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Gene Delivery to the Blood-Brain Barrier

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Drug- and gene delivery to the brain is highly restricted by the vascular barriers of the brain, denoted by the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (CSF) barriers. Among these barriers, BBB is the main limiting factor. It is composed by the brain capillary endothelial cells (BCECs). The BCECs barrier function is supported by astrocytes, pericytes and neurons to form the blood-brain barrier. BCECs are very tightly connected to each other by tight junctions. Apart from the essential substrates needed to nourish the brain, small and/or lipophilic molecules are free to diffuse into the brain. However most pharmacologically active drugs and gene fragments are too large to enter the brain. Various kinds of drug-carriers have been constructed for delivery of large substances to the brain.
Such drug-carriers have to be able to successfully carry their cargo through the BCECs and into the brain. For testing the ability of drug-carriers to deliver their cargo into the brain, investigators have constructed different in vitro BBB models, consisting of BCECs that express the main characteristics of the BBB in vivo.
In the first part of the thesis the ability of two drug-carriers, pullulanspermine and SPIOs, to mediate transfection of BCECs or transcellular transport through BCECs in vitro was studied.

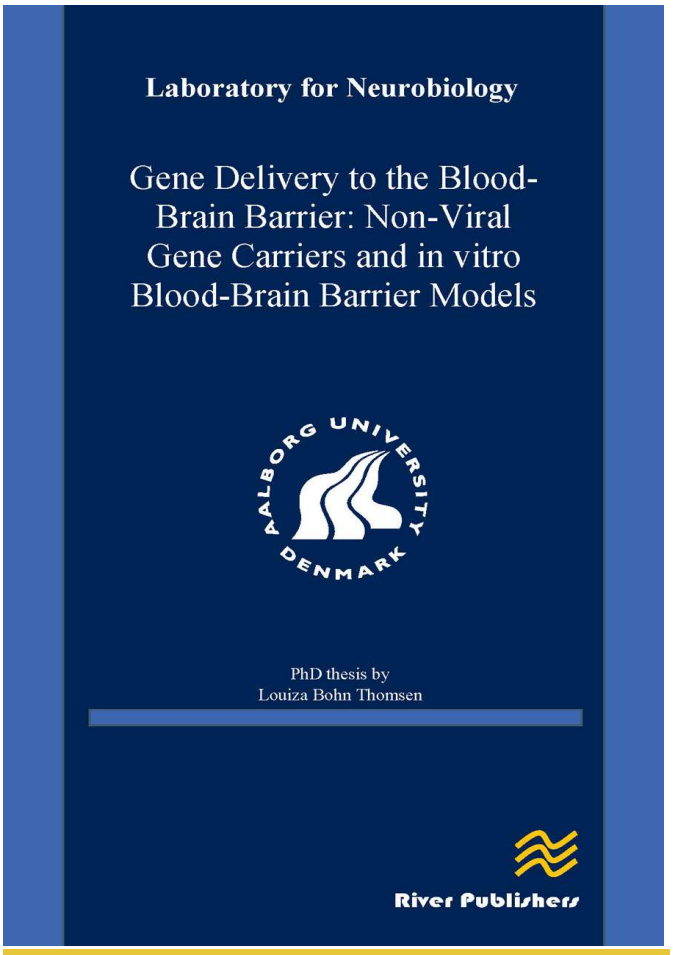
Pullulan-spermine is a polymeric complex consisting of the polysaccharide, pullulan and the polyamine, spermine. Pullulan-spermine formed a cationic complex shown to be able to bind plasmid DNA electrostatically.
Pullulan-spermine was conjugated with plasmid DNA encoding a red fluorescent protein, Hc-Red-1 C1, or human growth hormone 1 (hGH1). Pullulan-spermine complexed with Hc-Red-1 C1 cDNA led to the formation of a red fluorescent signal in human brain microvascular endothelial cells (HBMECs). Furthermore, pullulan-spermine complexed with hGH1 cDNA was not only able to transfect HBMECs but also led to secretion of the hGH1 into the culture media. Pullulanspermine-cDNA complexes could transfect non-dividing cells although the rate of transgene cells was higher in dividing cells. This indicated that the DNA is not only entering the cell nucleus under mitosis. Unfortunately, pullulan-spermine complexes proved incapable of transfecting HBMECs in the presence of serum in the growth media and additional studies are needed to enable its use for in vivo transfection.

Another potential drug-carrier, fluorescent iron oxide nanoparticles were also shown to enter HBMECs upon incubation. These nanoparticles were also able to pass though the HBMECs forming a BBB in a static in vitro BBB model. Furthermore, their passage was increased by the aid of an external magnetic field created by placing the cell culture plates with the SPIOs on a plate magnet. Two vitality tests showed no significant change in BCEC vitality after addition of

Non-viral delivery strategies into and across the brain capillary endothelial cells xiv
SPIOs or by dragging the nanoparticles through the BCECs in the presence of the
external electric field.

The results of the drug-carrier studies indicate that it is possible to deliver plasmid cDNA into BCECs and transfect these cells leading to their secretion of
encoded protein into the extracellular space. Moreover, SPIOs are potentially
potent carriers of attachable molecules trough cultured BCECs in vitro, which may
have high potential for drug-delivery to the brain in vivo.

In the second part of the thesis, two in vitro BBB models, a static and a dynamic model was investigated and compared. The static model consisting of
microporous membrane inserts in which immortalized BCECs is cultured. The
model induces many characteristics of the BBB in vivo, but lacks the tightness
induction factor of shear stress. Different experiments were performed with this
static model to monitor BBB integrity. Barrier formation by the BCECs was monitored by measuring transendothelial electric resistance (TEER) and the BCEC
monolayer was stained positive for zonula occludens 1 (ZO-1) a tight junction
protein. It was mainly found that the tightness of the BCECs was strengthened by
contact co-culture of the BCECs with astrocytes and addition of hydrocortisone to
the media. The dynamic in vitro BBB model however, did not lead to any reliable
results in this study and further investigation of barrier formation in this model was
not pursued. In consequence a comparison between the static and dynamic in vitro
models was not possible, but it could be concluded that the static model seems to
be the most reliable model



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