The Transferrin Receptor at the Blood-Brain Barrier - exploring the possibilities for brain drug delivery

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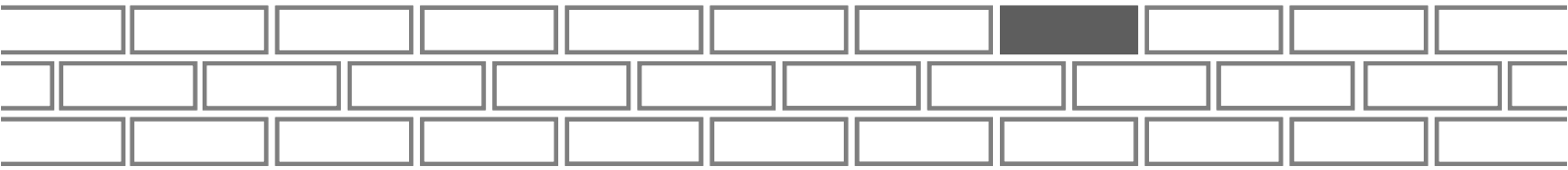
Chapter 1



Preface:

Scope and intent of the investigation

Background of the investigations

There are many diseases of the central nervous system (CNS), like Parkinson's disease, Alzheimer's disease, depression, schizophrenia, epilepsy, migraine headache, and HIV infection in the brain (1). However, treatment is difficult since many drugs cannot reach the brain in sufficient quantities due to the existence of the blood-brain barrier (BBB). According to Pardridge (2001), more than 98 % of all potential new drugs for the treatment of CNS disorders do not cross the BBB (1). Over the last years, pharmaceutical companies have focussed on the development of small drug molecules as therapeutic moieties. In general, small molecules should be lipid-soluble and have a molecular weight below 400 – 600 Da to be able to cross the BBB in therapeutically effective quantities (1). These characteristics often cannot be found in one molecule and, therefore, many of these small drug molecules will not cross the BBB in sufficient quantities without brain drug-targeting strategies. In addition, more and more larger molecules are generated by biotechnological means which constitute promising alternatives for the treatment of diseases of the CNS. These include proteins (neurotrophins, (2)) or genes (neprylysin gene, (3)) for Alzheimer's Disease, antisense therapy for Huntington's disease (4) and monoclonal antibodies for diagnostic purposes (5) and the treatment of brain metastasis of breast cancer (6, 7). These larger biotechnology therapeutics can not cross the BBB without using targeting and delivery strategies. Promising targeting strategies for drug delivery to the brain often focus on endogenous transporters at the BBB, such as the insulin receptor (8), the transferrin receptor (9, 10) or the hexose transport system for glucose and mannose (11). Targeting and delivery strategies include the use of pro-drugs, recombinant proteins, drug-targeting vector conjugates or liposomes tagged with a targeting vector.

Scope and outline of this thesis

The aim of this thesis is to explore the possibilities for drug targeting to the brain by using the transferrin receptor (TfR) at the BBB. These studies are conducted *in vitro*, with primary cultured bovine brain capillary endothelial cells (BCEC). Gaillard *et al* (2001) have developed an *in vitro* model of the BBB where BCEC are co-cultured with rat

astrocytes (12). However, we have not co-cultured the BCEC in the presence of astrocytes, but we used astrocyte-conditioned medium to be able to investigate the TfR more mechanistically. Bovine transferrin (Tf) is used as a targeting vector since the available bovine polyclonal antibodies are not specific enough for drug targeting purposes. Furthermore, by using the endogenous ligand a more mechanistic approach to explore the possibilities for drug targeting to the TfR was possible.

Chapter 2 gives an introduction to the biology and physiology of the BBB, with emphasis on the TfR. Furthermore, drug targeting and delivery strategies to the brain are discussed. *Section 2* of this thesis focuses on the TfR. **Chapter 3** describes the characterisation of the TfR on BCEC *in vitro*. In addition, the influence of several modulators, such as an excess of iron, an iron scavenger, astrocyte conditioned medium and lipopolysaccharide (LPS) on the expression and internalisation of the TfR is investigated. In **chapter 4** the validation of the TfR for drug targeting is described. For this research conjugates of horseradish peroxidase (HRP) and Tf were prepared. HRP is a 40 kDa protein, which normally does not cross the BBB. It was found that Tf-HRP conjugates were internalised by the TfR via a similar mechanism as endogenous Tf.

Section 3 focuses on Tf-tagged liposomes. By using liposomal drug carriers, the ratio of drug molecules per targeting vector (i.e. Tf) is increased for certain classes of drug molecules. Furthermore, it is not necessary to chemically modify the drug, as is the case for drug conjugates. The preparation of Tf-tagged liposomes is described in **chapter 5**. For this preparation it is essential that Tf retains its two iron atoms, since di-ferric Tf has a much higher affinity for the TfR than apo-Tf. Subsequently, in **chapter 6** the Tf-tagged liposomes are loaded with HRP to determine the association of liposomes by BCEC in vitro. This research showed that Tf-tagged liposomes were suitable for drug targeting to the brain. However, Tf-tagged liposomes had a different intracellular distribution than Tf-drug conjugates.

In *section 4* drug targeting and delivery under inflammatory disease conditions is described. For this research, Tf-tagged liposomes containing the free radical scavenger N-acetyl-L-cysteine were prepared. However, we found that liposomes themselves interacted with LPS. In **chapter 7** this interaction of liposomes and LPS is investigated and the influence of time of incubation, presence of serum and liposome composition are described.

To conclude this thesis, **chapter 8** summarises and discusses the results that were obtained and some future perspectives are presented.

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