Abstract

This thesis “Nanobody-functionalized liposomes for transferrin receptor 1-targeted drug delivery” focuses on the development and validation of a novel targeted liposomal system functionalized with the anti-TfR1 nanobody Q52c. Iron is an essential metal involved in numerous cellular and metabolic processes and must be tightly regulated to prevent the pathological consequences of both deficiency and excess.Transferrin receptor 1 (TfR1; CD71) plays a central role in iron homeostasis by mediating the uptake of transferrin-bound iron. Because TfR1 is frequently overexpressed in highly proliferative and metabolically active cells, including cancer, it represents an attractive and promising target for drug delivery applications.

Building on an extensive review of the literature, this thesis addressed both the therapeutic relevance of TfR1 and the limitations of conventional targeting strategies. The main objective was to investigate the potential of TfR1 as a biological target for nanocarrier-mediated drug delivery. The receptor-specific targeting approach adopted was Q52c, as a novel and previously unexplored ligand for liposome functionalization. Liposomes were selected as nanocarriers because of their biocompatibility, versatility, and ability to interact with biological barriers.The thesis aimed to assess whether Q52c-mediated TfR1 targeting could enhance the selective recognition of TfR1-expressing cells and promote receptor-mediated cellular uptake.

The production and in vitro studies of Q52c-functionalized liposomes were conducted at the Department of Pharmaceutics, Utrecht University. Cellular uptake experiments were performed under both static and dynamic flow conditions, with the latter designed to mimic physiological intravascular shear stress. Static uptake studies were conducted in human cerebral microvascular endothelial cells (hCMEC/D3), human breast cancer cells (MCF-7), and human lung adenocarcinoma cells (A549), allowing liposome uptake to be tested across different TfR1-expressing cellular models and enabling a comparison between tumor and endothelial cell models.

Dynamic perfusion experiments were performed in hCMEC/D3 cells, an established in vitro model of the blood–brain barrier (BBB). Under flow conditions, these cells are exposed to shear stress, providing a more physiologically relevant model for studying the uptake of liposomal nanocarriers. Q52c-functionalized liposomes displayed higher cellular uptake than both R2-functionalized control liposomes and non-targeted liposomes under static and dynamic conditions. These results support the use of Q52c-decorated liposomes as a promising strategy to enhance TfR1-mediated targeting and improve therapeutic precision while reducing off-target effects.

In parallel, non-functionalized liposomes were produced at the Department of Industrial Engineering, University of Salerno, using supercritical CO_2-assisted technology. This activity was not intended as a direct comparison with the nanobody-functionalized formulations developed in Utrecht, but rather as a manufacturing-oriented study aimed at exploring a potentially scalable and reproducible platform for future pharmaceutical translation.

This thesis provides experimental evidence that anti-TfR1 nanobodies can be effectively used to functionalize liposomes and enhance their selective uptake by TfR1-expressing cells. In addition, the preliminary production of non-functionalized liposomes by supercritical CO_2-assisted technology highlights the potential of this approach as a scalable and reproducible manufacturing platform for future liposomal formulations. The integration of receptor-specific nanobody targeting advanced production technologies may contribute to the future translation of TfR1-targeted liposomal systems toward pharmaceutical applications.