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Transcriptomic and Quantitative Proteomic Analysis of Transporters and Drug Metabolizing Enzymes in Freshly Isolated Human Brain Microvessels

Ramzi Shawahna,[†,‡,§] Yasuo Uchida,[§,∥] Xavier Declèves,[*,†,‡] Sumio Ohtsuki,[∥] Salah Yousif,[†,‡] Sandrine Dauchy,[†,‡] Aude Jacob,[†,‡] Francine Chassoux,[⊥] Catherine Daumas-Duport,[⊥] Pierre-Olivier Couraud,[#] Tetsuya Terasaki,[∥] and Jean-Michel Scherrmann[†,‡]

[†]Neuropsychopharmacologie des addictions (CNRS UMR 8206), Faculté de Pharmacie, Université Paris Descartes, Paris, France

[‡]Neuropsychopharmacologie des addictions, INSERM U705, Paris, France

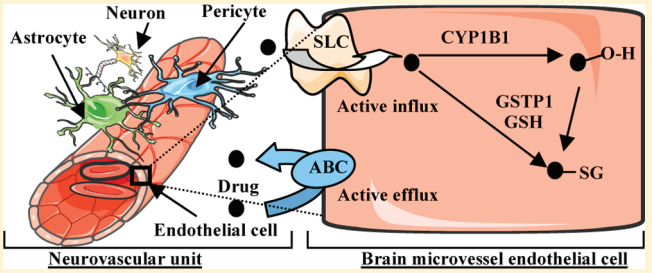
[∥]Division of Membrane Transport and Drug Targeting, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai, Japan

[⊥]Ste Anne Hospital, Paris, France

[#]Département de biologie cellulaire, INSERM U567, Institut Cochin, Paris, France

Supporting Information

ABSTRACT: We have investigated the transcriptomic and/or proteomic patterns of 71 solute carrier (SLC) and organic solute (OST) transporters, 34 ATP-binding cassette (ABC) transporters, and 51 metabolizing enzymes in human brain microvessels. We used quantitative RT-PCR and LC−MS/MS to examine isolated brain microvessels and cortex biopsies from 12 patients with epilepsia or glioma. SLC2A1/GLUT1, SLC1A3/EAAT1, and SLC1A2/EAAT2 were the main SLC proteins whereas ABCG2/BCRP, ABCB1/MDR1, ABCA2 and ABCA8 were the main ABC quantified in isolated brain microvessels; ABCG2/BCRP was 1.6-fold more expressed than ABCB1/MDR1, and ABCC4/MRP4 was 10 times less abundant than ABCB1/MDR1. CYP1B1 and CYP2U1 were the only quantifiable CYPs. Finally, GSTP1, COMT, GSTM3, GSTO1 and GSTM2 proteins were the main phase II enzymes quantified; UGTs and NATs were not detected. Our extensive investigation of gene and protein patterns of transporters and metabolizing enzymes provides new molecular information for understanding drug entry and metabolism in the human blood−brain barrier.



KEYWORDS: blood−brain barrier, transporters, drug-metabolizing enzymes, human, quantitative LC−MS/MS

INTRODUCTION

The blood−brain barrier (BBB) plays a critical role in the uptake and efflux of drugs from the blood to the brain, or vice versa, hence affecting their concentrations and effects in the central nervous system (CNS). The early concept of an anatomical barrier between the blood and the brain was supported by the finding of unique tight junctions between the brain endothelial cells so that they formed a continuous wall preventing the paracellular diffusion of solutes.[1] The BBB has more recently been defined as a pharmacological barrier since the endothelial cells were found to contain a range of metabolizing enzymes and transporters that control the rate and extent of drugs reaching the brain parenchyma via transcellular pathway.[2] Brain capillary endothelial cells express various transporters, or solute carriers (SLC) transporters, that facilitate brain uptake processes.[3] Some of the SLC transporters in the human BBB have been identified, but there is, as yet, no exhaustive absolute quantitative profile of SLC transporters. Solutes can also be extruded from the brain endothelium to blood by ATP binding cassette (ABC) efflux transporters.[4−6] The best example is the expression of P-glycoprotein (ABCB1/MDR1) by the luminal membrane, which limits the brain uptake of many CNS drug candidates.[7] Beside transporters, BBB expresses specific enzymes like monoamine oxidases (MAOs) and catechol *O*-methyltransferase (COMT).[8,9] Published reports on the presence of phase II enzymes at the BBB are conflicting[10] since metabolic activities were mostly investigated using brain homogenates instead of isolated brain microvessels.[11] For example, glucuronidation by UDP-glucuronosyl transferases (UGTs) was assumed to take place in the BBB while it was experimentally determined in the whole brain.[12−14] Similarly, little work has been done on the activities of other phase II drug metabolizing enzymes, particularly sulfotransferases (SULTs),

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