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Short communication

Insight into the efficacy of lipid nano-systems for brain delivery – uptake and internalization pathways in different cell culture lines

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Blood-Brain Barrier: from physiology to disease

The blood-brain barrier (BBB) represents high selective, semipermeable membrane which separates the circulation from the brain, due to the presence of endothelial cells and specialized tight junctions that prevents the transport of 100% of large neurotherapeutics and more than 98% of all small-molecule drugs (Daneman and Prat, 2015). There are different specific transporters on both sides of the BBB that are responsible for its selective transport role as well as certain enzymes released locally (secretory and metabolic role) (Abbott et al., 2006). BBB carefully protects the brain from neurotoxins and other harmful substances. Because of its neuroprotective role, the delivery of many potentially important agents intended for diagnostic or therapeutic purposes has been fully blocked or at least difficult and problematic. Moreover, many neurotherapeutics do not reach the adequate drug concentration in brain to be clinically effective (Sweeney et al., 2018).

There has been evidence that only several drugs such as morphine, methadone, diazepam, etc. could cross the BBB and express their pharmacologic effect in the brain. On the other hand, the delivery of most antibiotics, anti-tumor agents as well as the drugs for the treatment of Alzheimer's disease, Parkinson's disease, epilepsy, etc., has been quite challenging and there has been an urgent need for finding new approaches in order to enhance their transport through the BBB more easily and selectively (Khaledian et al., 2022). In this sense, a whole new generation of modern drug delivery systems has been designed and developed, where lipid based nano systems have been gaining much attention due to their biocompatibility and similarity to human cells.

Lipid nano-systems (LNS) as carriers for brain drug delivery

The ability to simultaneously encapsulate hydrophilic and lipophilic drugs as well as the possibility of particle surface modification with different ligands and polymers for stealth stabilization, make LNS one of the most used and evaluated drug delivery systems. When it comes to the effectively crossing the BBB and drug delivery to the brain, among various LNS, nanostructured lipid carriers (NLC) and liposomes stand out as the most important. In the literature, there are numerous research studies confirming their potential in brain drug delivery, starting from the dual-targeting doxorubicin liposomes that could selectively transfer the active component in brain glioma leading to reduction in the tumor size (Gao et al., 2013). Kong et al. (2020), evaluated the improvement of brain targeting when using transferring-conjugated liposomes. In another study the increased uptake of ribavirin loaded liposomes surface functionalized with glutathione and polyethylene glycol (GSH-PEG-liposomes) compared to non-functionalized liposomes, was investigated in three different cell lines (brain endothelial cells, human umbilical vein endothelial cells and human kidney epithelial cells), where it was revealed that the enhanced uptake of GSH-PEG-liposomes has been determined by endocytosis (Maussang et al., 2016). Various research studies also revealed the potential of NLC for enhancing the drug permeation through the BBB. For instance, the formulation of conventionally used active compound such

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as carbamazepine in the treatment of epilepsy, when encapsulated in NLC showed improved brain delivery and therapeutic outcome (Khan et al., 2020).

BBB uptake and internalization pathways of LNS

Several mechanisms to cross the BBB have been proposed that these carriers may follow, such as simple diffusion; paracellular transport; transcytosis, carrier-mediated transport and endocytosis, or a combination thereof (Ahn et al., 2020). Namely, the internalization of LNS into the cells is usually through lysosomal pathway when the active compounds are released into the cell upon nanoparticle degradation by the lysosomal components; or endosomal pathway where nanoparticles travel inside the endosomes from one side of the cell to the other without being degraded. When it comes to the uptake of NLC, research studies suggest that the process of endocytosis has been the most likely mechanism for particle internalization through the BBB. On the other hand, liposomes as one of the most attractive vehicles could fuse with endothelial cells and transport the active components via endocytosis or receptor-mediated transcytosis (Khaledian et al., 2022).

Literature data suggests that the most important factor that could affect the uptake of LNS is the particle size. As per the findings, the most used and effective nanoparticle size in brain drug delivery has been around 100 nm, which was found to be also significant for prolonged plasma circulation time. Additionally, the shape, charge and modifications of the nanoparticle's surface also play an important role in determining the amount and the rate of LNS uptake and passage through the BBB. LNS usually have spherical shapes which contributes for enhanced penetration through the cells. Even though it would be more natural positively charged particles to have higher uptake considering that the BBB is slightly negatively charged, however, literature data indicates that formulations with a negative zeta potential have been also effective and high drug concentrations cross the BBB. The possibility for particle surface functionalization represents an enormous advantage to design nano system with desirable properties that will be in accordance with the characteristics and requirements originating from the disease being treated. It has already been established that the process of PEGylation induces nonspecific cellular detachment resulting with prolonged bioavailability of the drugs in the central nervous system which is of particular benefit for targeted delivery (Shalabalija et al., 2021).

In this sense, in the last few years our research group is orientated towards design and development of LNS for brain drug delivery. Namely, the *in vitro* internalization efficiency of different formulations of liposomes and NLC was confirmed on hCMEC/D3 and SH-SY5Y cell lines as a model for BBB and neurons, respectively.

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