Lipid nanoparticles functionalization strategies to cross the blood-brain-barrier

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Brain diseases are a mayor public health concern worldwide [1,2]. The blood-brain-barrier (BBB) acts as a protective shield, preventing the direct exposure of the central nervous system (CNS) to pathogens and toxic substances [1]. While keeping the homeostatic environment of the brain, the BBB also prevents delivery of therapeutic substances. Therefore, it becomes an obstacle to treat many brain diseases [3-5]. This limitation becomes more critical with the shift in therapeutics from small molecules to macromolecules [1]. On the other hand, the BBB counts with several transport mechanisms for endogenous substances to satisfy the high metabolic requirements of the brain [6]. Nanotechnology-based drug delivery strategies have become an opportunity to take advantage of those mechanisms and target the CNS without compromising the BBB structure or functionality [4,5]. The modification of lipid nanoparticles (LNP) and other nanocarriers with surface moieties such as antibodies or peptides allows the stimulation and exploitation of transport mechanisms expressed in BBB [6,7]. A systematic review was carried out to compile the functionalization strategies used in academic works, and to evaluate which studies have provided more evidence on the effectiveness of brain targeting. This is part of a research project that aims to deliver small interfering ribonucleic acid (siRNA) through the BBB, with LNP. Figure 1 shows the schematization of the bibliographic review. In the screening phase other reviews and other pharmaceutical preparations instead of LNP were excluded. Experimental designs targeting the BBB through nasal route and research articles in which the nanoparticle crossing of the BBB was not objectively assessed (either using *in vitro* or *in vivo* models), were also excluded. Figure 2 shows the therapeutic goal of each of the 79 research papers included in the review. The largest group were applications for several types of brain cancer (29 research papers). Notably, eight studies worked on applications for Alzheimer's disease and only one publication had the final objective of developing treatments for Parkinson's disease. This relatively low academic production within this subject contrasts with the general academic interest and epidemiologic prevalence of Parkinson's disease [8]. Table 1 shows the active pharmaceutical ingredients incorporated to the LNP. There were 11 research works developed for no specific disease. These studies correspond to empty LNP developed as a model for brain delivery. Furthermore, Figure 3 shows the modification strategies used to facilitate the passage through BBB. An important group of papers did not identify a functionalization strategy (25), while another five use PEG or surfactant as a functionalization strategy (compared to the rest of formulations, these five could also be consider as LNP without active targeting modification). This is consequent with some literature reporting that unmodified LNP could cross the BBB due to its size and lipophilic nature [9]. The need for further modification could rely on an enhanced effect and specific targeting inside the BBB. Among the studies that included a surface modification, the most used were antibodies (10), proteins (12) and peptides (15). Figure 4 shows the distribution of the most used peptides. Finally, the evaluation strategies applied to determinate BBB crossing are classified in Table 2. The approach to incorporating controls among studies was not the same, specifically with respect to the inclusion of loaded/empty and functionalized/unfunctionalized combinations. Some authors did not include any comparisons in the study. Considering that the BBB crossing has been demonstrated without the need for any modification, the inclusion of controls is crucial to objectively determine the success of a given strategy. Despite the differences on evaluation strategies, composition and physical properties among the reviewed articles, the systematic review allowed us to identify:

- Therapeutic fields that represent research opportunities for brain delivery.
- The diversity of functionalization strategies and the predominance of peptide-based modification.
- Methodological practices that could provide greater robustness to experimental designs on the delivery of LNP through BBB.

This compilation of scientific literature in the field is an important input for research projects on LNP delivery across the BBB, and consequently, a contribution to the chain of development that would bring therapeutic solutions for brain diseases.

References

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Figures & Tables

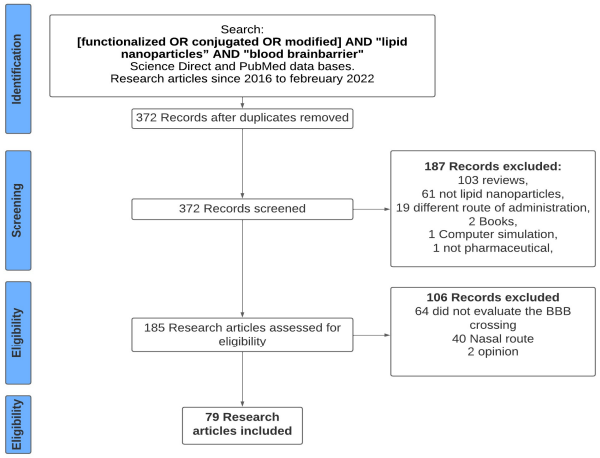

Figure 1. Flow diagram of the systematic review.

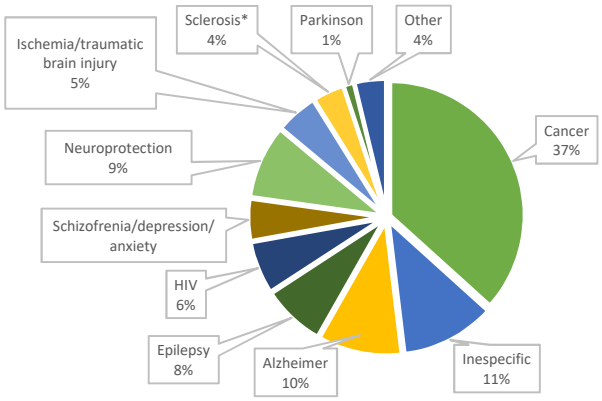

Figure 2. Therapeutic objective of the research articles included in the review. *Note: includes multiple and amyotrophic lateral sclerosis.

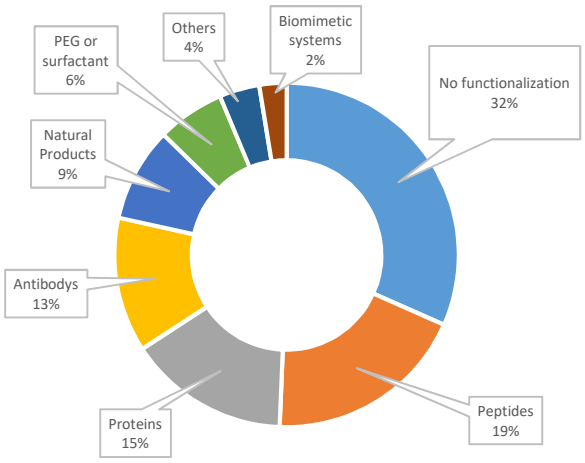

Figure 3. Lipid nanoparticle modification strategies to cross the blood-brain-barrier.

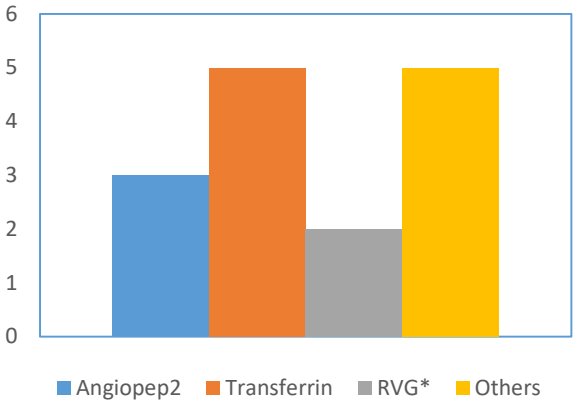

Figure 4. Peptide-based functionalization strategies to cross the BBB. *Note: rabies virus glycoprotein.

Table 1. Active pharmaceutical ingredients most commonly delivered for the therapeutic objectives described in Figure 2.

Drug	Number of papers[1]
Curcumin	7
Docetaxel, Resveratrol	5
Doxurrubicin, Etoposide	4
Quercitine	3
Dimetil fumarate, Nevirapine, Placitaxel	2

Note: 1- There are 34 more drugs appear in 34 different papers.

Table 2. Evaluation strategies for BBB crossing.

Type of study	Number of papers
In vitro studies	34
In vivo studies	35
In vivo + in vitro studies	10