Role of the blood brain barrier in the development of new therapies for the treatment of brain tumours

Dr Kieran Breen
is Director of Research at Brain Tumour Research which supports four dedicated centres of excellence across the UK.

Correspondence to:
E: Kieran@braintumourresearch.org

The blood brain barrier (BBB) refers to the membrane that exists between blood vessels and the brain, which plays a fundamental role in protecting and maintaining its privileged status. It was first described by Ehrlich in the 1880s when he injected specific dyes intravenously into animals, and found that they would stain all of the organs except for the brain. He therefore concluded that there was some form of separation between the blood and the brain. Since then, the complexity of the BBB has been confirmed with it consisting of specialised junctions and transport processes located within the membrane. In addition, there is a blood brain tumour barrier (BBTB) that is associated with tumour angiogenesis. A major challenge for neuro-oncology is to identify mechanisms by which drugs can cross the BBB to enter the brain and exert their therapeutic effects.

The BBB is composed of polarised endothelial cells connected by tight junctions in the cerebral capillary endothelium, resulting in an extremely low permeability for external molecules, including drugs. There are 5 sub-classes of membrane that combine to form the complete BBB, which include: (a) the meningeal barrier; (b) the blood-brain barrier; (c) the blood-CSF barrier; (d) the circumventricular organs, including median eminence, pineal gland, area postrema and the subfornical organ, and (e) the ependyma in the adult brain. All these differ according to their structure, composition and location, but combine to form the complete barrier. The BBB can also be modified by events, such as inflammation, ischemia and exposure to harmful substances, by modifying its structural makeup [1].

The targeting of existing transporter mechanisms within the membranes is one approach by which drugs could enter into the brain. The transporters are normally responsible for the passage into the brain of endogenous agents such as amino acids, hormones and fatty acids. However, these may be targeted to facilitate the transfer of drugs across the BBB. For example, the OAT transporter, which normally transfers small organic compounds across the membrane, has also been reported to transport drugs such as aspirin and ibuprofen into the brain. Furthermore, the drug L-DOPA, which is used for the treatment of Parkinson's disease, has an amino acid structure that allows it to enter entry via the amino acid transporter LAT1.

Receptor-mediated transcytosis involves the binding of cells to the specific components of the epithelial membrane of the BBB and cross over into the brain [2]. This involves the targeting of ligands, endogenous proteins or antibodies directed against cell surface receptors (e.g. transferrin or insulin receptors) to induce receptor-mediated transfer of drugs and small peptides across the BBB. However, the success of such a strategy will require a much greater understanding of the expression of cell surface proteins if one is to selectively target the BBB membrane.

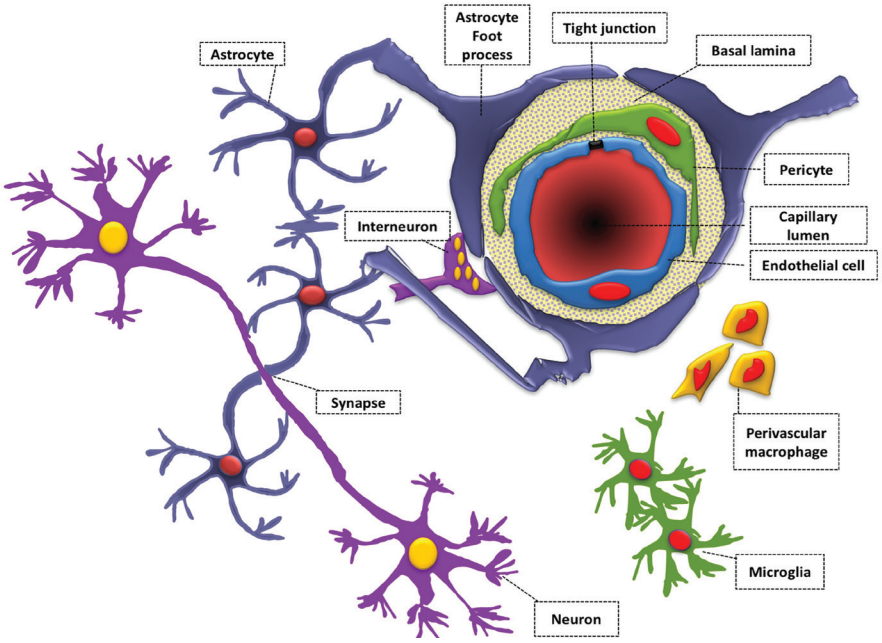
In addition to targeting the endogenous transport mechanisms, a number of alternative approaches are being developed to facilitate drug entry into the brain:

- The first is based on the direct injection of a drug into the site of the tumour. This is an invasive surgical procedure with associated risks. There are additional problems, including diffusion of the drug from the site of injection with potential damage to the brain tissue surrounding the tumour. This can be difficult to predict depending on the heterogeneity and density of the tumour mass. Reflux of the drug solution upwards along the side of the injection cannula will have similar effects.
- A new procedure, termed convection-enhanced delivery, is more accurate for the delivery of molecules to a specific site, with particular accuracy and minimal collateral damage due to diffusion.
- Another invasive procedure being developed is the permeabilisation of the BBB via nasal mucosal engrafting [3]. This procedure exchanges the normal mucosal membrane with one that is more permeable, which allows larger molecules to cross into the brain following nasal administration. However, this is still at the experimental stage and, as it is an invasive surgical procedure, its efficiency will need to be compared with existing surgical techniques and non-invasive approaches.

Physical approaches have been used to modify the structure of the BBB to establish transient permeabilisation to allow drug entry [4]. These include ultrasound disruption, reverse osmotic opening and electrical stimulation, all of which

REFERENCES

1. Saunders NR , Habgood MD , Møllgård K et al. *The biological significance of brain barrier mechanisms: help or hindrance in drug delivery to the central nervous system?* F1000 Faculty Rev 2016;313. doi: 10.12688/f1000research.7378.1.
2. Mager I, Meyer AH , Li J. *Targeting blood-brain-barrier transcytosis - perspectives for drug delivery. Neuropharmacology.* 2016;3908(16)30361-6. doi: 10.1016/j.neuropharm.2016.08.025.
3. Marianecci C, Rinaldi F, Hanieh PN et al. *Drug delivery in overcoming the blood-brain barrier: role of nasal mucosal grafting.* Drug Des Devel Ther. 2017;11:325-35. doi: 10.2147/DDDT.S100075
4. Dasgupta A, Liu M, Ojha T, et al. *Ultrasound-mediated drug delivery to the brain: principles, progress and prospects.* Drug Discov Today Technol. 2016;20:41-8. doi: 10.1016/j.ddtec.2016.07.007.
5. Saraiva C, Praça C, Ferreira R, et al. *Nanoparticle-mediated brain drug delivery: Overcoming blood–brain barrier to treat neurodegenerative diseases.* J Control Release. 2016;10;235:34-47. doi: 10.1016/j.jconrel.2016.05.044.
6. Vieira DB, Gamarra LF. *Getting into the brain: liposome-based strategies for effective drug delivery across the blood-brain barrier.* Int J Nanomedicine. 2016;18(11):5381-414.
7. Ha D, Yang N, Nadithe V. *Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges.* Acta Pharm Sin B. 2016;6(4):287-96. doi: 10.1016/j.apsb.2016.02.001.
8. Palmiotti CA, Prasad S, Naik P et al. *In vitro cerebrovascular modeling in the 21st century: current and prospective technologies.* Pharm Res. 2014;31(12):3229-50. doi: 10.1007/s11095-014-1464-6.
9. Hatherell K, Couraud PO, Romero IA, et al. *Development of a three-dimensional, all-human in vitro model of the blood-brain barrier using mono-, co-, and tri-cultivation Transwell models.* J Neurosci Methods.2011;199(2):223-9. doi: 10.1016/j.jneumeth.2011.05.012.



will impact on the membrane structure and function. The combined use of ultrasound with microbubbles has been proposed as a tool for delivering drugs across the BBB. This can be used directly for drug delivery, with the drug being contained within the microbubble vesicles, or indirectly by modifying the property of the membrane to allow drugs to cross the membrane directly. Microbubbles are 1-5μm sized gas-filled vesicles containing phospholipids, proteins or polymers. Their ability to cross the BBB following ultrasound stimulation depends upon both their composition and the frequency of the ultrasound being used. The nature of the ultrasound will also serve to localise the effect and therefore minimise damage to surrounding tissues. A current clinical study is underway to assess the use of this technique to deliver the chemotherapeutic agent doxyrubicin to the site of a glioblastoma [4].

There is increased interest in the direct use of carriers to enable drugs to cross the BBB. Nanoparticles (NPs) are colloidal carriers that can have a natural or synthetic origin, varying in size from 1 to 1000 nm [5]. Synthetic NPs may be prepared from polymeric materials such as poly(ethylenimine) or from inorganic materials such as gold or silicon dioxide. They can also be generated from natural polymers, e.g. polysaccharides, amino acid polymers or proteins. The carriers act by adsorbing, entrapping or covalently binding the drugs. Encapsulation of drugs into NPs can target specific transport processes to enhance their entry through the BBB. A combination of the NP constituents, their size and shape will influence whether they can mediate penetration across the BBB. While the majority of studies to date have been carried out on spherical nanoparticles, other studies have been investigating the potential of other forms of particle since their synthetic nature provides an excellent opportunity to modify their shape and size. For example, polysterene NPs with a rod-shape coated with an antibody against the transferrin receptor showed a 7-fold increase in brain accumulation compared to their spherical NP counterpart. However, inorganic NPs have disadvantages because they may not be degraded or eliminated through the kidneys, leading to potential toxic side effects associated with their pharmacokinetic profile.

Another form of nanoparticles are liposomes, which are synthetic vesicles with a phospholipid membrane that self-assemble into various sizes and shapes within an aqueous environment [6]. However, liposomes may have limitations associated with the activation of the host immune system, thereby increasing their toxicity. While inorganic polymeric nanoparticles may have better stability than liposomal systems, their biocompatibility and long-term potential safety remain concerns. Both of these delivery systems have been used to deliver different types of drugs into the brain, but further development is required before the most appropriate system can be identified for routine clinical use.

Exosomes are naturally-occurring small intracellular membrane-based vesicles containing various types of lipids and proteins involved in several biological and pathological processes. They are non-immunogenic and therefore have advantages compared to other nanoparticulate drug delivery systems [7]. They can be described as bilayer membrane "nanospheres" generated and secreted by all cell types, and can be found in most body fluids. They can be isolated from extracellular fluid by density gradient centrifugation and then filled with specific drugs. Because of their membrane composition, exosomes can be used to facilitate the transfer of drugs across the BBB. Studies using exoxomes derived from the U-87 glioblastoma cell line have demonstrated the encapsulation of drugs, such as paclitaxel and doxorubicin, which can be incorporated into exosomes that then cross the BBB (as shown in a zebrafish model).

Due to the cost of using *in vivo* models to assess the potential of drugs to cross the BBB, a number of *in vitro* and computational models have been developed to mimic the BBB and predict if, and indeed how, molecules may cross into the brain [8]. An appropriate model should allow for a detailed study of the role of the molecular processes that maintain membrane homeostasis, in conjunction with a demonstration that the model recapitulates the properties of the membrane in vivo, including the expression of appropriate transporter systems. One example is the development of a 3D model comprised of relevant human cell types and basal laminar proteins cultured in 24-well plates with polycarbonate membrane transwell inserts [9]. Systems can also be developed that model changes occurring in a number of disease states and therefore will be more accurate in their predictions of drug penetration into the brain. Other tools under development include in silico models, isolated brain microvessels, microfluidic models and 3D extracellular matrices-based scaffolds. All have their limitations and it is likely that a combination will be needed to fully model the *in vivo* situation.

While the BBB provides a particular challenge for the treatment of brain tumours, an increasing number of tools are being developed that hold promise for the delivery of therapeutic agents to the site of the tumour, and therefore increase the range of available therapies.