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Therapeutic Delivery

A gateway to the brain: shuttles for brain delivery of macromolecules

"The stakes in developing systemically active antibody therapeutics for CNS indications are exceedingly high ... The potential for opening CNS markets for a new class of therapeutics with high target selectivity and potency will continue to excite the development."

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Blood–brain barrier: is it insurmountable?

The blood–brain barrier (BBB) has been viewed as a main culprit for numerous failures of CNS-targeting drugs in preclinical and clinical development. The BBB identity of a virtually impenetrable fortress has recently been challenged through improved understanding of the BBB anatomy, physiology, molecular composition and function. It is no longer true that it requires potentially harsh and medically unsafe 'disrupting' strategies to deliver pharmacological levels of therapeutics into the brain. A current concept of a dynamic, fluid, polarized and interactive neurovascular unit offers opportunities to exploit naturally occurring passageways, pores and shuttles for therapeutic delivery across the BBB [1]. Parallel advancements in targeted protein engineering and screening have enabled the development and preclinical validation of the first generation of BBB-crossing macromolecules that exploit these physiological gateways into the brain [2]. Encouraged with these new developments, is the field on the brink of solving the BBB delivery hurdle? How will this change the outlook for CNS therapeutics, particularly biologics?

Shuttles & Trojan horses

The principal route for macromolecule delivery across the BBB is receptor-mediated transcytosis – a process in which ligands targeted to receptors found on the luminal side of brain endothelial cells are internalized, shuttled across the endothelial cytoplasm to the abluminal side of the barrier and exocytosed [3]. The precise mechanism of this transport is not known, although extrapolation to vesicular transport in other cell types implies transport through diverse sorting vesicles. The BBB receptors that undergo receptor-mediated transcytosis cycling have been referred to as 'BBB shuttles' whereas engineered ligands to these receptors are known as 'BBB carriers'. Attachment of drugs to BBB carriers enables their delivery across the BBB and these molecules have been termed molecular Trojan horses. The principal approach has been the development of bispecific antibodies, whereby the BBB carrier is an antibody against a BBB shuttle and the therapeutic cargo is an antibody against a central target. The preclinical proof-of-concept of this approach was established almost 2 decades ago with antibodies against TfR and IR [4,5]. These antibodies have been fused to therapeutic payloads targeting misfolded proteins, such as Aβ or tau, or the amyloid-processing enzyme BACE-1 [6,7]. These shuttle–Trojan horse pairs have proven effective, in some cases effecting pharmacologically meaningful central responses in preclinical species [4–9]. Another approach is re-engineering of natural protein ligands shuttled across the BBB by LRP-1 into smaller peptide fragments as BBB carriers [8]. However, the abundance of IR, TfR and LRP-1 in peripheral tissues, and their role in essential physiological func-

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tions such as glucose utilization, oxygen delivery and lipid exchange, respectively, may raise concerns about developability of ligands targeting these receptors.

Cautionary tales in preclinical development

The safety issues are exemplified in a few cautionary tales from the preclinical development of BBB carriers against IR and TfR. In the first example, the GDNF fused to a humanized antibody against IR (HIRMab), has been evaluated in Parkinsonian (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced) adult rhesus macaques [10]. Animals received twice weekly intravenous treatments of HIRmAb-GDNF for a period of 3 months. This did not improve Parkinsonian symptoms, but induced a dose-dependent hypersensitivity reaction, characterized with skin flushes, eyelid edema, vomiting, urticaria and in some cases respiratory distress; minimal to mild non-suppurative myocarditis was noted in majority of animals [10]. Circulating antibodies against the HIRmAb-GDNF have been detected in all treated animals [10]. Serious health risks were also identified in the form of focal pancreatic metaplasia likely caused by proliferative actions of GDNF targeted to IR in pancreatic tissues [10].

In the second parable, a TfR antibody has been found to induce hypersensitivity reaction in rodents, characterized by severe lysis of TfR-enriched reticulocytes due to complement-dependent cytotoxicity triggered by the effector-competent Fc [11]. The effect was mitigated by lowering the affinity of the antibody to TfR, which also extended antibody circulation half-life and reduced TfR degradation in the central compartment, and by removal of Fc effector function. Even a small level of effector function was sufficient to trigger reticulocyte lysis and an Fc domain devoid of all effector function was required [11].

Recently, another unintended effect of a bispecific anti-TfR/anti-β-amyloid DVD antibody – an increase in brain amyloid levels – has been communicated [12]. A potential explanation was that a high-affinity anti-Aβ antibody 'captures' circulating amyloid that is transported into the brain enhancing, rather than reducing, central Aβ deposits. This brings to a forefront another cautionary caveat – that bispecific antibodies are entities with unique pharmacologies, and may have actions that cannot be anticipated from the action of each separate antibody, or the actions from the co-administration of the two antibodies as separate entities.

The presence and abundance of BBB shuttle receptors in brain parenchymal cells could create a secondary safety risk associated with their actions on the unintended central target; therefore it is of paramount importance to achieve a degree of BBB shuttle selectivity against both peripheral and central compartments to improve safety margins.

New shuttles

A renewed search for alternative BBB shuttle–Trojan horse pairs can be attempted through the potential of 'omics' technologies and 'function first' screening of peptide and antibody libraries for BBB transmigrating species [13,14]. These methods may incorporate optimized BBB carrier formats as platforms for multiple therapeutic cargos and rational design of their functional properties based on chosen selection pressures; improved BBB selectivity, endothelial cell internalization, *in vitro* or *in vivo* BBB crossing, and species cross-reactivity. [13,14]. These approaches have yielded new BBB shuttle–Trojan horse pairs, including a single-domain antibody FC5 [14], which has delivered central pharmacological effects when incorporated in bispecific antibodies and antibody-peptide conjugates [2,15].

A way in & a way out: knowledge gaps

These recent advances have been focused on improving efficiency of brain delivery, however, a significant knowledge gap still remains in understanding mechanisms of transendothelial migration and central disposition and elimination.

The efficient transcytosis of anti-TfR antibodies requires their escape from endosomal–lysosomal trafficking system, a process facilitated by lower affinity and pH sensitivity of antibody binding to TfR [16]. Studies with FC5 suggested that the transcytosis pathway may involve formation of intraendothelial exocytosing multivesicular bodies and shedding of exosomes containing a portion of transmigrating antibody [17]. The extravasated bispecific antibody is challenged with several secondary barriers on its diffusion path to the target in the neuropil; these include vascular and parenchymal basement membranes, cellular layers associated with neurovascular unit, interactions with extracellular matrix and the tortuosity of the brain extracellular space [18]. Target engagement by the antibody 'drug' arm leads to target-specific antibody disposition – cell surface receptor targets could lead to antibody internalization and intracellular degradation, whereas antibody complexation of soluble targets could result in complex elimination via circulation of brain fluids. The target-mediated disposition of BBB-crossing bispecific antibodies is additionally complex because the BBB carrier arm itself often binds the central target; such is the case with TfR and IR antibodies. To determine (therapeutic) target exposure of such antibodies, it is also important to determine their brain residence time, governed by multiple and still poorly understood elimination pathways including reverse transport across the BBB, convection of brain interstitial fluid along perivascular routes, its exchange with the cerebrospinal fluid and drainage into deep cervical nodes [19]. What are the dynamic rates of

these processes? How long does a bispecific antibody remain intact and active in the brain? How does each of the target-specific 'arms' affect its brain residence time and elimination? Filling in these knowledge gaps will be fundamental for understanding the true brain (target) exposure for each BBB-enabled bispecific fusion protein and for managing its safety.

Path to the clinic

Two BBB carrier technologies have entered clinical trials; ANG1005, a peptide BBB carrier–paclitaxel conjugate for primary and metastatic brain tumors, and HIRmAb-iduronate sulfatase fusion protein [20] for the rare lysosomal storage disease, Hunter syndrome. Both these indications lack effective treatment options and are tolerant of a lower safety margin. However, as yet there are no reports of bispecific antibody strategies targeting cell signaling mechanisms through inhibition of receptor–ligand interactions, an area where antibodies have excelled in targeting peripheral disease mechanisms.

The future selection of 'ideal' shuttle–BBB carrier pairs will depend on the intended therapeutic disease indication. BBB shuttles with fast transport rates would be appropriate when fast onset of drug action is needed, for example, in arresting intractable seizure activity; Trojan horse molecules with long serum half-life that augments the extent of brain exposure may be appropriate for treating chronic neurodegenerative and pain conditions. The path forward will have to include a rational selection of 'fit-for-indication' BBB shuttles, improved formats, translational pharmacokinetic/pharmacodynamic models tailored to bispecific biologics, and very rigorous safety/toxicology studies.

The stakes in developing systemically active antibody therapeutics for CNS indications are exceedingly high, given the vast unmet medical need of many neurological disorders. The potential for opening CNS markets for a new class of therapeutics with high target selectivity and potency will continue to excite the development. Despite the risks, the field is steadily edging toward realizing this opportunity.

Financial & competing interests disclosure

CI Webster is an employee of MedImmune, an orginisation with financial interest in the subject matter of this manuscript, and therefore has a potential conflict of interest. DB Stanmirovic is employed by the Government of Canada (National Research Council of Canada) who have several Sponsored Research Agreements with industrial partners to develop blood–brain–barrier carriers, including FC5. DB Stanmirovic does not have financial or managerial interests in these companies. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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