Blood-brain barrier functionality, disease and drug targeting

Visser CC, Voorwinden LH, Van Bloois L, Gaillard PJ, Crommelin DJA*, Danhof M, *De Boer AG*
LACDR, Leiden University, Division of Pharmacology, Blood-Brain Barrier Research Group, Leiden, *UIPS, Utrecht University, Department of Pharmaceutics, Utrecht

Introduction. The blood-brain barrier (BBB) regulates the homeostasis of the brain. In addition, it limits the transport of many drugs to the CNS. Due to its specific features, such as tight junctions between endothelial cells, a continuous basal membrane, low pinocytotic activity and a lack of fenestrae, only small lipophilic drugs or drugs that are substrates for a specific transporter, can pass the BBB. Particularly under disease conditions such transport systems are needed to treat brain diseases. Our research focuses on i) the characterisation of the transferrin receptor (TfR) at the BBB and ii) the use of this receptor for drug targeting to the brain. Specifically, the objective is to deliver drugs tagged with transferrin (Tf) to the BBB. These studies are conducted in an *in vitro* BBB model, consisting of primary cultured bovine brain capillary endothelial cells (BCEC) and astrocytes conditioned medium (1-3).

Results and Discussion. The level of TfR expression on BCEC was determined by radioligand binding studies. For endothelial cells cultured in the presence of astrocytic factors a B_{max} of 0.18 ± 0.06 fmol/mg protein and a K_d of 3.2 ± 1.0 μg/ml was found. The addition of saponin, a cell permeabilising agent, increased the B_{max} to 2.3 ± 0.6 fmol/mg protein, while the K_d was unaffected. Endocytosis studies at 37°C revealed that Tf is actively endocytosed by the TfR on BCEC. Furthermore, uptake of Tf was inhibited by phenylarsineoxide, an inhibitor of the clathrin-mediated pathway associated with the TfR. In contrast indomethacin, an inhibitor of caveolae mediated endocytosis, did not change Tf uptake.

In order to study the uptake of carge coupled to Tf we have first chosen to couple horseradish-peroxidase (HRP) to Tf. This compound can be easily measured in cells following cell lysis. The results showed that the uptake of Tf-HRP by BBB endothelial cells at 37°C increased linearly following application of 0.25 - 10.0 μg/ml. In addition, binding at 4°C increased linearly but was about 4 times less than at 37°C.

Subsequently the rate of uptake was estimated following incubation with a concentration of 3 µg Tf-HRP/ml. Equilibrium was attained following 1 h indicating that uptake was a fast process. In addition, binding at 4°C was again about a factor 4 smaller than uptake at 37°C.

Selective/specific transport of Tf-HRP was investigated in the presence of excess Tf (which competes for transport by the TfR) and bovine-serum-albumin (BSA) that competes for a-selective binding sites. 500 fold excess of unconjugated Tf decreased the uptake of Tf-HRP at 37°C by a factor of about 5 while 500 fold excess of BSA did not had any effect at uptake. This illustrates that the uptake occurred by a selective transport process, particularly via the TfR.

Conclusion.These results show that the TfR is present on BCEC and that it is actively endocytosing Tf via a clathrin-mediated pathway. In addition, Tf-conjugated compounds (Tf-HRP) are selectively taken up via the Tf-R at the BBB. This indicates that targeting drugs to the Tf-R at the BBB is a promising way for drug delivery to the brain.

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A(bert) G. de Boer, Dicision of Pharmacology, LACDR, Univ Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands, t 071 5276215, e-mail b.boer@lacdr.leidenuniv.nl
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