Editorial

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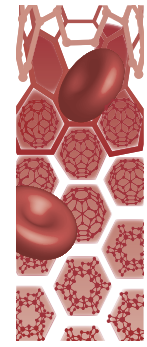

Immuno-nanocarriers for brain delivery: limitations from *in vitro* to preclinical and clinical studies

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"Despite the promising results verified in *in vitro* studies, most of these NPs fail to further proceed to animal studies or clinical trials."

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The treatment of several neurological disorders, such as brain tumors and Alzheimer's, Parkinson's and Huntington's diseases, remains a challenge due to the blood–brain barrier (BBB). This barrier presents a major obstacle in the treatment of neurological diseases as it prevents the delivery of most therapeutic agents to the brain, thus impeding effective therapies. Therefore, innumerous approaches have been envisaged in the last decade to transport therapeutic drugs into the brain.

One such strategy is the nanoencapsulation of drugs, most particularly using nanoparticles (NPs) decorated with targeting moieties, which can direct them to the brain. These targeting molecules are linked to the NPs' surface to be recognized by the targeted tissue receptors. These nanocarriers exhibit controlled properties and the ability of delivery the drug in the target tissue.

Monoclonal antibodies (mAbs) are one of the most promising approaches, having demonstrated promising results in *in vitro* studies. These types of nanosystems display an advantageous property since only a few mAbs are necessary to achieve high levels of drug targeting. Also, therapies using these immuno-nanosystems more efficiently direct the drug to the target tissues, avoiding the delivery of excessive amounts of therapeutic drugs into the blood circulation, reducing toxic effects. However, the use of these immuno-targeting nanocarriers presents some limitations, such as the species specificity.

Recent advances in the immuno-nanocarriers for brain targeting

The therapy of CNS diseases remains a huge challenge. In recent years, several research groups have developed drug-delivery systems to transport therapeutic molecules into the brain [1–4]. That way, therapeutic molecules could be transported to their site of action without modification of their physicochemical properties. Several types of NPs have been developed, such as lipidic (liposomes, solid lipid nanoparticles), polymeric (as is the case of poly(lactic-co-glycolic) acid [PGLA] NPs) and metallic NPs [5]. There are already some clinically available NPs. For example, different liposome-based carriers can be found in the market such as: Ambisome® (Gilead Sciences, CA, USA), Myocet® (Cephalon/TEVA Pharmaceutical Industries, NJ, USA), and Daunoxome® (Gilead Sciences). Ambisome has the antifungal amphotericin B encapulated, Myocet transports an anticancer agente, the doxorubicin and Daunoxome have other anticancer agent encapsulated, the daunorubicin. Also, some PLGA nanoformulations are commercially available, for example, Trelstar® (Pfizer, NY, USA) that encapsulates an anticancer drug and Nutropin Depot® (Genentech, CA, USA) that is used for long-acting dosage of recombinant human growth hormone (rhGH).

In vivo experiments demonstrate that the use of NPs to transport the active molecules decrease the potential toxicity of the drugs [6].

With the aim of directing these NPs to the brain, surface modifications with targeting moieties are a promising approach [7]. Different molecules can be used as active targeting moieties to the BBB receptors, allowing the passage of drugs through this barrier via receptor-mediated endocytosis. This transport mechanism is regulated through the interaction between a ligand and its specific receptor at the surface of the BBB endothelial cells. It is important that these receptors are found in higher expression in the capillary endothelial cells at the BBB than in other cells, to meet the requirements of a 'directed Trojan horse'. Insulin and transferrin receptors are two of the most abundant receptors at the BBB [8]. So, using these transferrin and insulin ligands as targeting moieties are promising approaches.

However, antibodies against these receptors are preferable to the ligand molecules. In fact, it has been proven that mAb for transferrin receptors does not compete with the transferrin molecules existent in the bloodstream [9]. Other receptors present in the brain endothelial cells are IGF, leptin, Fc-like growth factor, scavenger type B1, low-density lipoprotein, lactoferrin, IL-1 and folic acid receptors [10–12]. They could be also targeted by antibodies able to recognize them.

Thus, immuno-nanocarriers have been studied to overcome the BBB. Most particularly, mAb molecules are being extensively studied for brain drug targeted delivery due to their advantageous features, such as exhibiting high specificity, long half-life and their ability to be mass produced [13]. In fact, several *in vitro* studies using mAbs for brain targeting have been conducted and have proved that the use of this targeting strategy increased the permeability of the nanocarriers through BBB models [14–18].

Immuno-nanocarriers limitations

Despite the promising results verified in *in vitro* studies, most of these NPs fail to further proceed to animal studies or clinical trials. The prediction of NP's *in vivo* behavior remains the major limitation once it is difficult to mimic biological systems. Also, in some cases, the use of targeting moieties coupled with the NPs does not confer a significantly increased brain accumulation of NPs/drug. Sometimes, it results in low therapeutic efficacy and toxicity of the nanosystem.

Thus, only one clinical trial using immuno-NPs for the treatment of brain diseases is registered (either ongoing or completed) in the clinical trials database. This study follows a previous clinical trial conducted by SynerGene Therapeutics, where cationic liposomes modified with anti-transferrin receptor single-chain antibody fragment showed promising results for the treatment of different types of cancer [19]. Due to theses successful results, this trial is moving forward to Phase II, where patients with recurrent glioblastoma will be participating [20]. However, although a few ongoing trials studying the efficacy of imuno-nanocarriers for different diseases exist, transferring the use of these NPs for brain targeting remains a challenge.

Nevertheless, the use of antibodies increases the production of immune-NPs costs, making it difficult to scale up to an industrial level, a crucial step in creating a therapeutic product available commercially. The optimization of antibodies production is one of the goals in the immunotherapy. Since the mAbs introduction to the pharmaceutical market in 1986, several efforts by different research groups and industries have been made to optimize the process. Antibody production requires the use of very large cultures of mammalian cells and extensive purification steps under GMP conditions. This type of protocols leads to very high manufacture costs.

In addition, the species specificity of antibodies is a serious limitation in experimental studies. The oldest mAbs tested in humans were murine molecules. When administrated in humans, they were eliminated by the immune system and, consequently, their biological efficacy was strictly limited. So, the transition from *in vivo* experiments in animals to clinical trials in humans remains a challenge. Antibodies that work in animals could not work in humans and *vice versa*.

Future perspective

All the aforementioned reasons explain why at this moment no immuno-nanocarriers for brain delivery have been approved by the EMA and US FDA. Therefore, it is crucial to develop new strategies for the targeted delivery of drugs for the treatment of neurological diseases.

For example, an effective route to deliver drugs into the brain is via intranasal delivery, since drugs can enter directly into the brain through the olfactory mucosa, bypassing the BBB. However, due to the reduced dose volume administration allowed by the nasal cavity, the drugs therapeutic efficacy is compromised. Thus, parametric administration routes remain a preferable strategy.

Although the use of immuno-nanocarriers for brain delivery is far from reaching a clinical application, these nanotools have been proven to be efficient and safe since they preserve the integrity of the BBB, proving to be a promising strategy for systemic administration. However, it is still necessary to further expand the knowledge of the scientific community in the fields of neuropharmacology and brain disorders to improve the development of immuno-nanosystems for the treatment of brain diseases.

Even though further research on the distribution of the immuno-nanocarriers in the brain tissue is required, they display a notable potential for the therapy of neurological disorders.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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