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Research review paper

# BBB-targeting, protein-based nanomedicines for drug and nucleic acid delivery to the CNS

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ABSTRACT

The increasing incidence of diseases affecting the central nervous system (CNS) demands the urgent development of efficient drugs. While many of these medicines are already available, the Blood Brain Barrier and to a lesser extent, the Blood Spinal Cord Barrier pose physical and biological limitations to their diffusion to reach target tissues. Therefore, efforts are needed not only to address drug development but specially to design suitable vehicles for delivery into the CNS through systemic administration. In the context of the functional and structural versatility of proteins, recent advances in their biological fabrication and a better comprehension of the physiology of the CNS offer a plethora of opportunities for the construction and tailoring of plain nanoconjugates and of more complex nanosized vehicles able to cross these barriers. We revise here how the engineering of functional proteins offers drug delivery tools for specific CNS diseases and more transversally, how proteins can be engineered into smart nanoparticles or 'artificial viruses' to afford therapeutic requirements through alternative administration routes.



**Contents**



## 1. Introduction

The maintenance of the central nervous system (CNS) homeostasis is essential for its normal function. The limits of the CNS tissue are

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established by the astrocytic glia limitans facing the meninges and the blood vessels, and by the ependymocytes of the choroid plexus where the cerebrospinal fluid is produced (Fig. 1A). Astrocyte end-feet wrap the meningeal fibroblasts and the endothelial cells (ECs) of the capillaries, leaving between them the basement membrane. Brain capillaries display a large surface area (~20 $m^2$ per 1.3 kg brain), and thus possess a predominant role in regulating the brain microenvironment. The blood–brain-barrier (BBB) limits the entry of blood-derived molecules and circulating leukocytes, protecting the CNS from fluctuations in plasma compositions or circulating agents such as neurotransmitters and xenobiotics. It is composed of specialized ECs held together by multiprotein complexes known as tight junctions, astrocytes, pericytes and basement membrane (Abbott et al., 2006; Reese and Karnovsky, 1967) (Fig. 1B). CNS ECs display more efficient cell-to-cell tight junctions than other ECs (Wolburg and Lippoldt, 2002), rest on a continuous basement membrane and express a series of transporters responsible for the regulated exchange of nutrients and toxic products. These characteristics make the CNS ECs a continuous and selective physical barrier for hydrophilic substances, and a key player in the regulated trafficking of molecules into the CNS (Abbott et al., 2006) (Fig. 2). Interestingly, the Blood Spinal Cord Barrier (BSCB) displays similarities to the BBB, but it also has some unique properties, among them being slightly more permeable (Bartanusz et al., 2011). Transit restrictions imposed by the BBB (and at lesser extent by BSCB) represent the most important barrier to overcome in the drug delivery to the CNS. In the context of emerging neurological diseases, targeting drugs to the CNS is under strong pushing demands, but vehicles for BBB crossing are still in their infancy, with a long run until full tailoring.

## 2. Cross-transportation through BBB

The BBB gradually develops in humans during the first postnatal year (Adinolfi, 1979) and it's nearly complete in rats after the second postnatal week (Stewart and Hayakawa, 1987). This highly differentiated EC phenotype is induced and maintained in the long term by interactions with the surrounding cells, mainly astrocytes and pericytes but also perivascular macrophages and even neurons (Abbott et al., 2006; Alvarez et al., 2011; Arthur et al., 1987; Janzer and Raff, 1987). For instance, in vivo, astrocytes secrete Sonic Hedgehog (Shh), that will act on endothelial cells and promote BBB integrity (Alvarez et al., 2011). In addition to the role in long-term barrier induction and maintenance, astrocytes and other cells can release chemical factors that modulate local endothelial permeability over a time-scale of seconds to minutes. Thus, both natural stimuli for BBB leakage and pharmacological compounds acting on endogenous BBB induction pathways like Shh inhibitors (Alvarez et al., 2011) can be used to transiently increase the entrance of molecules into the CNS parenchyma. Moreover, the phenotypical characteristics of the BBB ECs include both uptake mechanisms (e.g. GLUT-1 glucose carrier, L1 amino acid transporter, transferrin receptor) and efflux transporters (e.g. P-glycoprotein), and thus transporter/receptor-mediated transit across the BBB has also been used to deliver molecules of pharmacological interest into the CNS parenchyma (Fig. 2). In this case, specific transcellular receptor-mediated transcytosis transports molecules from the luminal membrane, lining the internal surface of the vessels, to the abluminal membrane on the external CNS-lining surface. In addition, less specific adsorptive-mediated transcytosis can also be used for the delivery of molecules, but CNS ECs show a lower rate of transcytosis activity than peripheral ECs (Rubin and Staddon, 1999), making this a less efficient process for the incorporation of circulating molecules.

A final consideration regarding potential limiting steps for the delivery of hydrophilic substances into the CNS across the BBB is that both intracellular and extracellular enzymes provide an additional barrier. Extracellular enzymes such as peptidases and nucleotidases are capable of metabolizing peptides and ATP respectively. Intracellular enzymes, that are involved in hepatic drug metabolism, have been found in the

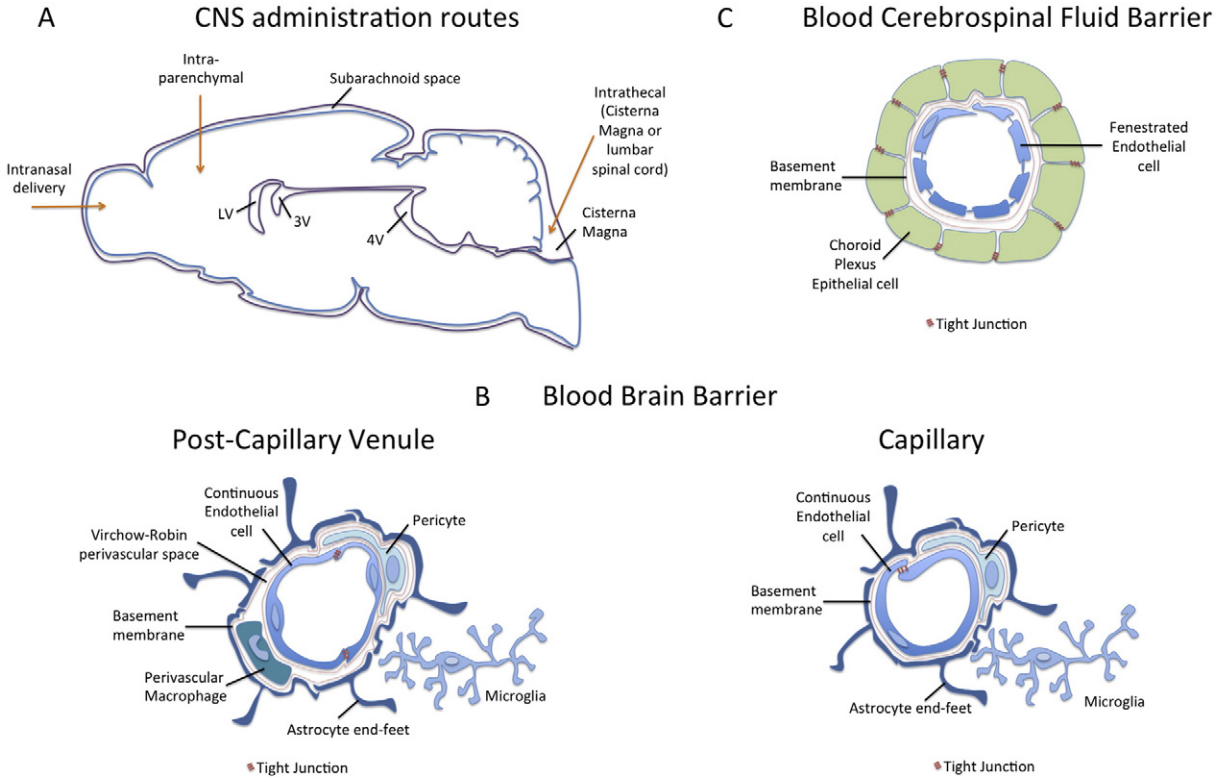


**Fig. 1.** Anatomical basis of the BBB. Boundaries of the CNS tissue contacting the blood vessels, meninges and the cerebrospinal fluid are depicted (A), and also alternative routes for administration of substances to the CNS to bypass the BBB. The intimate relationship between ECs, continuous basement membrane, astrocytes, pericytes and perivascular macrophages contributing to various degrees to the BBB formation and maintenance can be observed (B). Moreover, ependymocytes of the choroid plexus produce the cerebrospinal fluid and conform, in addition, the Blood Cerebrospinal Fluid Barrier (BCFB) (C).