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Review article

Revisiting nanoparticle technology for blood–brain barrier transport: Unfolding at the endothelial gate improves the fate of transferrin receptor-targeted liposomes

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ABSTRACT

An unmet need exists for therapeutic compounds to traverse the brain capillary endothelial cells that denote the blood–brain barrier (BBB) to deliver effective treatment to the diseased brain. The use of nanoparticle technology for targeted delivery to the brain implies that targeted liposomes encapsulating a drug of interest will undergo receptor-mediated uptake and transport through the BBB with a subsequent unfolding of the liposomal content inside the brain, hence revealing drug release to adjacent drug-demanding neurons. As transferrin receptors (TfRs) are present on brain capillary endothelial, but not on endothelial cells elsewhere in the body, the use of TfR-targeted liposomes — colloidal particulates with a phospholipid bilayer membrane — remains the most relevant strategy to obtain efficient drug delivery to the brain. However, many studies have failed to provide sufficient quantitative data to proof passage of the BBB and significant appearance of drugs inside the brain parenchyma. Here, we critically evaluate the current evidence on the use of TfR-targeted liposomes for brain drug delivery based on a thorough investigation of all available studies within this research field. We focus on issues with respect to experimental design and data analysis that may provide an explanation to conflicting reports, and we discuss possible explanations for the current lack of sufficient transcytosis across the BBB for implementation in the design of TfR-targeted liposomes. We finally provide a list of suggestions for strategies to obtain substantial uptake and transport of drug carriers at the BBB with a concomitant transport of therapeutics into the brain.

Contents

Abbreviations: 6-OHDA, 6-hydroxydopamine; ABC, ATP-binding cassette; ATP, adenosine triphosphate; BBB, blood–brain barrier; BCEC, brain capillary endothelial cell; bFGF, basic fibroblast growth factor; CHE, cholesteryl hexadecyl ether; CNS, central nervous system; CSF, cerebrospinal fluid; Da, Dalton; DMT1, divalent metal transporter 1; DNA, deoxyribonucleic acid; DOTAP, 1,2-dioleoyl-3-trimethylammonium-propane; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine; EEA-1, early endosome antigen 1; EGFR, epidermal growth factor receptor; FAK, focal adhesion kinase; GDNF, glial cell line-derived neurotrophic factor; GLUT1, glucose transporter 1; HIV, human immunodeficiency virus; ID/g, injected dose per gram; IGFR, insulin-like growth factor receptor; IgG, immunoglobulin G; JAM, junctional adhesion molecules; LDLR, low-density lipoprotein receptor; LRP, LDLR-related protein; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; NVU, neurovascular unit; PDGFRß, platelet-derived growth factor receptor ß; P-gp, P-glycoprotein; PECAM, platelet endothelial cell adhesion molecule; PEG, polyethylene glycol; PI3K, phosphoinositide-3-kinase; TEER, transendothelial electrical resistance; TfR, transferrin receptors; TGF-ß, transforming growth factor-ß; ZO, zonula occludens.

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1. Introduction

Disorders of the central nervous system (CNS) affect millions of people worldwide. Their severe nature results in a large number of hospitalizations every year, which poses a substantial cost on the healthcare system. In the United States alone, the estimated annual spending on treatment of CNS disorders is around $650 billion [1]. Inadequate treatment opportunities are often caused by the challenges imposed by the blood–brain barrier (BBB), which results in reduced uptake of drugs and subsequent failure to undergo transport into the brain parenchyma [1]. Therefore, there is an obvious need for the development of new therapeutic compounds or drug delivery vehicles that can traverse the BBB and deliver effective treatment to affected brain areas. In order to secure high concentrations of drug reaching the brain, many research groups use advanced drug carriers like liposomes that are colloidal particulates with a phospholipid bilayer membrane encapsulating an aqueous core. This composition is attractive, because it allows for encapsulation and loading of both hydrophilic and hydrophobic drugs. The liposomes can be coated with specific molecules or constructs that ensure stealth-like properties, hence allowing for long-time circulation in plasma or targeting of the liposomes towards accumulation in specific tissues [2,3]. Liposomes can target the brain via different transport molecules present on the surface of brain capillary endothelial cells (BCECs). Research efforts devoted to the study of transferrin receptors (TfRs) demonstrated efficient uptake of drug-loaded liposomes targeting this receptor, revealing uptake into BCECs in a degree much larger than when attempts were made to target receptors like the low-density lipoprotein receptor (LDLR), LDLR-related protein (LRP), or insulin-like growth factor receptor (IGFR) [4–6]. Moreover, the use of nanoparticle technology enable targeted delivery to the brain implies that targeted liposomes encapsulating drug of interest will undergo receptor-mediated uptake and transport thorough the BBB with a subsequent unfolding of the liposomal content inside the brain. Although encouraging preclinical data suggest that liposomes targeting the TfR are of high relevance for brain drug delivery, no formulations have yet reached the clinical stages of drug development.

In this review, we critically evaluate current evidence on the use of TfR-targeted liposomes for drug delivery based on a thorough investigation of all available studies within this field of research. We focus on important issues with respect to experimental design and data analysis that may provide an explanation to conflicting reports. We illustrate the current knowledge of the intracellular trafficking of liposomes taken up by BCECs, and we discuss possible explanations for the current lack of sufficient transcytosis across the BBB for implementation in the design of TfR-targeted liposomes. Our running hypothesis of this discussion is that the binding mode between the targeted liposome and receptor is of great importance to predict the intracellular sorting. We also highlight key structural challenges present beyond the BBB for consideration to obtain sufficient distribution of the drug inside the brain parenchyma, and we bring our suggestions for providing substantial uptake and transendothelial transport of drug carriers at the BBB.

2. The structure and function of the blood–brain barrier and neurovascular unit

The BCECs are characterized by the presence of a membrane specialization denoting the BBB. This BBB is mainly composed of a tightly sealed monolayer of BCECs that prevents free exchange of molecules larger than 400 Da, thereby precluding the entrance of many nutrients, ions and neuroactive drugs into the brain (Fig. 1) [7]. The tight association between individual BCECs is maintained by the expression of different tight junction proteins at the cellular interfaces. In concert, the tight junction proteins are able to create an impermeable membrane with high values of transendothelial electrical resistance (TEER) reaching up to 1800 $\Omega * cm^2$ in the human brain [8,9]. The most prominent tight junction proteins are occludin and the claudins (especially claudin 5), all integral membrane proteins capable of binding to counterparts on adjacent cells [7]. The strength of the cell-to-cell interaction is also enforced by the occludins and claudins binding via adaptor molecules (ZO-1, ZO-2, ZO-3 and cingulin) to the actin cytoskeleton [10,11]. Activation of the actin cytoskeleton as a consequence of increased intra- and extracellular concentrations of calcium ions therefore modulates the barrier properties by interfering with the tight junction assembly [12,13]. In addition to the contribution from occludins and claudins, the BBB integrity is also regulated by the expression of adherens junction proteins (VE-cadherin, caveolin-1 and PECAM-1) and junctional adhesion molecules (JAMs) [7].

Due to the low permeability of the BBB, the brain parenchyma depends on different molecular transport systems to maintain homeostasis. Thus, the surface of BCECs is characterized by the expression of a vast variety of transport molecules. One of the main transport molecules is GLUT1 that transports glucose across the endothelial layer in a manner tightly regulated by the metabolic needs of the brain parenchyma, as is evidenced by the asymmetrical distribution of this transport protein between the luminal and abluminal BCEC membranes [14]. Two transport systems exist for amino acids that differ in their dependency of sodium as a co-factor. Many amino acid transporters are abundantly expressed on both the luminal and abluminal membranes, whereas others are exclusively expressed on the abluminal membrane to facilitate removal of potentially neurotoxic amino acids from the brain [7]. The ionic balance of the brain parenchyma is regulated by the expression and activity of ion transporters at the BBB, such as the sodium–potassium ATPase and the sodium–potassium-two chloride cotransporter [15,16]. Importantly, the BCECs also express a number of efflux transporters including ABC transporters like the P-glycoprotein (P-gp) that prevent the uptake of harmful substances and many drugs by releasing them back into the circulation instead of allowing continued transport across the BCEC layer [17,18]. The combined effects of these transport systems keep the microenvironment of the brain parenchyma tightly regulated, which secures proper functioning of the CNS [7].

A basement membrane surrounds the BCECs and contributes to the maintenance of BBB [7,19,20]. This membrane is bipartitioned into two sub-membranes; an inner, endothelial cell-associated basement membrane and an outer, parenchymal-associated membrane. The inner membrane consists of one sheet of collagen and one sheet of laminin interconnected by small extracellular matrix molecules called perlecan and nidogen [21]. The collagens of this membrane are mostly isoform IV, and they are known to be highly important for maintaining the integrity of the BBB, since deletion of the COL4A1 gene leads to extensive cerebral hemorrhage [22]. Laminins denote a group of trimeric proteins each with a single α-, β-, and γ-chain. The laminins of the inner endothelial basement membrane are mostly laminin411, although with laminin511 co-expressed in a patchy pattern. This organization is thought to facilitate leukocyte entry, since deletion of laminin411, and the subsequent compensatory increase in laminin511