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Brain safety concerns of nanomedicines: the need for a specific regulatory framework

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Teaser: mRNA–lipid nanoparticle vaccines have been authorized in recent months. These nanomedicines can undergo *in vivo* a phenomenon called protein corona, which could affect their biodistribution. Current regulations evaluating their brain toxicity are still limited.

There is growing interest in using nanomaterials as carriers for the delivery of drugs in diseases such as cancers and central nervous system (CNS) disorders. Although several nanomaterial-based products have been approved, the regulatory framework for their use in humans remains limited. Nanomedicines (NMs) are usually not designed to cross the blood–brain barrier (BBB). Given the lack of a comprehensive set of standardized methods to assess their *in vivo* fate, there is an urgent need to characterize NM biodistribution as well as the toxicity that could result from their interaction with the CNS. Here, we discuss the risks of potential unwanted BBB crossing and brain toxicity of nanocarriers (NCs), along with the safety assessment and current regulatory challenges related to NMs.

Introduction

The use of NCs in human medicine is part of NM vectorization strategies designed to facilitate the passage of diagnostic and therapeutic agents through biological structures such as the BBB.¹ NMs are usually formed via the encapsulation, trapping, or adsorption of molecules of interest within a lipidic, polymeric, or inorganic NC less than 100 nm in size.² NC surface functionalization, allowing tissue-specific drug delivery, can be used to target brain tumors, such as glioblastomas,³ or to treat neurological disorders, such as Alzheimer's disease or Parkinson's disease.⁴ Nevertheless, targeted drug delivery does not exclude that NMs might be directed toward regions of the body in which their localization is unwanted.^{5,6} This might be concerning for therapies or diagnostic agents not designed to be located in the brain, especially when they are intended to be used in vulnerable populations, such as children or older patients. Although NMs can be delivered through different routes, such as intranasal administration,⁷ they remain administered mainly intravenously. Therefore, NMs need to go through the BBB to access the brain. This barrier, which covers 99% of the surface of cerebral capillaries, acts as a selective filter blocking between 98% and 100% of micro- and macromolecules, respectively.^{8,9} NM biodistribution is influenced by various factors that also have a role in the passage of NMs through the BBB.^{10–12} Even though several NMs have already been approved, the *in vivo* fate and BBB interaction of these systems need to be further investigated, given the increasing and diversifying number of applications using NCs (Table 1).^{13–15}

Over the past few years, initiatives have been undertaken to provide specific evaluation criteria for this new class of drug. These initiatives have established initial methodological and regulatory frameworks, but these remain incomplete and mostly focused on lipid NCs.^{16,17} The importance of the distribution of NCs within the organism and the ability of these systems to interact with biological barriers, such as the BBB, require better characterization of both the mechanisms used to pass into the brain and the influence of biological environments on NC behavior.

Nanomedicines, nanocarriers, and the blood–brain barrier

The brain has a central role in the cognitive development of individuals, even though it accounts for only 2% of the total body mass.⁵ The passage of molecules from the bloodstream to the brain depends on three barriers: BBB, blood cerebrospinal fluid barrier (BCSFB); and blood–arachnoid barrier (BAB).¹⁸

Given that the BBB is the main barrier among the three, it explains why cerebral capillaries, which account for 85% of the vascular network of the brain, are mainly lined with BBB endothelial cells.^{8,18,19} These cells, which form an inner layer connected by tight junctions, are completed by an outer layer that includes a basement membrane, astrocytes, pericytes, and neurons.^{5,8,19} The BBB forms a selective barrier that limits the passage of molecules to the cerebral parenchyma. NMs can cross the BBB in different ways. In targeted vectorization, intracellular uptake depends mainly on receptor-mediated transcytosis.²⁰ For some inorganic NCs, such as gold nanoparticles (AuNPs), passive transcellular diffusion might also occur.²¹ In addition, inorganic NCs can facilitate paracellular diffusion transport by disrupting the tight junctions of endothelial cells.^{22,23} Paracellular diffusion is usually only present on

0.006% of the BBB surface.²⁴ The disorganized vascular architecture of tumors implies the existence of large fenestrations between endothelial cells.²⁵ This leaky vasculature allows NMs to cross the BBB through the openings between endothelial cells via a phenomenon known as the enhanced permeation and retention effect.^{25–27}

The ability of NMs to cross the BBB is influenced by the size, shape, and charge of the NC, as well as the functional groups on its surface.²⁸ Certain biological phenomena, such as the protein corona (PC), can also influence BBB crossing by NMs.²⁹ The adsorption of serum proteins at the NC surface, also known as the PC, can facilitate the passage of NMs through the BBB.³⁰ PC formation is a dynamic phenomenon that involves a change in the type of protein adsorbed at the NC surface, based on the circulation time and interaction with the immediate biological environment (Figure 1).^{31,32} The composition of the PC also varies with the size and surface charge of the NC.³² Table 2 provides a list of proteins that can be found in the PC. Indeed, some of these proteins might be common to several NC categories, whereas others can only be found in one type of NC.³³ Some of these proteins, such as human transferrin or apolipoproteins, are known to cross the BBB.^{34–36} Additionally, there may be preferential adsorption of proteins specialized in transport through the BBB, such as afamin, on certain categories of functional groups used for targeting.³³ In active transport, such as transferrin receptor-mediated endocytosis, different intracellular trafficking routes can be followed by NMs. They can either cross the entire BBB or be redirected toward different intra-endothelial compartments for further processing.^{37,38}

The potential diffusion of NMs in brain parenchyma should raise questions about the risks associated with the localization of NMs not intended for the CNS, given the large number of applications using NCs and which might involve vulnerable populations (Table 1).

Thus, it becomes obvious that, during the development of a NM [quality (Q) in manufacturing, preclinical safety (S) tests, and clinical efficacy (E) studies], the manufacturer must be able to combine different analytical methods to also study its NM in complex biological environments. It is similar to collecting Q/S/E data for the future Common Technical Document (CTD) in a real situation, without excessive approximations. Min *et al.* recently reviewed the tools used to explore the PC phenomenon observed with NMs, such as transmission electron microscopy (TEM), surface-enhanced Raman scattering, and small-angle X-rays scattering.³⁹

Brain safety concerns

The range of potentially detrimental effects on the brain caused by NMs reflects the diversity of structures used for their vectorization. Cationic lipid NPs (LNPs) are frequently used as drug delivery systems because they can enter cells more easily compared with anionic NCs.^{40,41} However, cationic LNPs are in general more toxic than anionic LNPs.⁴¹ Positively charged LNP are likely to increase brain vascular volume, to cause BBB damage, and potentially lead to the formation of cerebral edema.^{42,43} Some NMs, such as mRNA vaccines, contain optimized LNPs with an ionizable surface charge that is supposed to remain neutral at physiological pH.⁴⁴ However, it cannot be ruled out that this surface charge might change in individuals with conditions that can cause pH variations. The surface charge of nanocarriers has an influence on NM biodistribution, but biological phenomena, such as PC, can also influence their *in vivo* fate.^{29,45} Indeed, the PC can facilitate the passage of NMs through the BBB.⁴⁶ The nature of the PC can differ before and after NM passage through the BBB.⁴⁷ The evolution of the PC composition during transport through endothelial cells suggests that proteins that are not initially found in the cerebral parenchyma might enter and disrupt brain homeostasis.⁴⁷ In addition, some metabolic imbalances, such as altered autophagy or lysosomal activation, can be observed in endothelial cells or in the CNS after inorganic NC interactions with the BBB.⁴⁸ Autophagy is a mechanism that might be involved in the development of neurodegenerative diseases, such as Alzheimer's disease or Parkinson's disease.⁴⁹ Oxidative stress resulting from NPs has been identified as one possible mechanism for the disruption of autophagy.⁵⁰ Inorganic NCs used in therapeutics are often responsible for the production of reactive oxygen species involved in oxidative stress mechanisms.⁵⁰ For example, silver NPs can generate a significant oxidative stress, which can cause neurotoxicity.⁵¹ Thus, toxicity risks resulting from NM interactions with the BBB are not negligible. Given the lack of specific methods for investigating the central toxicity of NMs, especially *in vivo*, it is important that the regulation provide a standardized methodological framework, which will help to better characterize the behavior of these NMs in different biological environments.

Regulatory challenges

Since 1970, more than 600 applications for nanomaterials have been submitted to the US Food and Drug Administration (FDA), with an increase in submissions over the past 10 years.⁴⁰ Although several NMs are already on the market, there is still no specific regulatory framework for the study of their toxicity in human.^{52,53} The main challenge faced by regulatory agencies is the development of an appropriate classification that includes the variety of platforms used for the development of nanomaterials for preventive, therapeutic, and diagnostic purposes.⁵⁴

During the second half of the 2000s, international bodies, such as the FDA, the European Commission, and the Organisation for Economic Co-operation and Development (OECD), undertook initiatives to standardize the safety evaluation process of drug products containing nanomaterials. In particular, they issued several works that support the development of NMs.^{55–57} For example, under the NANoREG project, the Joint Research Centre from the European Commission proposed to manufacturers and regulators an overview of available tools (e.g., test methods and decision trees) for the safety assessment.⁵⁸ Among all documents made available by the FDA,⁵⁵ there is the guidance referring to 'drug products, including biological products, that contain nanomaterials'.¹⁷ As it notes, the biological fate of the NC and its potential impact on safety might need to be assessed in addition to those of the

active pharmaceutical ingredient because of a risk of increased crossing of biological barriers. Therefore, it becomes necessary to realize *in vivo* biodistribution studies using labeled nanomaterials in animals. Regarding clinical development, further nonbinding recommendations are listed, such as the evaluation of pharmacokinetic studies of the NC.¹⁷

Although these recommendations are not specific to the study of brain toxicity for brain- and nonbrain-targeting NMs, several insights can be derived from existing documents to identify potential impacts on brain structures. For example, a mass balance study of a drug substance labeled with a radioactive isotope in a liposomal formulation and in a nonliposomal formulation could be completed by confocal microscopy and TEM to assess qualitatively and quantitatively the distribution of NM at subcellular level.¹⁶ Likewise, in the case of drugs containing nanomaterials developed from a reference drug product, the use of single and multiple dose pharmacokinetic–pharmacodynamic studies for medium and high-risk categories should provide further indication of tolerability compared with a simple bioequivalence study.⁵⁹ This transposition of generic procedures into a particular setting helps to increase specificity, but there are still limitations.

Therefore, the applicability of traditional analytical methods to all NMs remains questionable. For example, although guidelines recommend the use of at least two different analytical techniques to characterize *in vitro* and *in vivo* NMs, there are still no conventional or alternative analytical techniques validated specifically for NMs. Thus, NM marketing authorization mainly relies on traditional evaluation methods that are not well suited to take into account the specific characteristics of NMs.⁶⁰ In addition, as previously reported, the PC confers singular properties to NMs. Current guidelines provide limited insights on how to assess NM behavior *in vivo*.

In July 2020, the FDA published a new report retracing the history of the progress as well as the commitments undertaken over the past two decades, but this document has answered questions on how to address the lack of specificity of NM toxicity assessment.⁴⁰ These elements underline the importance of quickly defining an appropriate regulatory framework able to respond to the safety challenges of NMs, especially with the arrival of NMs intended for preventive applications, such as mRNA vaccines.¹⁵ The efforts undertaken by the FDA, the European Union, and the OECD must lead to the standardization of the safety validation processes to reduce the use of a case-by-case approach.⁵²

Concluding remarks

The variety of approaches used in the development of NMs in human health complicates the implementation of a rational classification of these drugs and the elaboration of a normative framework that can respond to the issues related to their use in humans. This issue, combined with the lack of specific methods for evaluating the *in vivo* fate of NM, should encourage regulatory authorities to consider the challenges regarding the safety of products on the market or in development. The magnitude of the current Coronavirus 2019 (COVID-19) health crisis has prompted governments and industrials to quickly find solutions capable of curbing the spread of severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2). However, the main consequence of the global rush has been a dramatic shortening of preclinical and clinical development times as well as an increase in the use of emergency approval procedures (e.g., rolling review by the EMA and emergency use authorization request to the FDA).

Part of the response to stop the pandemic includes the use of vaccine categories for which LNP serve as drug delivery systems. Apart from the massive use of NMs in an unprecedented health crisis, the need to define the evaluation criteria specific to this new category of products is explained by the arrival of NMs designed for the prevention and treatment of vulnerable populations, such as infants or older patients, for whom the undesired location of NMs in the brain could have a significant clinical impact.

Declaration of interests

The authors have no conflicts of interest to declare.

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Figure 1. *In vivo* protein corona on nanoparticles. After administration, bare spheres can gather serum proteins and then forming nanoparticle-protein corona complexes in the blood stream. After crossing the blood–brain barrier (BBB), qualitative and quantitative changes of corona are observed. The evolution of the percentages of the four proteins after crossing the BBB was calculated from ⁴⁷.

Table 1. Example of authorized/approved NMs in Europe and the USA

NM	Company	Particle type/active moiety	Indication	MA (year of issue)	Refs
mRNA-1273	Moderna	LNP/mRNA	COVID-19	FDA ^a (2020); EU ^c (2020)	[15]
BNT162b2	Pfizer/BioNTech	LNP/mRNA	COVID-19	FDA ^a (2020); EU ^c (2020)	[15]
Patisiran; ALN-TTR02; ONPATTRO [®]	Alnylam Pharmaceuticals	LNP/RNAi	Transthyretin-mediated amyloidosis	FDA (2018); EU (2018)	[13]
CPX-351; VYXEOS [®]	Jazz Pharmaceuticals	Liposome/cytarabine:daunorubicin	Acute myeloid leukemia	FDA (2017); EU (2018)	[13]
Paclitaxel; ABRAXANE [®]	Celgene	Albumin/paclitaxel	SNCLC, breast cancer, pancreatic cancer	FDA (2005); EU (2008)	[13]
AmB; AmBisome [®]	Gilead Sciences	Liposome/AmB	Fungal infections, cryptococcal meningitis, VL	FDA (1997); France (1998) ^d	[13]
Doxorubicin; CAELYX [®]	Janssen	Liposome/doxorubicin	Ovarian cancer, HIV-associated Kaposi's sarcoma, MM	FDA (1995); EMA (1996)	[13]
NBTRX3 (radioenhancer); HENSIFY [®]	Nanobiotix	Phosphate coating/hafnium oxide	Soft-tissue Sarcomas	CE mark (2019)	[14]

^aAbbreviations: AmB, amphotericin B; MA, market authorization; MM, multiple myeloma; VL, visceral leishmaniasis.

^bThis medicine received an emergency use authorization.

^cThis medicine received a conditional marketing authorization (via a centralized procedure).

^dThis medicine was approved via a national procedure.

Table 2. Examples of proteins found in the PC^a

Proteins found in corona	Preferred NC fixation (when available) ^b	Refs
Serotransferrin	8D3-PLGA NPs (4), 8D3-PLGA-Drug (8), PLGA-Drug (15); 8D3-PLGA NPs (1), 8D3-PLGA-Drug (2), PLGA-Drug (4)	[33]
Serum albumin	Bare PLGA NPs (4); 8D3-PLGA-Drug (5), PLGA-Drug (13)	[47]
Apolipoprotein E	Bare PLGA NPs (19); bare PLGA NPs (2), PLGA-Drug (3)	[33]
Apolipoprotein A1	8D3-PLGA NPs (7), 8D3-PLGA-Drug (7); 8D3-PLGA-Drug (1), 8D3-PLGA NPs (2), PLGA-Drug (2)	[33]
Apolipoprotein B-100	Bare PLGA NPs (3); PLGA-Drug (10), bare PLGA NPs (12)	[33]
Prothrombin	8D3-PLGA-Drug (19), 8D3-PLGA NPs (18); PLGA-Drug (1), Bare PLGA NPs (1)	[47]
Complement C3	8D3-PLGA-Drug (3), 8D3-PLGA NPs (3); bare PLGA NPs (16)	[33]
Plasminogen	PLGA-Drug (6), 8D3-PLGA-Drug (10)	[47]
β -2-glycoprotein 1	Bare PLGA NPs (13); 8D3-PLGA NPs (na)	[47]
Afamin	na	[33]
Proteasome subunit beta type 5	na	[31]
Annexin A1	8D3-PLGA-Drug (16)	[31]
Alpha-2-macroglobulin	na	[33]
Ferritin light chain	na	[47]

^aAbbreviations: na, not available; 8D3-PLGA-Drug, anti-transferrin receptor antibody-poly(lactic-co-glycolic acid)-thiazolidinedione (as drug model).

^bNumbers refer to order of abundance: most abundant (1) to less abundant (20).

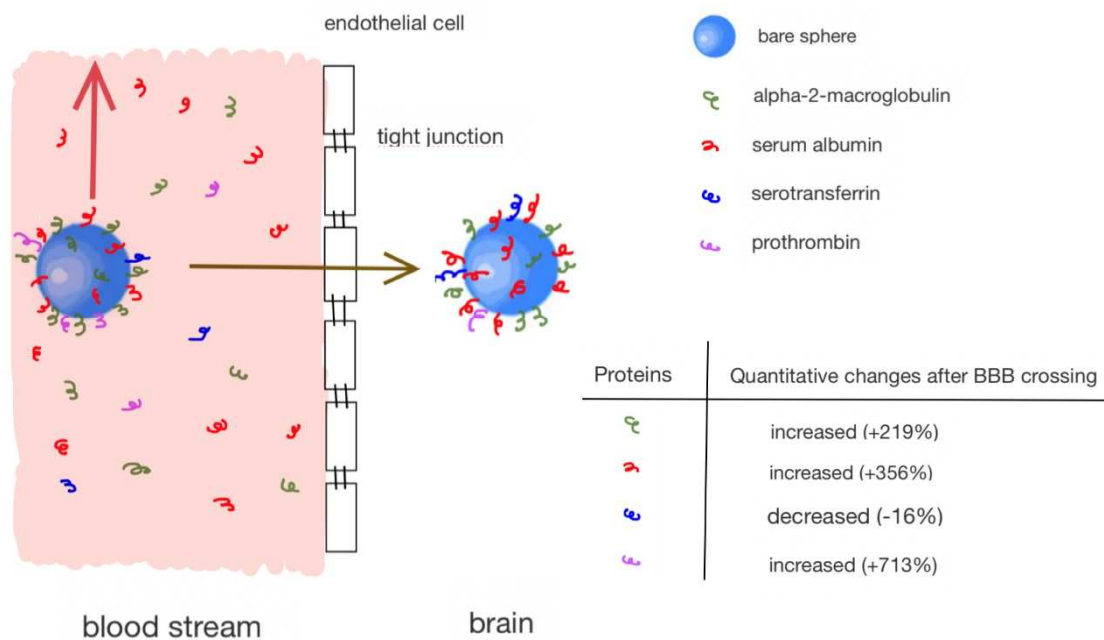


FIGURE 1

In vivo protein corona on nanoparticles. After administration, bare spheres can gather serum proteins and then forming nanoparticle-protein corona complexes in the blood stream. After BBB crossing, qualitative and quantitative changes of corona are observed. After the passage of the BBB, the evolution of the percentages of the four proteins was calculated from the reference [47].