CAMO (COMPARING ANIMAL MODELS TO ORGANOIDS) - TE STING MEDICAL COUNTERMEASURES WITH MICROPHYSIOLOGICAL SYSTEMS AND COMPARING TO TRADITIONAL ANIMAL MODELS AND CLINICAL TRAILS

A Syngenic Porcine In Vitro Blood-brain Barrier Model Predicts in vivo Brain Delivery Of Therapeutic Antibodies.

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Background
A reliable and predictive in vitro model of the blood brain barrier (BBB) suitable for high through-put screening has proved historically difficult to establish. In vitro BBB models using immortalized cell lines can be modified to form a tight barrier, but do not maintain crucial in vivo characteristics like the expression of tight junction proteins, uptake transporters and receptors, or efflux transporters.

We have optimised a primary porcine in vitro BBB model, simplified for semi-high through-put screening, comprising porcine brain capillary endothelia co-cultured with porcine astrocytes, the syngenic model. Additional cell types of the neuro-vascular unit (e.g. pericytes) do not further improve the model. The syngenic model has low passive permeability, expresses BBB characteristic tight junction proteins (claudin-5, ZO-1), and BBB transport systems encompassing receptor mediated transcytosis (transferrin, LRP-1) and solute carriers (e.g. GLUT1), which are polarized functionally favouring blood-to-brain transport.

Purpose
The model was used to screen BBB penetration of non-ionic synthetic vesicles (NISVs) with a mAb or small molecule cargo, and a range of ligands to target BBB transporters and optimise BBB penetration. These data were then compared to in vivo brain delivery of the cargo in mice.

Preliminary Results
The in vitro screen predicted that ligands targeting either BBB solute transporters or receptor mediated endocytosis, increased BBB penetration 2-3 times over that of the pristine NISV, or non-BBB targeting ligands. Overall, BBB targeted NISVs improved mAb transport 7-12 times compared to free mAb, while pristine NISVs improved mAb transport across the BBB 4 times.

In vivo murine tissue distribution and infection studies, using NISVs carrying either the dye Hoechst or an anti-VEEV mAb, correlated well with the in vitro screen for brain delivery. The most effective in vivo BBB-targeted ligands were glucosamine (GLUT1 target) and Angiopep-2 (LRP-1 target), as predicted by the in vitro screen, and the least effective coating was mannosamine (CD206 target) which is not predominantly expressed on the brain endothelia. Importantly the enhanced targeting to the BBB does not come at a cost of other tissues, with benefits of NISV encapsulation also seen in lung, liver and spleen.

Preliminary conclusions
In summary, the syngenic in vitro BBB model successfully predicts brain targeted delivery of nanoparticle NISV cargo, correlating well with in vivo assays. This model is a useful tool for screening potential CNS therapies and allowing pre-in vivo optimisation of delivery systems in an affordable and timely manner.

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