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EDITORIAL



Nanoparticles crossing blood–brain barrier need specific design for normal, neurodegenerative or cancerous brain conditions

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Central nervous system (CNS) diseases including depression, stroke, brain infection, brain cancer, neurodegenerations, meningitis, neuropathic pain, amyotrophic lateral sclerosis, migraine, schizophrenia, epilepsy and multiple sclerosis are a serious burden on the public health system. More recently, COVID-19 has contributed to mental health crisis, exacerbating neurological diseases and increasing the risk of developing new neurological disorders [1,2]. However, during the last 15 years, many pharmaceutical companies have been downsizing CNS-related drug projects, with a 48% reduction in 2009–2014 and a further 34% decrease in 2014–2023 [1].

Several physiological and pharmacological factors present roadblocks in CNS drug development. A major problem is poor CNS drug penetration through the blood–brain barrier (BBB). It is made of special endothelial cells with tight junctions connections that preclude many drugs from entering the CNS tissues [3]. Compared with normal CNS BBB, common neurodegenerations such as Alzheimer's (AD), Parkinson's (PD), multiple sclerosis [3,4] or brain tumors [5] show pathological changes in the BBB. Similarly, the blood-retinal barrier is compromised in vision-threatening diabetic retinopathy [6].

Recent data show that BBB changes in various pathologies are different. This necessitates accurate choice of delivery vehicles and targets depending on whether the drugs need to reach CNS tissues with normal, neurodegenerative or tumor BBB structure. In the last two decades, nanomedicine opened novel avenues in overcoming limited BBB permeability, which was not possible to achieve with small molecule drugs or biological therapeutics such as peptides or antibodies effective for the treatment of other organs or systems [5,7].

1. Normal BBB

Several biological factors influence drug transport to the normal brain including vasculature, pericytes, extracellular matrix and type of brain cells. The endothelial BBB has tightly sealed tight and adherens junctions resulting in high transendothelial electrical resistance and low permeability, both paracellular and transcellular. The BBB also regulates brain homeostasis by ions channels, transporters and efflux pumps [3,4]. Tight junctions are composed of occludin, various claudins and membrane-associated guanylate kinase proteins ZO1, ZO2 and ZO3. Adherens junctions are composed of several cadherins, junctional adhesion molecules and PECAM-1. The endothelial cells are ensheathed in capillaries by pericytes and in arterioles and arteries by vascular smooth muscle cells, in addition to astrocyte end-feet [3,4]. Several brain endothelial receptors for insulin, folate, transferrin and low-density lipoprotein can potentially mediate transcytosis through BBB to the brain parenchyma by nanodrugs targeting these receptors. These receptor systems have been used to cross BBB in tumors, but the efficiency of normal BBB penetration by nanoconstructs is usually rather low unless some special methods are used such as nasal delivery or focused ultrasound [3]. We have identified some receptor-targeting peptides that can to some extent penetrate BBB as parts of nanoconstructs. Angiopep-2 (AP-2) peptide binding to the LRP-1 could transcytose the complex through the normal BBB when attached to the β -poly(L-malic acid) (PMLA) scaffold, but mostly to the perivascular space [8]. Our experiments in three different BBB types (normal, AD and tumor-glioblastoma) have shown better BBB penetration in normal mice by PMLA-trileucine-attached D-configuration peptides D1 and ACI-89 also binding

to LRP-1 [9]. Overall, efficient systemic nanoconstruct delivery through normal BBB needs further optimization and enhancement.

2. Pathological changes of BBB in aging & neurodegeneration

Aging and brain pathologies including neurodegeneration and tumors, are associated with blood flow alterations, inflammation, edema and increased intracranial pressure, metabolic perturbations, BBB changes and altered gene and protein expression [4,10,11]. For instance, 80% of patients with frequent Lewy body dementia present with vascular alterations such as cerebrovascular disease, lacunae and multiple microinfarcts, hemorrhages, atherosclerosis, arteriolosclerosis and cerebral amyloid angiopathy [12]. BBB alterations in AD involve reduced capillary length and density, pericyte degeneration, disrupted transporter expression, low expression of tight and adherens junction proteins claudin-5, ZO1, occludin and VE-cadherin, as well as increased perivascular IgG leakage [4]. Similar abnormalities were reported in PD and diabetic retinopathy [4,6]. These alterations may enable toxic blood molecules, cells and pathogens to reach the brain and are associated with pathological immune and inflammatory responses that can contribute to neurodegeneration [3].

3. BBB in primary or metastatic brain tumors

The brain tumor vasculature is characterized by multiple branching of the newly formed leaky tumor vessels (neovascularization). Tumor BBB has altered endothelial tight junctions (decreased expression of occludin and claudins) and astrocytic contacts in the tumor-affected areas. The most frequent type of primary brain tumors is glioblastoma (GBM), with very poor survival rate and treatment response. The GBM BBB has impaired signaling with astrocytes and pericytes. Normal brain astrocytes secrete VEGF and other angiogenic factors to maintain vascular growth. Brain tumors use VEGF to promote neovascularization to grow and survive when they become hypoxic. Brain tumors also present with hyperplasia of α -SMA expressing pericytes that overexpress CD248, which may help in the formation of tumor neovasculature. Astrocytes additionally secrete a trafficking molecule Mfsd2a, important for the BBB development. In the tumor BBB, Mfsd2a is downregulated due to decreased signaling from astrocytes to endothelial cells [2]. Tumor microenvironment is also changed including abnormal shift of laminin isoforms to “malignant” laminin-411 that activates Notch pathway and cancer stem cells. This contributes to tumor invasion and higher

leakiness of tumor BBB, and correlates with poor patient prognosis [10,13].

4. Multifunctional nanosystems for neural drug delivery & treatment

The known complexity of BBB dictates designing multifunctional drug delivery systems to cross each specific BBB type. Such a system should ideally consist of specific BBB delivery vector; moiety targeting the pathological cell type; drug payloads capable of endosomal release. The system should be biodegradable and non-toxic, undergo a thorough physico-chemical and pharmaceutical evaluation required by FDA/EMA, and be effective.

New multimodal systems based on biodegradable polymers have been developed using adhesive dextran-dendrimer hydrogels. They can deliver various therapeutic agents to solid tumors, including small molecules, nucleic acids and antibodies. Such hydrogels capable of intracranial delivery of GBM therapies and activating the stimulator of interferon genes (cGAS-STING) pathway are promising inhibitors of immunosuppression typical for GBM [5].

Recently introduced nanorobotics or nanobots [14] are specifically engineered with sensing, decision making and actuation properties for BBB crossing for theranostic use by utilizing computer modeling and technological gaps. Nanorobots are biocompatible entities of 1–1000 nm size and are synthesized with lipids, polymers, metals and crystals.

We developed fully biodegradable nanopolymers for I.V. injection with measurable blood clearance without any immunogenic toxicity. These polymers had PMLA as a scaffold with a covalently attached BBB crossing (transcytosing) moiety such as peptides AP-2, MiniAP-4 (M4), or transferrin receptor (TfR) ligands cTfRL and B6, or anti-TfR antibody. To provide endosome escape and imaging capability, other attached moieties included trileucine (LLL) and a fluorescent marker, respectively. Thirty min after a single iv. injection, the optimal nanoconjugates already showed significant accumulation in the parenchyma of cortical layers II/III, the midbrain colliculi and the hippocampal CA1-3 cellular layers. Control variants lacking AP-2 or LLL showed poor BBB penetration. Trileucine apparently stabilized the nanoconjugates, whereas AP-2 provided BBB permeation. Nanoconjugates with attached peptides M4, cTfRL and B6 showed little infiltration of brain parenchyma, likely due to low BBB crossing. The PMLA/LLL/AP-2 nanoconstruct can be functionalized with intra-brain targeting and various drugs (e.g., antisense oligonucleotides) directed to molecular regulators implicated in neurological disorders [8].

Various peptides were recently tested side by side in different conditions for the ability to allow nanodrugs to pass through BBB. These included AP-2, B6, MiniAP-4 and D-configuration peptides D1, D3 and ACI-89, ensuring specific transcytosis by binding to LRP-1, TfR, bee venom-derived ion channel and $\text{A}\beta$ /LRP-1 related transcytosis complex, respectively. BBB crossing followed a two-step mechanism, with binding of PMLA/LLL to endothelial membrane and allosteric exposure of transcytosis receptors by specific peptides. *In vivo* nanoconjugate delivery properties were determined in normal, tumor (GBM), and AD mouse models. BBB crossing in brain tumors was the most efficient, followed by normal and AD-like brain. In tumors and normal brain, AP-2 provided the most efficient BBB crossing, although low in normal brain. However, D-peptides, especially D3, were clearly superior in AD model. The TfR vector B6 was equal in normal and AD brains [9]. In AD mice, D3 peptide provided specific delivery of morpholino miRNA-186 (inhibitor of β -site amyloid precursor protein-cleaving enzymes, BACEs) to brain neurons with downregulation by RNA-seq of *BACE-2* and upregulation of important genes (*IGF-1* and *GDF2*) suppressed in AD [15]. D3-conjugates provided targeted systemic delivery of functional miRNA and AONs to brain neurons in AD. For GBM treatment, AP-2 BBB-crossing nanoconjugates were used for brain delivery of checkpoint inhibitor antibodies anti-PD-1 and anti-CTLA-4 to activate general and tumor local immune system [16,17] and to inhibit mouse GBM targets, c-Myc and EGFR/EGFRvIII, with prolonged animal survival [18].

5. Future perspective

Given the complexity of treatment of CNS pathologies, the condition-specific selection of multifunctional/multimodal nano systems is a promising way for future success in neuro nano drug delivery through BBB. Artificial intelligence and machine learning to predict selective BBB penetrating peptides (B3PPs) are emerging for neurotherapeutics development for brain-related disorders. For instance, a new efficient scoring card-based predictor (SCMB3PP) has been developed for improving B3PP identification and characterization. Overcoming the limitation of black-box computational approaches, SCMB3PP predictor could automatically estimate amino acid and dipeptide propensities important for B3PPs. Validation and independent testing confirmed that SCMB3PP outperforms popular machine learning-based methods on multiple independent datasets. SCMB3PP-derived amino acid propensities were utilized to identify informative biophysical and biochemical properties needed for the characterization of B3PPs:

(<http://pmlabstack.pythonanywhere.com/SCMB3PP>).

Novel computational approaches are anticipated to facilitate high-throughput identification of potential B3PP candidates for subsequent experimental validation [12,19,20] and significantly speed up evaluation *in vitro* and *in vivo* to find the best effective delivery vehicles and treatment targets for CNS disorders.

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