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**Functionality of P-glycoprotein in the Blood-Brain Barrier Mimicking Cell Line PBMEC/C1-2**


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The blood-brain barrier (BBB) maintains the homeostasis between the central nervous system and the blood circulation [1]. One of the main efflux transporter proteins at the BBB is P-glycoprotein (P-gP) also known as ABCB1 or MDR1. Recently, transport studies with antidepressants suggested presence and functional activity of P-gP in an *in vitro* model of the BBB based on porcine cell line *PBMEC/C1-2* [2]. Due to the important role of P-gP for the transport barrier function of the BBB, the presence and functionality of P-gP was investigated in cell line *PBMEC/C1-2* maybe leading to an *in vitro* BBB model suitable for P-gP substrate screening.

Firstly, presence of P-gP was confirmed on the protein level by western blotting with two different primary antibodies and immunofluorescence microscopy as well as on the mRNA level by RT²-PCR. Functional assessment was accomplished by an established 96-well uptake assay using Rhodamine 123 as a P-gP substrate and Verapamil as a moderate P-gP inhibitor. In this regard, fluorescence microscopy confirmed a significant greater uptake of Rhodamine 123 into *PBMEC/C1-2* cells when supplemented with Verapamil. Furthermore, functional knock-down of P-gP by antisense oligonucleotides revealed an increase of Rhodamine 123 uptake indicating decreased P-gP functionality. Finally, transport studies in a Transwell system with the antihistaminic drug Fexofenadine showed a significant increase of the permeability coefficient after addition of Verapamil.

In summary, the presence and functionality of P-gP in the immortalised cell line *PBMEC/C1-2* was proven with several techniques and assays. Thus, this cell line could be used for P-gP substrate screening in the context of BBB relevant issues.
