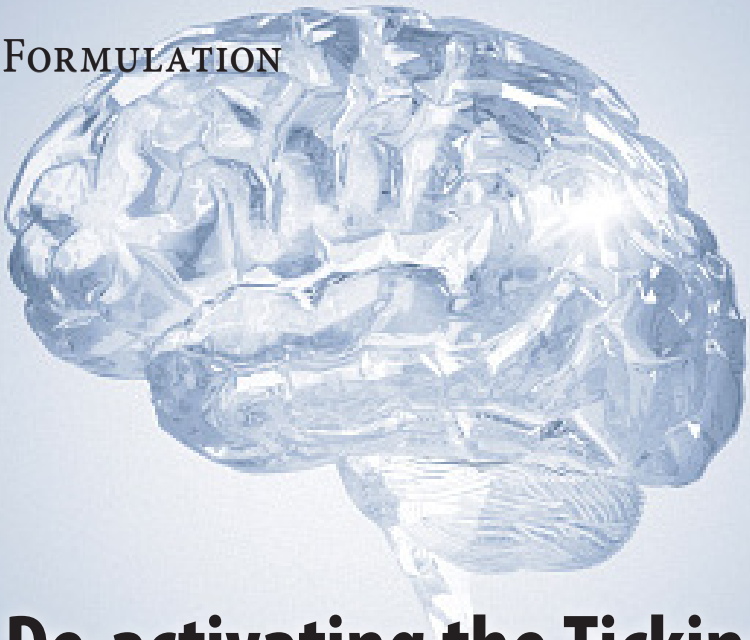

De-activating the Ticking Bomb of Brain Diseases with Nanomedicines

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Nanoparticulate technologies are showing promising potential in delivering both small-molecule drugs and biomacromolecules across the blood brain barrier.

Brain diseases varying from neurodegenerative, cerebrovascular, psychiatric diseases, epilepsy, pain, and cancers are on the rise. In 2010, the European Brain Council (EBC) showed that, one third of all Europeans (179 million people) suffered from at least one brain disorder resulting in a cost of EUR 798 billion (approximately $902 billion) annually and 35% of Europe's total disease burden (1). In this respect, brain disorders can represent a ticking bomb under Europe's economy due to an ageing population and the enormous societal costs requiring better understanding of underlying brain physiology and development pathways of brain diseases, better diagnostic tools and drug-delivery systems combined with clinical brain research.

Most brain diseases remain untreated due to the inability of many candidate drugs to cross the blood-brain barrier (BBB) in adequate quantities. The hypothesis that the cerebral capillaries provide the anatomical basis for a physiological barrier between the brain and the remaining body resulted in the important concept of barrier system maintaining the brain homeostasis, which was termed as the BBB by Lina Stern in 1921 (1).

Some molecules with appropriate polar surface area (< 80 Å), low molecular weight (< 450 Da), a weak hydrogen bonding potential (< 6 hydrogen bonds), and lipophilicity (Log D > 2) along with the absence of free rotatable bonds and low affinity of binding to plasma proteins can permeate across the BBB by diffusion (3). Nonetheless, it is estimated that more than 98% of drug molecules do not cross the BBB (3), because of the distinctive characteristics of this barrier (see **Figure 1**). A lack of fenestrations and the presence of tight junctions between adjacent endothelial cells, formed by an intricate complex of transmembrane proteins (occludin, claudins and junctional adhesion molecules-1) with cytoplasmic accessory proteins (zona-occludens-1 and -2), is reflected in a high transendothelial electrical resistance (1500–2000 Ω.cm^2) that restricts paracellular transport across the BBB. In addition to the physical barrier, the expression of several ATP-binding cassette (ABC) membrane-associated transporters plays a significant role in restricting the permeability of pharmacological agents. In particular, the P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRP1, -2, -4, -5) and breast cancer resistance protein (BCRP) restrict the permeability of even lipophilic drugs, including colchicine, vinblastine, paclitaxel, and cyclosporine A, across the BBB, thus restraining the treatment of brain diseases.

Penetrating the blood-brain barrier with nanomedicines

Nanoparticle technologies are not widely used in medicine despite notable exceptions such as Ambisome (liposomal Amphotericin B), Doxil (liposomal doxorubicin), and Abraxane (paclitaxel albumin bound nanoparticles). These multicomponent, three-dimensional constructs require careful engineering, detailed analytical testing,

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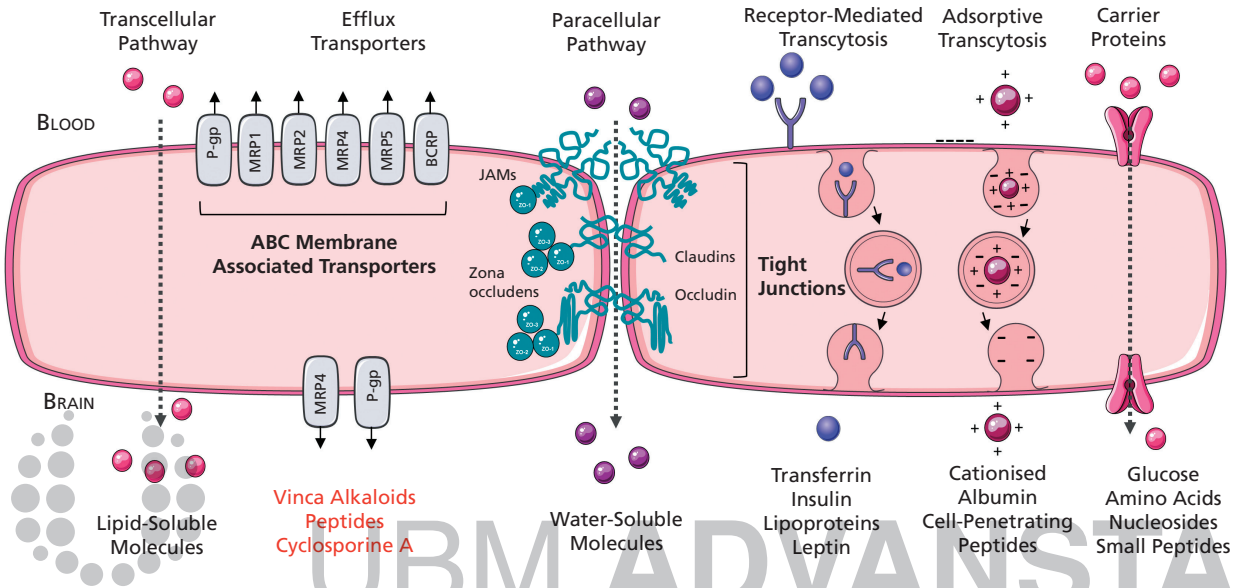
Figure 1: Transport routes across the blood-brain barrier and specific localization of the tight junctions and the ATP-binding cassette (ABC) membrane-associated transporters on brain microvessel endothelial cells. Lipophilic molecules may passively diffuse through the large surface of the lipid cell membrane across the endothelium via a transcellular pathway while hydrophilic molecules are transported through a paracellular pathway that is strictly restricted by the presence of the tight junctions. Receptor-mediated transcytosis can transport essential polar macromolecules (peptides, proteins) and adsorptive-mediated transcytosis appears to be induced by positively charged molecules resulting in the transport into the brain. Transport proteins can transport essential polar molecules into the central nervous system. Arrows indicate the direction of substrate transport.



and reproducible manufacturing scale-up to ensure a consistent product with the intended physicochemical, biological, and pharmacological properties (3). The lack of clear regulatory standards in the examination of nanomedicines frustrates their clinical translation. Preclinical studies of nanoparticulate technologies, however, have shown significant promise in meeting the challenge of brain-targeted delivery for both small-molecule drugs as well as biomacromolecules (3) such as antibody-drug conjugates, carbohydrate nanoparticulate systems and inorganic nanoparticles.

Angiopep conjugates or decorated nanoparticles

Angiochem (Montreal, Canada) has engineered a family of 19-amino acid, BBB-permeable peptides derived from the kunitz domain of aprotinin known as Angiopeps, that have a high transcytosis rate via the lipoprotein receptor-related protein 1 (LRP-1) and LRP-2 receptors (3). The LRP receptors are highly expressed on the BBB and they enable the entry of more than 40 natural ligands into the brain. These high capacity LRP receptors are difficult to saturate because of their rapid transport and recycling time (~30 seconds) (3). LRP-1 is also upregulated in multiple cancer cells comprising malignant glioma and metastatic cancers in the brain. Chemical conjugation of the peptide vector (Angiopep-2) to three molecules of paclitaxel (ANG1005) resulted in a 100-fold greater delivery of the drug to the brain in an *in-situ* rat brain perfusion assay leading to increased survival in a mouse intracranial tumour model. Two Phase I, multicenter, sequential cohort, dose-escalation studies (NCT00539344 and NCT00539383) were carried out with ANG1005 in patients with malignant glioma (4) and with advanced solid tumors with brain metastases (5). Because the anticancer activity of ANG1005 was encouraging in patients with brain metastases from breast cancer (NCT01480583) in a Phase II, multicenter, open-label study, two additional Phase II trials have been performed to further verify its clinical activity in patients with recurrent high-grade glioma (NCT01967810) and breast cancer patients with recurrent brain metastases (NCT02048059). The latter study, which was recently completed in June 2016, demonstrated clinical benefits both intracranially and extracranially in pretreated breast cancer patients with recurrent brain metastases and leptomeningeal carcinomatosis (6).

Angiochem has also developed a novel brain penetrant Angiopep-2 congugated to a monoclonal antibody (mAb) similar to Herceptin (trastuzumab, brain impermeable), which targets the HER2 receptor (ANG4043) in order to treat HER2+ breast cancer metastases in the brain (6). Angiopeps can be further conjugated with anticancer drugs (docetaxel and maytan-

sine). Angiopep-2 covalently linked via a polyethylene glycol (PEG) spacer has been also be studied for nanoparticle delivery (7) or gene delivery (8). Strong efficacy and excellent safety have been demonstrated with no evidence of central nervous system (CNS) toxicity or immunogenicity, even after repeat dosing of up to 22 cycles.

Molecular envelope technology (MET)

Nanomerics Ltd. (St Albans, United Kingdom) is using technology based on an engineered amphiphilic chitosan polymer (N-palmitoyl-N-monomethyl-N,N-dimethyl-N,N,N-trimethyl-6-O-glycol chitosan-GCPQ) tailored to form nanoscale polymeric aggregates that are able to package or specifically interact with peptides (3). In preclinical studies, the technology has been successful in delivering leucine5-enkephalin (an endogenous opioid peptide with a plasma half-life of 3 min) across the BBB, with enhanced and prolonged antinociceptive activity demonstrated in a rodent acute pain animal model after both intravenous and oral administration (9–11). Good laboratory practice (GLP) testing on MET has been completed for the repeat administration of MET via the oral and intranasal routes and significantly high no observed adverse effect levels (NOAELs) have been documented by prestigious external contract research organizations. The toxicology tests were conducted in two major tranches of activity and include safety pharmacology tests on the CNS and respiratory systems as well as repeat-dose, 28-day toxicology tests in two species (12). MET ensures that the peptide is protected from degradation in the intestines, nanoparticles are taken up by the enterocytes, absorbed particles stabilize the peptide and peptide prodrug against plasma degradation, and that the particles adhere to the endothelial cells of the BBB, enabling the peptide to cross the BBB (3). Lomustine-loaded MET nanoparticles have been shown recently to improve the survival in an orthotopic U-87 MG glioblastoma model without major bone marrow toxicity (13).

Gold nanoparticles (AuNPs)

Gold nanoparticles are receiving interest as carriers for drug delivery to the brain. They are easily synthesized and functionalized, maintaining good biocompatibility (14). Recent studies have also illustrated the spontaneous permeability of gold nanoparticles (2.5 nm) across the BBB. This permeability, however, is reduced by 50% after injection of calcium (Ca^{2+}), sodium (Na^{+}) and potassium (K^{+}) ion channel blockers. It is thought that either ion channels play a key role in the BBB permeability mechanism of small AuNPs or that the ion channel blockers influence the penetration of AuNPs through regulation of tight junctions resulting from changes in ion balance (14). Glucose-coated gold nanoparticles (4 nm) have shown a transfer rate across primary human brain endothelium that was at least three times faster than across non-brain endothelia (15). Spherical nucleic acid (SNA) nanomedicines comprised of gold nanoparticles, covalently functionalized with densely packed, highly oriented small interfering RNA duplexes have also been shown to result in intratumoral apoptosis, and decreased tumor burden and progression in xenografted mice, without adverse side effects after intravenous administration (16).

Nanomedicines are the only technologies today that have shown significant promise in brain delivery without comprising the integrity of the BBB in preclinical and early clinical phase trials. The size of the particles is a crucial parameter. Particles less than 70 nm have shown higher percentages of dose levels in the brain compared to larger particles when they are not functionalized with peptides or proteins for endocytosis across the BBB (3). Nanoparticles conjugated with ligands able to interact with BBB receptors at a relatively low density (low avidity) have the best performance. With an aging population worldwide, there will always be a demand for more effective therapies to treat brain diseases, and nanotechnology can provide versatile and translatable platform technologies for overcoming biological barriers such as the BBB.

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