Expression of ABC transporters and CYP enzymes in an in vitro human BBB model: approximation possibilities?

Ramzi Shawahna†‡, Jean-Michel Scherrmann†‡, Xavier Declêves†‡

†Neuropsychopharmacologie des addictions (CNRS UMR 8206), Faculté de Pharmacie, Université Paris Descartes, Paris, France
‡Neuropsychopharmacologie des addictions, INSERM U705, Paris, France

In pharmaceutical industry, low permeability across the blood-brain barrier (BBB) is a classic motive to discontinue 98% of the candidates investigated to treat brain diseases and/or correct brain abnormalities. Recently, the hCMEC/D3 cell line has been validated as a promising in vitro tool in the study of various signaling as well as drug transport mechanisms at the BBB. Since a while, our laboratory is devoted to characterize the gene and protein expression of important influx and efflux transporters as well as metabolizing enzymes at this promising in vitro model in comparison with those observed in freshly isolated human brain microvessels (FIHBMV). We used quantitative real-time PCR (qRT-PCR) to establish and compare the gene expression of ATP-binding cassette (ABC) transporters and cytochrome P450 (CYP) in hCMEC/D3 and FIHBMV. In accordance with FIHBMV, the hCMEC/D3 cells expressed ABCB1/MDR1, ABCG2/BCRP, ABCC1/MRP1, ABCC4/MRP4, and ABCC5/MRP5. When quantitatively compared, ABCB1/MDR1 and ABCG2/BCRP were at least 10 and 100 fold less expressed in hCMEC/D3 cells than in FIHBMV, respectively. However, MRPs were approximately 2 to 10 fold more expressed in hCMEC/D3 cells. In line with FIHBMV, hCMEC/D3 cells expressed the CYPs 1A1, 1B1, 2B6, 2D6, 2E1, 2J2, 2R1, 2S1, and 2U1. Both FIHBMV and hCMEC/D3 cells expressed the transcriptional factor AhR. When quantitatively compared, the gene expressions for the CYPs 2U1, 2S1 and 2R1 were approximately similar. However, with the exception of CYP 2J2, the gene expressions of the rest of enzymes were less in hCEMC/D3 cells. Recently, we established a comparative gene expression profiles for ABC and CYPs in hCMEC/D3 cells cultivated under different conditions (Transwell filter inserts and plastic flasks). Interestingly, culturing on Transwell inserts induced the gene expression of ABCG2/BCRP up to 3-fold, whereas no effect was observed for the rest of transporters. In our efforts to approximate the gene expression in hCEMC/D3 cells to those observed in FIHBMV, we treated hCMEC/D3 cells with a Wnt/β-catenin activator (LiCl). LiCl treatment induced the expression of ABCG2/BCRP and CYP1A1 by 6 and 9 fold, respectively. Previously, we treated hCMEC/D3 with a potent AhR ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). We observed induction of gene expression of CYP1A1 and CYP1B1 from 8-72h, which was 26 and 28 fold after 24h, respectively. Recently, there has been a lively debate on the representative character of existing in vitro BBB models. From a maximalist point of view, despite the considerable expression of BBB phenotype by models like hCEMC/D3, no any model represents the BBB in vivo situations so far. Our results could be interesting from a minimalist point of view, since expression of ABC transporters and metabolizing enzymes is often induced in malignancy. However, our efforts also showed promising opportunities for approximation of gene expression in a versatile in vitro BBB model.