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

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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/D3cells 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.