of orally administered steroidal alkaloids derived from tomatoes, tomatidine and esculeogenin A, was examined since P-gp preferably recognizes steroidal compounds as substrates. The plasma concentration of a typical P-gp substrate fexofenadine was increased following oral administration of P-gp inhibitors elacridar and tariquidar, whereas $AUC_{0\text{-}6h}$ and C_{max} of tomatidine was not significantly changed. On the other hand, these inhibitors significantly increased brain-to-plasma concentration ratio ($K_{p,brain}$) of tomatidine by 2.38- and 2.41-fold, respectively. Plasma concentration and $K_{p,brain}$ of esculeogenin A were not changed by coadministration of elacridar. $AUC_{0\text{-}6h}$ and C_{max} of tomatidine were significantly increased in *Abcb1a/1b/Abcg2*$^{-/-}$(TKO) mice compared with wild-type mice by 2.81- and 2.52-fold, respectively, whereas $K_{p,brain}$ of tomatidine in TKO was also significantly higher than wild-type mice by 2.98-fold. ATP-dependent uptake of tomatidine was observed in P-gp expressing membrane vesicles, and its uptake was reduced by elacridar and tariquidar. On the other hand, ATP-dependent uptake of tomatidine was not observed in the BCRP-expressing membrane vesicles. These results suggest P-gp may play some roles in distribution of tomatidine to the brain, but tomatidine may not be a useful biomarker to assess P-gp inhibition by the drugs. In conclusion, isoflavone sulfates were proposed as the first evidence to be a surrogate marker of BCRP inhibition, whereas further studies are required to assess a surrogate marker of P-gp inhibition.

Key words: Metabolomic study, BCRP, P-gp, biomarker

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