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Invited review

Targeting blood-brain-barrier transcytosis – perspectives for drug delivery

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ABSTRACT

Efficient transcytosis across the blood-brain-barrier (BBB) is an important strategy for accessing drug targets within the central nervous system (CNS). Despite extensive research the number of studies reporting successful delivery of macromolecules or macromolecular complexes to the CNS has remained very low. In order to expand current research it is important to know which receptors are selective and abundant on the BBB so that novel CNS-targeting antibodies or other ligands could be developed, targeting those receptors for transcytosis. To do that, we have set up a proteomics- and transcriptomics-based workflow within the COMPACT project (Collaboration on the Optimization of Macromolecular Pharmaceutical Access to Cellular Targets) of the Innovative Medicines Initiative (IMI) of the EU. Here we summarise our overall strategy in endothelial transcytosis research, describe in detail the related challenges, and discuss future perspectives of these studies.

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Contents

Acknowledgements 7
Supplementary data 7
References 7

Reaching macromolecular drug targets in the brain has remained a great challenge in biomedical research and clinical sciences despite rigorous efforts for more than two decades. This is because the blood-brain-barrier (BBB) represents one of the most tightly regulated and complex biological barriers in mammals with a function to protect the brain from damage of unwanted blood-borne molecules. This is essential for maintaining correct neurological functions. However, in case of neurological disease, the delivery of therapeutic bioactive macromolecular compounds to affected areas of the central nervous system (CNS) is highly desired but notoriously difficult to achieve, especially when the general protective barrier function of the BBB is to be maintained. This explains the failure of clinical trials of numerous biologicals targeted to the CNS (Pardridge, 2015a).

To overcome this problem, many attempts have been made to find specific targeting ligands, endogenous proteins or antibodies against certain receptors (e.g. transferrin receptor (TFRC) and insulin receptor (INSR)) that could be used to induce receptor-mediated transcytosis of therapeutic biologicals across the BBB. For example, TFRC antibody chimeras have been successfully used in animal models of Parkinson's disease, stroke, Alzheimer's disease and lysosomal storage disease for enhancing brain accumulation of therapeutic proteins (Watts and Dennis, 2013; Pardridge, 2015b;

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Lajoie and Shusta, 2015; Niewoehner et al., 2014). INSR targeted antibody and therapeutic protein fusions have shown great potential in non-human primates for treating mucopolysaccharidosis type I (MPS-I) and Alzheimer's disease, and also in brain delivery of GDNF, TNFR, erythropoietin and paraoxonase-1 (Lajoie and Shusta, 2015). To deliver drugs across the BBB independently of specific targeting ligands, the barrier can be temporarily opened in some conditions by using microbubbles and focussed ultrasound. Initial safety and efficacy data, incl. the treatment of a brain tumour patient, are promising and indicate that concerns about risks related to the loss of BBB integrity could be managed (Piper et al., 2016; Lipsman et al., 2014; Sunnybrook). However, the advantages and disadvantages compared to the receptor-mediated BBB transcytosis need further thorough evaluation.

Receptor-mediated transcytosis across the BBB is an attractive strategy for reaching targets within the CNS for various reasons. For example, it could enable the transport of macromolecules or macromolecular complexes that would be unfeasible in carrier-mediated transport systems (i.e. channels and carriers) (Pardridge, 2012). Furthermore, alternative CNS entry routes such as via injection into the cerebrospinal fluid (CSF) are generally regarded as unfavourable because it requires hospitalization of the patient, because of the risk of infection, and due to the rapid drug export from the CSF back to blood circulation (Pardridge, 2012).

The architecture of brain microcapillary network is favourable to receptor-mediated transcytosis too. The distance between adjacent vessels in the brain is in the range of 40–60 μm (Pardridge, 2015a; Nicholson, 2001; Duvernoy et al., 1983). Taking account the size of neurons, the cell body of a neuron is no further than 10–20 μm from the closest capillary on average (Wong et al., 2013; Schlageter et al., 1999). This means that the diffusion distance of the traversed macromolecular compounds is short enough to diffuse to neurons in the time scale from seconds to tens of minutes (but could be longer in a crowded environment), depending on the molecular weight of the compound (Atkins and de Paula, 2006). However, achieving transcytosis across the BBB requires thorough understanding of brain microendothelial cells surface proteins.

One of the crucial questions is how to find receptors which are (a) selective to the brain microvasculature, (b) expressed at sufficiently high levels and (c) could be used for transcytosis. Furthermore, once these receptors have been identified, it is an additional challenge to find ligands/antibodies capable of taking advantage of transcytosis of these receptors for drug delivery. These questions address the issue how to reach the interstitial space of the brain tissue. However another layer of complexity is added when the drug target is in the intracellular environment of neurons, requiring cell uptake and delivery to required cell compartment.

Within the COMPACT project (Collaboration on the Optimization of Macromolecular Pharmaceutical Access to Cellular Targets) of the Innovative Medicines Initiative (IMI) of the EU (http://www.imi.europa.eu/content/compact), we have developed and set up a workflow to identify brain microvascular cell surface specific receptors (Fig. 1) with an aim to use them for drug delivery across the BBB. The workflow is based on analysing the cell surface proteome and whole cell transcriptome.

The need to thoroughly characterise individual molecular constituents of the BBB for finding novel BBB-specific receptors for drug delivery has been recognized by many. Recent advances in proteomics and transcriptomics have enabled detailed comparisons of human and small animal model BBB properties, which in turn helps to select human relevant brain-specific receptors for drug delivery with a possibility to study them in animal models (Ohtsuki et al., 2014; Enerson and Drewes, 2006; Zhang et al., 2014). Even though the gene expression profile of BBB endothelial cells is heterogeneous (Macdonald et al., 2010), by analysing brain microvascular endothelial cell transcriptome, it is possible to find leads for brain-specific transporters and other proteins with drug delivery potential (Daneman et al., 2010). Recent data indicate that by combining transcriptomic analysis and proteomic profiling of brain endothelial cells in mice, new target receptors can be discovered that mediate therapeutic antibody transcytosis to brain more efficiently than TFRC and INSR binding antibodies in mice (Zuchero et al., 2016). For exploiting unknown receptors for BBB transcytosis, assays such as phage display panning has been used (Jones et al., 2014; Muruganandam et al., 2002; Smith et al., 2012).

In order to identify brain microvasculature selective receptors it is essential to profile microvascular cells also from other tissues for comparison. Because many nanoparticle drug delivery formulations accumulate in liver and lung, the first choice is to include microvascular cells from these organs to the comparison (Fig. 1). However, it must kept in mind that drug formulations can accumulate in those vessels not only in receptor-specific manner; nonspecific physical capture can be important too. There are two principal strategies for profiling the cells, comparing either (a) cell transcriptomes, e.g. by next generation sequencing (NGS) (Fig. 1, A), or (b) cell surface proteomes by LC-MS/MS (Fig. 1, B). Both strategies have clear advantages and disadvantages. NGS is sensitive enough for allowing the analysis of RNA from freshly isolated primary microendothelial cells without requiring prior cell culture, a process that potentially impacts the gene/protein expression profile of isolated primary cells. Freshly isolated mRNA samples are even available from commercial vendors. However, NGS based mRNA expression profiles may not fully match in a qualitative and quantitative manner the expressed cell proteome. Furthermore, it is also unclear whether membrane proteins, even when effectively translated, are sorted to the plasma membrane for being exposed to potential targeting ligands or targeting antibodies.

These drawbacks can partially be addressed by performing a cell surface proteomics experiment. Cell surface proteins can be labelled, for example with biotin by using appropriate kits, and pulled down using magnetic beads. By analysing protein composition of these samples it is possible to claim with high confidence which receptors are presented on cell surface and therefore accessible to targeting ligands and antibodies. However, in order to obtain enough material for proteomics it may be necessary to subculture the isolated primary microendothelial cells first, before extracting the proteins. It is well documented that this process can considerably change the gene/protein expression profile of cells, for example the loss of certain tight junction proteins (Cecchelli et al., 2007; Abbott et al., 2006; Nakagawa et al., 2009). Furthermore, proteomic profiling does not aid in the discovery of non-protein receptors such as gangliosides and/or glycans. All these aspects must be taken into account when interpreting the cell surface proteomics data.

In order to minimise risks associated with the both strategy it is important to conduct mRNA sequencing and proteomics experiments in parallel. Understanding similarities and differences of identified receptors is highly informative and can help selecting as robust targets as possible for the drug delivery systems (DDSs) being developed. However, these technical challenges are not the only challenges that need to be met, as summarised below (Fig. 2).

Long term goals of most investigations related to drug delivery systems are associated with their applicability to treat human diseases (Fig. 2, A). This means that already in the first instance it is important to include human primary tissue samples for the transcriptomic and proteomic analyses. However doing that is related to several practical questions, such as the availability of mRNA and cells samples from commercial providers. Even if readily available, there might be great differences in terms of the age, sex and health/disease status of donors of the samples, possible contamination