

Time to open the blood–brain barrier gate for biologics?



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Ever since Ehrlich and colleagues described the existence of the blood–brain barrier (BBB), it has been appreciated that the BBB presents a real challenge in drug development for numerous brain disorders [1]. This is especially true for biologics such as antibodies that are large in size and unable to cross the BBB efficiently. As biologics become increasingly important as human therapeutics [2] novel systems that promote their access to the brain are desperately needed. One of the most promising approaches to delivering biologics efficiently into the brain is based on the Trojan horse principle [3]. The Trojan horse in the present context is a protein that naturally crosses the BBB via a receptor and is coupled to a protein of therapeutic interest. This concept, which makes use of a cellular process known as receptor-mediated transport (RMT), has been refined over the years by using antibodies instead of natural proteins as ligands to BBB receptors [4,5]. In parallel with increasing understanding of the complex biology of the BBB [6] and the advancement in antibody

engineering [7] the field is now ready to take the next essential step in translating this brain delivery technology into clinical use. In this editorial, we will briefly discuss what we believe is crucial not only for advancing this delivery concept forward to human use but also a number of important observations made in the BBB field.

In order to progress the BBB field as a whole and brain delivery of biologics in particular, an important aspect is development of truly predictive *in vitro* BBB models. There are basically two major reasons why *in vitro* BBB models are extremely useful. First, the investigation of intracellular sorting mechanisms of receptors requires methods, especially microscopy, at subcellular resolution, which is not easily applicable for *in vivo* investigations. The identity, composition and kinetics of vesicular compartments involved in transcytosis are still largely unknown, hampering our understanding of productive intracellular pathways through the BBB and the search for novel transcytosis receptors. Second, screening of a larger number of substances

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for BBB penetration cannot be done in animals owing to practical and ethical considerations. The main functions of the BBB are to maintain brain homeostasis, regulate influx and efflux transport, and protect the brain from harmful substances. This is achieved by a specialized multicellular structure composed of endothelial cells, pericytes and astrocytes. Numerous attempts have been made to recreate BBB functionality *in vitro* using different cell types [8], including human induced pluripotent stem cells [9]. Many model systems recapitulate some aspects of the BBB. However, reconstruction of a *bona fide* BBB is a complicated task that most likely requires precise geometry and multicellular interactions to prevent the strong phenotypic drift when these cell types are withdrawn from their native environment [10]. At present, no validated *in vitro* BBB model exists that can effectively replace *in vivo* investigations. Thus, great caution should be exercised in the analysis and interpretation of the results obtained with cultured cells in any form and with extrapolating to the native BBB *in vivo*. We believe that too many conclusions are based on inadequate *in vitro* models that lack any supporting *in vivo* data. Ultimately, this is counterproductive for advancing BBB research. The most fruitful way forward for studying BBB transport of biologics is to use validated *in vivo* models with reliable and robust readouts. For the time being *in vitro* models should be used only to generate supportive mechanistic data to complement key findings in animal models. Nevertheless, the field still needs to invest in next-generation *in vitro* BBB models but critically examine the progress to be successful long term.

A further limitation of BBB research is the methodology used to measure brain exposure of biologics. Many studies assessing BBB crossing and brain levels of biologics are based on the use of trace amounts of radiolabeled protein and a method called capillary depletion. The aim of capillary depletion is to remove blood vessels from brain homogenate in order to assess how much of a biologic truly reaches the brain parenchyma. In this procedure, not all small capillaries are completely removed though, and the fractionation procedure may allow free diffusion of molecules from the vessel fraction into the brain fraction, making brain quantifications imperfect. Furthermore, some studies use fluorescently labeled proteins and quantification is based on a fluorescent signal independent of any interaction

with a specific target within the brain. In order to avoid these pitfalls, a relevant therapeutic dose of the protein should first be used to determine the transport capacity of the RMT system to ensure that sufficient quantities can be transported for meaningful *in vivo* potency. Next, quantification of target engagement or, alternatively, direct measurement of pharmacodynamic activity within the brain should be used to provide a reliable estimate of BBB crossing and brain exposure.

A conventional Y-shaped antibody is bivalent, meaning one antibody can bind two receptors simultaneously. Bivalent binding can induce dimerization or higher order oligomerization of the receptor at the cell surface and/or inside the cell and is likely to change the natural arrangement and density of the receptor. Interestingly, recent studies showed that bivalent binding of an antibody to its cognate receptor leads to trapping of the antibody inside the brain endothelial cells of the BBB [11,12]. Consequently, the fraction of the antibody that actually gets through the BBB and into the brain parenchyma is very small. What has recently become evident in relation to BBB receptor engagement is the importance of understanding intracellular trafficking of the transporting receptor inside the brain endothelial cells and how its biology is influenced by the interaction with the carrier [13,14]. In agreement with earlier work our results show that the valency of the brain shuttle (i.e., number of modules interacting with the BBB receptor) is crucial for effective transport of the construct from the circulation into the brain parenchyma [13]. Furthermore, the interaction between the carrier and the BBB receptor must follow certain rules to ensure efficient BBB transport. As a rule of thumb, the interaction between the carrier and the BBB receptor should mimic the binding mode of the natural ligand on the receptor, but use a different binding site to minimize the risk of affecting the ligand/receptor homeostasis. Also, receptors with cell signaling functionalities should be avoided if possible as it might lead to unforeseen consequences unrelated to the transport function of the receptor. Another very important aspect is the expression pattern of the BBB receptor in organs other than the brain as this will affect the degree of delivery and the peripheral pharmacokinetics. The most widely used receptors for RMT approaches [15] are also expressed outside the brain so the research field should strive to identify other potential receptor pathways that might be more brain specific.

As far as safety is concerned, special attention has to be paid to the biological function of the transporting receptor as well as to the way in which a carrier engages with it. A recent study highlighted safety issues related to the Fc effector functionality of a bispecific antibody and underscored the importance of understanding Fc-mediated effects on the interaction between the carrier and transporting receptor [16]. In another study, some unexpected safety issues were encountered in a Trojan horse approach using the insulin receptor (IR) as a transporter in parkinsonian monkeys [17]. In this study, a standard Y-shaped antibody was used against the IR. This format could potentially explain some of the findings since a Y-shaped antibody may cause receptor dimerization [18]. This would lead to downstream cell signalling and potentially also downregulation of the IR. Taken together, any approach based on RMT over the BBB needs to be carefully evaluated in well-designed toxicology studies before clinical recommendations can be made.

As mentioned in the beginning of this editorial, the concept of delivering biologics over the BBB using an RMT approach has now

advanced to a point where first clinical investigations are seriously being considered. It is reasonable to suggest that the first attempt should be based on biologics that have a proven therapeutic effect but lack efficacy in the CNS due to poor exposure and target engagement behind the BBB. Even though delivery of biologics to the brain remains a formidable challenge, the reward in the form of novel medicines to treat devastating brain disorders is huge. Treatment modalities such as antibodies, growth factors, enzymes, peptides and antisense oligonucleotides, that inherently possess functionalities not found in small molecules, are now taking the stage as promising drug candidates for CNS disorders.

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