Nanoparticle crossing of blood–brain barrier: a road to new therapeutic approaches to central nervous system diseases

Valentín Ceña^{*,1,2} & Pablo Játiva^{1,2}

¹Unidad Asociada Neurodeath, Universidad de Castilla-La Mancha, Albacete, Spain

²CIBERNED, Instituto de Salud Carlos III, Madrid, Spain

*Author for correspondence: valentin.cena@gmail.com

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Diseases of the CNS constitute a significant burden in terms of both personal suffering of the patients and also in economic terms for the health systems. It has been estimated that the cost of brain diseases in Europe amounted to €798 billion in 2010 [1]. The cost in the USA was similar, about US\$789 billion in 2014 [2]. Moreover, the increase in lifespan in Western countries predicts that CNS diseases will have a much more profound impact on both their society and economy.

One of the main problems that both treatments and diagnostic tools for CNS diseases face is the inability of most therapeutic and contrasting agents to adequately cross the blood–brain barrier (BBB). The BBB is a specialized part of the vascular system which comprises the basal lamina, containing different extracellular matrix proteins such as laminin, heparan sulfate or collagen, which in turn encompasses microvessel endothelial cells and pericytes. In addition, astrocyte endfeet and interneurons also contribute to the BBB structure. The BBB also plays an important metabolic role by disposing waste products, metabolizing different chemical compounds, both drugs and toxics, and protecting the brain from changes in the ionic composition of the cerebrospinal fluid. One of the main characteristics of the BBB is the presence of a very high electrical resistance across itself. This high resistance is caused by the presence of intercellular tight junction complexes that keep together adjacent endothelial cells, and which are formed by different proteins including claudins, ZO-1 and occludin [3]. These structures prevent toxic substances or pathogens from entering the CNS, also markedly limiting in turn the brain uptake of therapeutic and diagnostic compounds. Moreover, the presence in BBB endothelial cells of ATP-binding cassette transporters (ABC transporters) [4], expelling back to the blood stream some compounds that might cross the BBB, further limits the availability of drugs and imaging probes in the CNS. Accordingly, very few molecules are transported efficiently into the brain.

Since new therapeutic compounds are needed for many CNS diseases where mostly symptomatic treatments are available, different approaches have been used to overcome the limits that BBB poses for compounds, intended for CNS diseases diagnostic and therapeutic procedures, to reach their target sites. One of the most promising alternatives is the use of nanoparticles to deliver such compounds through the BBB into the brain. Several reviews have been focused on this subject in recent years [5–7].

Nanoparticles have the same limitations as other xenocompounds to cross the BBB and, accordingly, to access the brain. Several strategies have been devised to improve BBB crossing by nanoparticles taking advantage of the different physiological mechanisms underlying this process. The main approaches that have been used to overcome this problem are:

- Provoking a transient permeability increase in the BBB paracellular pathway. The disruption of the tight junctions between adjacent endothelial cells is generally achieved by using ultrasound/microbubbles [8], and

osmotic pressure both of which locally enhance BBB permeability allowing an increase in nanoparticle entry. However, this approach entails some risks since it would allow an uncontrolled entry of many compounds into the brain due to the loss of the homeostatic function of BBB, which could lead to cerebral toxicity.

- Taking advantage of the transcytosis pathway by favoring the binding of nanoparticles to the surface of endothelial cells making use of two different mechanisms:
 - Adsorptive transcytosis, where the surface properties of the nanoparticles facilitate the binding of the nanoparticle and its cargo to the luminal plasma membrane of endothelial cells. Positively charged nanoparticles are more prone to undergo this mechanism when compared with neutral or negatively charged ones, since endothelial cell plasma membrane is negatively charged. In addition, nanoparticles decorated with compounds such as wheat germ agglutinin can be taken up by nerve terminals and retrogradely transported by the axons to the CNS [9].
 - Receptor-mediated transcytosis, where nanoparticles decorated with different ligands on their surface are able to bind specific receptors and thus promote endocytosis. Several receptors and transporters have been used as targets, such as GLUT1, lactoferrin (Lf), transferrin (Tf) [10] or different peptides including angiopep-2 or Seq12 [11].

The initial mechanism involved in transcytosis is nanoparticle uptake by endocytosis, which consists in the production of endocytotic vesicles, generated from plasma membrane invaginations, which in turn allow the incorporation of extracellular substances into the cells. It is a complex process involving many components such as receptors and different signaling pathways. There are two main routes for endocytosis:

- Clathrin-mediated endocytosis, which takes place in clathrin-enriched areas of the cell membrane, forming endocytotic vesicles up to 200 nm in diameter. Once inside the cell, clathrin-coated vesicles fuse each other generating an early endosome that progress to late endosomes while its intravesicular pH decreases and, finally, fuse with lysosomes causing degradation of their cargo. In order to be a successful delivery agent, the nanoparticle should facilitate its cargo escape from the endosomes before they fuse with lysosomes, thus avoiding the possible cargo degradation.
- Caveolin-mediated endocytosis, which takes place in lipid rafts where plasma membrane invaginations of about 80 nm in size are produced. After this initial step, caveolin vesicles fuse with other caveolin vesicles generating caveosomes, which avoid the lysosomes and whose final fate will vary depending of the cell type.

The interaction of the different nanoparticle types with these two endocytotic mechanisms plays a relevant role on the final ability of a given nanoparticle to successfully cross the BBB.

One recurrent question when designing nanoparticles intended to cross the BBB is whether there is a discernible pattern in those nanoparticles reported to successfully achieve their goal, which could help predict that a significant amount of a given nanoparticle might deliver its cargo to the brain parenchyma. Although there is no clear pattern, several characteristics of the nanoparticle itself might contribute to a successful BBB crossing, such as the following:

- Size. As indicated above, the final pathway of nanoparticle entry into the endothelial cells by transcytosis is determined by the initial step of endocytosis. Nanoparticles with a size smaller than 200 nm (the estimated limiting size for a nanoparticle to undergo endocytosis through a clathrin-mediated mechanism) have more chances to efficiently cross the BBB [12].
- Charge. Positively charged nanoparticles can use the adsorptive transcytosis pathway more easily than their neutral or negatively charged counterparts due to the negative charge of the endothelial cell external membrane. On the other hand, neutral or negatively charged nanoparticles show a reduced protein adsorption, leading to longer circulation times [13]. However, longer circulation times may also be achieved by coating the nanoparticles with compounds such as PEG. PEGylation generates a steric barrier around the nanoparticles which avoids opsonization and their subsequent elimination by the mononuclear phagocyte system. Different zwitterionic molecules can be also used, which may overcome the low cell uptake efficiency observed for PEG-coated nanoparticles [13].
- Surface modifications. Nanoparticle surface decoration with different ligands mainly aims to increase BBB crossing by the nanoparticle by means of:
 - Taking advantage of transport- and receptor-mediated transcytosis, as indicated above. In this sense, ligands for targets such as GLUT1 or albumin transporters [14], Lf receptors, LRP1 (targeted by angiopep-2) or Tf

receptors [10] have been used. One important concept when dealing with incorporating different ligands onto a nanoparticle to increase receptor-mediated transcytosis is avidity [15]. Basically, placing too many affinity ligands (high avidity) on the surface of a nanoparticle can hinder endocytosis by endothelial cells, and make the nanoparticle and its cargo remain attached to the endothelial cell plasma membrane. This issue can be overcome by placing fewer ligands with increased affinity for its receptor [16].

- Decorating the nanoparticles with cell-penetrating peptides that avoid the endocytotic pathway, and deliver the nanoparticle and its cargo directly in the cell cytoplasm [17].
- Increasing nanoparticle circulation time that leads to an enhanced brain uptake using ligands such as PEG that also decreases nanoparticle opsonization and sequestering by the reticuloendothelial system [13].

The usual method for nanoparticle administration to achieve an effect in the CNS is intravascular (generally intravenous) administration. However, nanoparticles face rapid clearance from circulation, leading to a limited circulation time and thus to a reduced BBB crossing. It is estimated that, at best, less than 5% of the initially administered nanoparticle dose can be found in the brain [18]. This has led to the search for additional ways to deliver nanomaterials to the CNS. One alternative is intraventricular administration of nanoparticles, since the mammalian paraventricular zone has a more permeable BBB than other brain areas that would facilitate BBB crossing. However, while it might be useful for research purposes, it is a too aggressive technique to be routinely used in a clinical setting. Another alternative that is being explored is intranasal administration that uses retrograde transport of nanoparticles through olfactory and trigeminal neural pathways to deliver nanoparticles to the CNS. This approach has been mainly used to deliver natural polymers, such as chitosan and its cargo to the CNS [19]. One problem that this delivery pathway faces is the nose ciliary movement which will tend to remove the nanoparticle from the nose, thus limiting the time the nanoparticle is available to be taken up by the nerve terminals.

In addition to the above mentioned, there is another point that should be borne in mind when testing BBB crossing by nanoparticles aimed to treat CNS diseases. Generally, nanoparticles are tested in different strains of wild-type rodents. However, it must be considered that when it comes to test them on an animal model of disease for some of the main CNS pathologies (i.e., Alzheimer's disease, Parkinson's disease or stroke), an alteration in BBB permeability may be expected as it has been described in these human diseases [20]. This would lead to a different BBB crossing efficiency than that observed in wild-type animals. For this reason, nanoparticle BBB crossing tests should be also carried on in the corresponding animal model of disease.

Summing up, besides certain advances in the design of nanoparticles able to efficiently cross the BBB, we still need to increase our knowledge of the precise interactions among the different factors, related to a nanoparticle structure, leading to a successful BBB crossing. A better understanding of these interactions will contribute to a more precise and successful design of nanoparticles that would be able to efficiently deliver therapeutic and/or diagnostic molecules to the CNS.

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