Novel in-vitro models for assessing transport across the human blood-brain barrier (BBB)

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The blood brain barrier (BBB) is a protective layer to limit or completely to avoid passage of components present in blood to the brain. These components could be therapeutic drugs or chemical toxins. BBB is characterized with a transporter mechanism which is effective in moving chemicals inside and outside of the brain area. These transporters are either efflux (pumping chemicals out of the brain) or uptake (taking chemical into the brain).

The aim of this project was to establish a cell model expressing brain cancer resistant protein (BCRP) (efflux transporter) or organic anion transporting peptide 2b1 (OATP2B1) (uptake transporter). In order to do that, we introduced these constructs (transfect) in to an established dog kidney cell line known as MDCK. We continued growing and keeping healthy the surviving cells until they were all examined. We performed transport experiments to find out which one of our cells have successfully sustained these transporters. For BCRPs we used transwell experiment and for OATPs we used uptake experiments. In the transwell experiment, cimetidine has been used as a test compound. We calculated the permeability of our test compound to identify how much of the drug is being pumped out (i.e. effluxed). Those cells which showed enough efflux ratio (ER) were considered as positive and were further investigated with an inhibitor to see if the ER would reduce. Hence, any cell which showed the ER reduction, identified to be the most promising one.

In order to screen OATPs, we used three test compounds, Atorvastatin, Rosavastatin and estrone-3 sulphate (E3S). In addition, uptake experiment was performed and the data were compared with the negative control (MDCK cells without any transporter) and cells which showed significant increase in uptake transportation were considered as positive.

In this study six BCRP- introduced clones (identical set of cells) showed acceptable level of ER and further showed reduction after treatment with the inhibitor. Four OATP2B1- introduced clones showed increase uptake with at least two OATPB1 test compounds. These clones have been identified for further investigation.

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