Drug metabolism at the human epileptic blood-brain barrier: \textit{In situ} and \textit{in vitro}

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Abstract

\textbf{Rationale:} Drug delivery to the drug resistant epileptic brain may be hampered by overexpression of multiple drug transporter protein at the blood-brain barrier (BBB). We hypothesize that the BBB may also act as metabolic barrier. While cytochrome P450 (CYPs) enzymes play a major role in the metabolism of drugs by the liver, it is not clear whether these enzymes are also functionally expressed in human epileptic BBB, regulated by cerebral blood flow and possibly involved in drug response or resistance.

\textbf{Methods:} Primary endothelial cultures were obtained from brain specimens of drug resistant epileptic subjects (EPI-EC). Corresponding brain and serum samples were used for HPLC analysis. A primary human-derived brain endothelial cells (HBMEC) was used as control. CYPs transcript levels were assessed by qDNA microarrays. Results were confirmed and quantified by western blot on endothelial cells and by immunohistochemistry on resected brain epileptic specimens. The metabolism of carbamazepine (CBZ) metabolism was assessed by HPLC-UV using a dynamic in vitro (DIV) model of human epileptic BBB and directly on corresponding brain homogenate and serum.

\textbf{Results:} mRNA levels of CYP1A1, 1B1, 2A6, 2B6, 2C, 2C9, 2C19, 2D4, 3A4, 4A11 and 11b were significantly elevated in EPI-EC. CYP3A4 protein, involved in the metabolism of anti-epileptic drugs, was overexpressed in EPI-EC (290 ± 30%) compared to control and significantly increased in region of reactive gliosis in the drug resistant epileptic brain. In vitro, the amount of CBZ metabolized by EPI-EC was similar to the one metabolized by human hepatocytes (41.6% and 39.3% respectively). Interestingly, small levels of CBZ were detected in the abluminal side of the epileptic BBB throughout the duration of the experiments (1 to 72 hours) compared to the fraction that permeated in the abluminal space when using control endothelium. When comparing drug penetration/metabolism in the epileptic DIV-BBB with brain samples, a similar pattern of CBZ metabolism were discovered by mass-spectrometry.

\textbf{Conclusions:} CYP3A4, an AED metabolic enzyme, is expressed by drug resistant epileptic endothelium especially in regions of neurovascular malfornations.

\textbf{CBZ} metabolizer to form a stable fragment/minostilbene (194:10 m/z) and an interesting candidate is observed (additive form of CBZ; 249.06 m/z) in the brain side of both in situ and in vitro system which has yet to be characterized.

The "humanized" DIV-BBB can reproduce the pharmacokinetic blood-to-brain drug behavior as observed in drug resistant patients.

A novel experimental approach to determine qualitative and quantitative profile of AED penetration/metabolism in the human epileptic brain.