Breaching the Blood-Brain Barrier for Drug Delivery

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Molecular "hitchhiking" through receptor-mediated transcytosis at the blood-brain barrier is a CNS drug delivery strategy. In this issue of *Neuron*, Niewoehner et al. (2014) describe a modular anti-transferrin receptor F_{ab} approach for shuttling therapeutic antibodies into the brain.

The blood-brain barrier (BBB) is lined with brain endothelial cells, sealed with paracellular protein complexes, bound by extracellular matrix, and maintained through pericyte and glial interactions (Zlokovic, 2008). Through its ability to restrict penetration of biomolecules, the BBB regulates the chemical composition of the CNS required for proper neuronal function. While vital for health and normal physiology, the BBB remains an obstacle for delivery of therapeutics into the brain. In particular, large biologics including peptides and antibodies exhibit a restricted ability to cross the BBB (Pardridge, 2012; Yu and Watts, 2013). Therefore, the development of noninvasive strategies to enhance macromolecule delivery across the BBB has been a long-sought objective for academic and biopharmaceutical research. A variety of approaches including nanoparticles, liposomes, vesicles, peptide conjugates, viruses, and antibodies are being pursued, most with mixed results (Pardridge, 2012; Ramos-Cabrer and Campos, 2013; Yu and Watts, 2013). Although considerable preclinical data exist using these BBB transport strategies, none of these approaches are known to be efficacious in treating human CNS disorders.

The main barriers that regulate molecular exchange between blood and brain include choroid plexus and arachnoid epithelium for exchange between blood and CSF, and the BBB separating blood from brain parenchyma. While epithelial barriers permit passive transport, large molecules in CSF are rapidly cleared into blood via bulk flow and diffuse poorly into the brain parenchyma (Pardridge, 2012). Therefore, due to the substantial surface area for molecular transport, the BBB is considered the primary interface for drugs to effectively penetrate the brain. While small lipid-soluble molecules can enter the CNS through passive transport, specialized carriers and receptors actively mediate transport of small water-soluble, polar molecules and macromolecules across the BBB (Zlokovic, 2008). For example, carrier-mediated transport pathways exist for glucose, amino acids, and nucleosides (e.g., adenosine), whereas receptor-mediated transcytosis (RMT) pathways carry macromolecules such as insulin, leptin, and transferrin into the brain. Transcytosis is a process by which macromolecules are transported within membrane bound vesicles between apical and basolateral domains of polarized cells (Tuma and Hubbard, 2003). Among transcytotic cargo of the cerebrovascular endothelium, the transferrin receptor (TfR) has been particularly well studied as a means to target drug delivery into the CNS. Whereas most studies have utilized a TfR antibody carrying therapeutic cargo (Pardridge, 2012) or a bivalent antibody in which one arm binds TfR and the other a disease target (Yu et al., 2011), in this issue of *Neuron*, Niewoehner et al. (2014) developed an anti-TfR F_{ab} to mediate BBB transcytosis of an attached immunoglobulin. To test the therapeutic potential of this "Brain Shuttle," Niewoehner et al. (2014) re-engineered a monoclonal antibody (mAb) against Aβ, the toxic peptide that accumulates in Alzheimer's disease (Bohrmann et al., 2012), by fusing the anti-TfR F_{ab} to the C terminus of the anti-Aβ mAb in a monovalent fashion (Figure 1A). Notably, this Brain Shuttle-modified anti-Aβ showed significantly enhanced brain penetration and amyloid plaque reduction in a transgenic mouse model of Alzheimer's disease.

As early as 1984, Jefferies discovered the abundance of TfR on brain capillaries that functions to deliver iron-bound transferrin into the brain (Jefferies et al., 1984). Nearly a decade later, experiments using radiolabeled tracers demonstrated that TfR antibodies can cross the BBB (Pardridge et al., 1991) and deliver therapeutic payloads such as methotrexate (Friden et al., 1991). Since then, significant effort has been made to identify the molecular mechanisms and therapeutic potential of TfR or other RMT pathways for delivering biologics into the brain. Transferrin or transferrin mimetic peptides fused to a therapeutic cargo are minimally effective in BBB transport due to high levels of competing endogenous transferrin in blood. However, antibodies against TfR that do not disrupt transferrin binding have been developed and shown to transport macromolecules including glial-derived neurotrophic factor (GDNF), erythropoietin (EPO), tumor necrosis factor receptor II (TNFR-II), and various enzymes across the BBB in preclinical models (Pardridge, 2012). Recently, a bispecific antibody with one arm that binds TfR and another arm that binds BACE1 was described (Yu et al., 2011). BACE1 is a membrane-associated aspartyl protease that mediates initial cleavage of the amyloid precursor protein (APP) required for generation of Aβ. This bispecific antibody significantly reduced central Aβ levels even after a single dose (Yu et al., 2011), presumably by inhibiting or targeting BACE1 for degradation. Notably, Yu et al. found that lower affinity anti-TfR antibodies showed increased brain uptake, whereas antibodies with high affinity to TfR remained inside the neurovasculature. The efforts described above established a foundation for developing RMT therapeutic delivery strategies for treatment of CNS disorders, such as the approach reported from

Niewoehner et al. (2014) in this issue of *Neuron*.

Niewoehner et al. (2014) began by generating brain shuttle constructs fusing one (sF_{ab}) or two (dF_{ab}) anti-TfR F_{ab} fragments to the C terminus of a full-length monoclonal anti-Aβ antibody (mAb31) to mediate monovalent and bivalent binding to TfR, respectively (Figure 1B). Both constructs maintained high-affinity binding to Aβ and were taken up in endothelial cells via TfR endocytosis. This is where the similarities between the two constructs diverged. Whereas the monovalent sF_{ab} fusion mediated effective uptake, transcytosis, and TfR recycling, the presence of two F_{ab} fragments on mAb31 (dF_{ab}) resulted in uptake followed by trafficking to lysosomes and an associated reduction in TfR levels (Figure 1B). The intracellular sorting and trafficking pathways for recycling versus degradation were shown in vitro in endothelial cells and in vivo in the PS2APP transgenic mouse model of Alzheimer's disease. Niewoehner et al. (2014) hypothesize that the presence of two anti-TfR F_{ab} fragments on mAb31 results in TfR dimerization and sorting to lysosomes (Figure 1B). It will be important for future studies to define the molecular basis for this differential sorting in brain endothelium as it could help optimize future design strategies for transcytotic delivery.

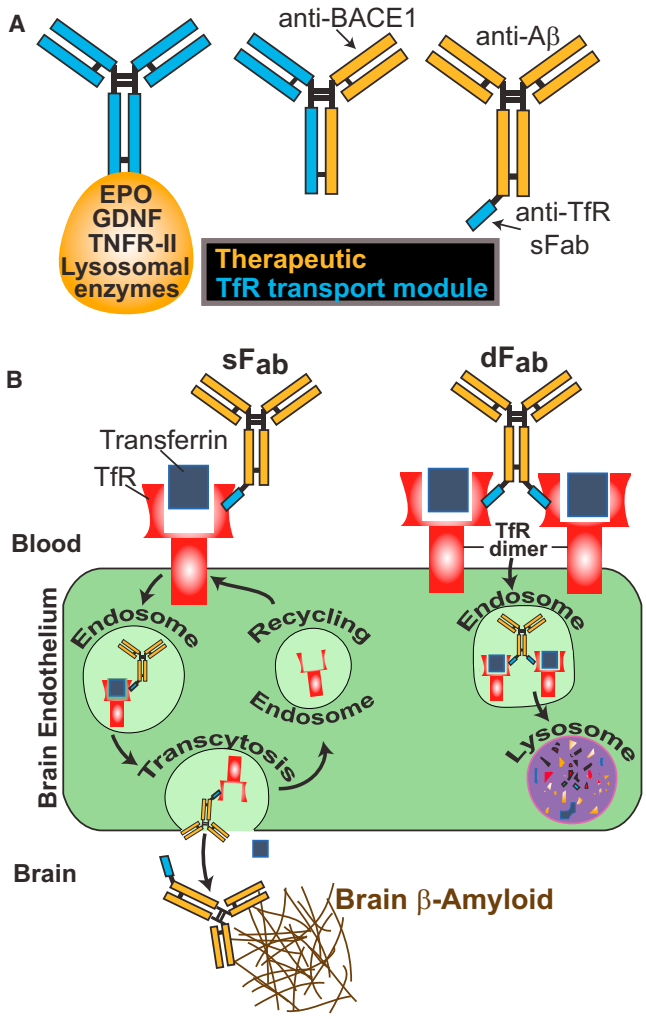

Figure 1. Anti-TfR Vehicles for CNS Drug Delivery

(A) TfR-based drug delivery strategies including IgG-based molecular trojan horse fusion proteins (left) (Pardridge, 2012), a bispecific anti-TfR/BACE1 antibody (middle) (Yu et al., 2011), and the anti-TfR sF_{ab} Brain Shuttle (right) (Niewoehner et al., 2014).

(B) Proposed pathway for differential intracellular sorting of monovalent and bivalent anti-TfR F_{ab} fusions. Whereas monovalent anti-TfR sF_{ab} fusions undergo transcytosis across the BBB, bivalent anti-TfR dF_{ab} fusions leads to TfR dimerization and lysosomal degradation.

Next, Niewoehner et al. (2014) examined the ability of the monovalent TfR F_{ab}-fused Aβ antibody (mAb31-sF_{ab}) to access brain parenchyma and reduce Aβ. Remarkably, mAb31-sF_{ab} exhibited a 55-fold increase in amyloid plaque engagement compared to unmodified mAb31 or bivalent mAb31-dF_{ab} in the brain of PS2APP mice. Moreover, treatment with mAb31-sF_{ab} for 3 months significantly reduced amyloid plaque burden even at a relatively low dose when compared to treatment with unmodified mAb31. Overall, the sF_{ab} anti-TfR brain shuttle module enhanced the delivery and potency of a plaque reducing Aβ antibody and could potentially be expanded to the delivery of other therapeutic cargo.

While this study from Niewoehner et al. (2014) provides mechanistic insight and hope for the delivery of macromolecule therapeutics across the BBB, additional studies will be required. Fundamental questions regarding pharmacokinetics of TfR-based drug delivery approaches remain. The quantification of mAb31-sF_{ab} brain penetration is limited to immunofluorescent-based signal amplification imaging. Detailed neuropharmacokinetic analysis of the percent injected dose in the brain will be important. Moreover, understanding how affinity versus valency influences RMT and cell sorting will be important for optimizing TfR drug delivery strategies. It will also be interesting to examine the role of extracellular matrix, pericyte, and glial interactions and varying disease pathology in modulating the transcytotic delivery of macromolecules. Indeed, in the case of Alzheimer's disease, Aβ deposition occurs within cerebrovasculature, which could alter BBB function (Zlokovic, 2008). Niewoehner et al. (2014) found that the Brain Shuttle strategy does not damage the BBB, but it is important to consider whether utilization of endogenous RMT pathways interferes with physiological processes. For example, TfR-based transport modalities have been associated with loss of reticulocytes (Couch et al., 2013). Understanding what cells and tissues will be targeted with any given RMT-based strategy is essential when determining toxicity risks. For instance, delivery of GDNF with an anti-insulin antibody in nonhuman primates led to pancreatic lesions, presumably due to abundance of insulin receptors and adverse proliferative consequence of GDNF in pancreatic cells (Ohshima-Hosoyama et al., 2012).

Finally, the most effective RMT pathway at the human BBB remains to be determined. Currently, antibodies

against insulin receptors do not exist for BBB transport studies in rodents, so anti-TfR approaches have been the primary strategy for preclinical testing. Whether or not this approach will have the best translational potential in humans has yet to be confirmed. Variable expression of small molecule efflux transporters such as PgP and BCRP in different species has been established, and it will not be surprising if species differences exist for RMT transport pathways. Acquisition of data in humans or human cell models will be required to reveal the expression and kinetics of TfR and other RMT pathways at the BBB.

Routine delivery of large biomolecules across the human BBB remains a holy grail for CNS therapeutics. More than $1 billion has been spent on clinical development of peripherally administered Aβ antibodies that exhibit limited CNS penetration (Yu and Watts, 2013). The exciting finding by Freskgård and colleagues that fusion of a single anti-TfR F_{ab} improves brain penetration of antibodies by transcytotic delivery points toward a general strategy for CNS delivery and may help define the basic cell biology of membrane trafficking in the cerebrovasculature. By identifying a monovalent, modular means of moving molecules into the CNS, Niewoehner et al. (2014) provide a potentially powerful procedure to pierce through the blood-brain barrier.

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Neural Signatures of Modified Memories

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Censor et al. (2014) combine behavioral, TMS, and neuroimaging to identify task-free neural signatures that relate to modification of motor memories. Modulation of memories using TMS may provide a powerful approach to improve human brain function in neurorehabilitation and cognitive neuroscience.

Modification of existing memories after their reactivation may result in behavioral outcomes that can be beneficial or maladaptive. Numerous studies have provided evidence that when an already consolidated memory is reactivated upon retrieval, it becomes susceptible to modification before it is reconsolidated again into a stable form (Nader and Hardt, 2009; Dudai, 2012). The outcomes of this modification can be degradation (Nader et al., 2000), stabilization, or strengthening of the original memory (Lee, 2008; Walker et al., 2003; Censor et al., 2010). Substantial advances in the field have been achieved using animal models, by injecting protein synthesis inhibitors to the relevant brain regions, upon reactivation of the memory. Progress has been also made in humans, pointing to similar mechanisms (Chan and LaPaglia, 2013; Schiller et al., 2010; Schwabe et al., 2012; Censor et al., 2010; Walker et al., 2003). Overall, modification of existing memories after their reactivation may play an important role in learning and skill acquisition and, furthermore, can be of special relevance in rehabilitation after brain injury or in treating chronic neurological conditions. What has been missing to date is evidence for task-free neural signatures of modified human memories at a systems level.

In this issue of *Neuron*, Censor et al. (2014) start to address this question by focusing their interest in the corticostriatal loop, under the working hypothesis that activity in this loop might relate to interindividual differences in the ability to modify