

**The complexity of the blood-brain barrier and the concept of age-related brain targeting: challenges and potential of novel solid lipid-based formulations**

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## **ABSTRACT**

Diseases that affect the Central Nervous System (CNS) are one of the most exciting challenges of recent years, as they are ubiquitous and affect all ages. Although these disorders show different etiologies, all treatments share the same difficulty represented by the Blood-Brain Barrier (BBB). This barrier acts as a protective system of the delicate cerebral microenvironment, isolating it and making extremely arduous delivering drugs to the brain. To overtake the obstacles provided by the BBB it is essential to explore the changes that affect it, to understand how to exploit these findings in the study and design of innovative brain targeted formulations. Interestingly, the concept of age-related targeting could prove to be a winning choice, as it allows to consider the type of treatment according to the different needs and peculiarities depending on the disease and the age of onset. In this review was considered the prospective contribution of lipid-based formulations, namely Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs), which have been highlighted as able to overcome some limitations of other innovative approaches, thus representing a promising strategy for the non-invasive specific treatment of CNS-related diseases.

## INTRODUCTION

Diseases afflicting the Central Nervous System (CNS) represent a major challenge for medical and pharmaceutical research. In parallel to the neurological disorders that arise with aging, there are a considerable number of disorders that are mostly juvenile, such as traumatic brain injury (TBI), which is identified as one of the major pediatric public health problems. In fact, TBI can have a variable degree of severity in the vulnerable and delicate brain of children, and in mild cases, a restorative treatment of brain physiological condition is required.<sup>1</sup>

The treatment of these pathologies is really challenging to implement with the classical methods, because the presence of the blood-brain barrier (BBB) makes it almost impossible for the drugs administered to reach a therapeutically active dose into the brain. In addition, the use of more invasive methods, such as direct administration of medication by intrathecal injection, is not practicable in all those situations that require a continuous and prolonged treatment over time. Thus, there is the need of innovative non-invasive formulations capable of conveying the drug to the CNS in a safe and controlled way.<sup>2</sup>

Furthermore, it is imperative to focus attention on the concept of age-related targeting: not only it is necessary to obtain innovative formulations able to cross the BBB, but these must be specially structured according to the peculiarities and needs of the target tissue, which can vary depending on the age and the pathological condition affecting it.

In fact, it is reasonable to think that the BBB undergoes changes in these situations, thus highlighting the need to study these phenomena in order to understand how to exploit these pathophysiological characteristics to obtain a targeting as focused and specific as possible for the pathology treated.

This review is structured to provide an overview of the BBB, both in health and disease, considering that it represents one of the most significant obstacles to drug delivery in the CNS. In particular, the BBB will be described in its anatomical-physio pathological features according to the different biological characteristics of the subjects involved, to identify a system of targeted administration of the drug, able to cross the BBB without giving a direct action on biological collateral structures.

It explores some of the most significant pathological changes associated with acute and chronic disorders that may affect the CNS from childhood to old age. This insight is essential to understand how to exploit these differences to obtain more targeted and selective formulations based on the need or deficiency of the tissue under investigation.

The latest part of this review is focused on the great contribution that pharmaceutical technology could provide for the above mentioned problems. Specifically, the attention has been focused on Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs), which are promising lipid-based formulations. From what has been shown in the literature, SLNs and NLCs have been particularly studied in recent years as they offer a number of advantages over the innovative non-invasive formulations of the previous generation. In fact, they are less toxic than polymeric nanoparticles, biodegradable, biocompatible and non-immunogenic. Moreover, given their small size, they are able to penetrate into the brain via BBB passive diffusion, allowing the encapsulated drug to reach easily the site of action, to be protected from degradation and to be released slowly over time. In addition, it is possible to functionalize the surface of the carrier, thus obtaining an active targeting system; therefore, this paper provides a more detailed overview of the advantages and disadvantages associated with the use of these formulations and their potential use in the treatment of age-related diseases.<sup>3</sup>

### 1. THE BBB: CONCEPT AND FUNCTIONS

The CNS represents the control center of the human body and it is able to manage all the stimuli arriving to the organism, both those internal ones originated by the organs and those determined by the external environment, generating appropriate responses.<sup>2</sup>

All of these mechanisms are made feasible because, inside the CNS, the “neural” cells and the spinal cord maintain a close communication due to the presence of chemical and electrical signals. These signals involve the displacement of small molecules such as neurotransmitters and modulators, and a constant flow and concentration of ions for the genesis and maintenance of synaptic and neuronal potentials.<sup>4</sup>

In order to achieve a precise and reproducible signaling among the nervous cells, it is fundamental that the composition of the CNS internal microenvironment remains finely controlled and protected by the blood flow rich in ions, peptides, xenobiotics which could alter this fragile balance.<sup>4</sup> More specifically, there are neural cells, such as neurons, astrocytes, oligodendrocytes and microglia cells, that are actively involved in the homeostatic maintenance of the interstitial fluid, while cell barriers at the interfaces between blood circulation and CNS are responsible for regulating the flux of substances into and out.<sup>5</sup>

This delicate control is due to the presence of three physical barriers opposing molecular flux: the endothelium of the brain microvessels (BBB) between blood and the brain interstitial fluid (ISF), the choroid plexus epithelium between blood and ventricular cerebro-spinal fluid (CSF), and the arachnoid epithelium between blood and subarachnoid CSF.<sup>6</sup> The choroid plexus and arachnoid form the blood-CSF barrier (BCSFB).<sup>5</sup> Despite the presence of various barriers, the BBB represents the one with the greatest control over molecular traffic and homeostatic maintenance of cerebral environment.<sup>7</sup>

In the BBB, the presence of tight junctions throughout all cellular endothelium makes molecular trafficking very difficult, due to the absence of fenestrations and low occurrence of pinocytic activity,<sup>8,9</sup> while for what concerns the capillaries of the choroid plexus, these allow the movement of substances via intracellular gap and fenestrations.<sup>10</sup> Although the presence of tight junctions on the apical side of the endothelium, these are more permeable than those on BBB, making it less difficult solutes entry.<sup>11</sup>

Foremost, the BBB exists as a selective diffusion barrier at the level of cerebral microvascular endothelium;<sup>12</sup> the tight junctions between adjacent cells prevent free movement of polar solutes via paracellular pathways, but the presence of solute carriers on the apical and basal membranes allows small molecules entry and efflux. Large molecules such as peptides and protein can be carried in and out via mechanism of adsorptive and receptor-mediated transcytosis.<sup>2</sup>

The BBB performs a set of essential functions for the physiological condition of the CNS; it supplies brain with nutrients from blood and regulates the clearance of waste products deriving from metabolism of brain<sup>7</sup> and, by keeping toxins out, it guarantees the protection of neurons and neural network connectivity.<sup>2</sup>

The BBB regulates the production of the ISF, which is the extracellular fluid that fills the “interstices” of tissues and bathing cells;<sup>7</sup> to finely regulate its composition, the BBB limits fluids flow from blood while allowing ions movement due to the presence of ionic channels and specific transporters.<sup>6</sup> Although the ISF's composition seems very similar to plasma, it is necessary for it to maintain a lower  $Ca^{2+}$  and  $K^+$  concentration but a higher  $Mg^{2+}$  level to preserve synaptic and axonal signalling.<sup>13</sup>

Moreover, this barrier restricts protein entry to limit brain's innate immune response and controls the presence of leukocytes allowing immune surveillance with minimal inflammatory and cell damage. Finally, the BBB acts by separating central and peripheral neurotransmitter pools by reducing “cross-talk” interference between signaling networks that use the same agents.<sup>2,14,15</sup>

Thus, this interface endothelium layer acts as physical, transport, metabolic and immunological barrier; the BBB (Fig.1) is a dynamic structure, able to manage any impulse it receives adequately.<sup>2</sup> This plasticity is the result of the combined work of cerebral endothelium and the Neurovascular Unit (NVU), which is a complex cellular system modulating and supporting BBB's activity.<sup>16</sup>

(INSERT FIGURE 1)

## 1.1 The neurovascular unit (NVU)

As mentioned above, the BBB is a dynamic entity able to supervise brain condition; this goal is achieved due to the activity of various cell types that collaborate in close contact forming the NVU<sup>4</sup> (Fig.2). It has been defined as a complex formed by endothelium, neurons, astrocytes, pericytes, basal lamina and extracellular matrix.<sup>17</sup>

The definition of NVU is constantly evolving, and it has recently been extended to include macrophages and microglia involved in immune responses and circulating inflammatory cells for their role in immune surveillance of CNS;<sup>18</sup> in addition, the importance of brain endothelial luminal surface glycocalyx in the leukocyte-endothelial interaction has been clarified by “prefiltering” the access to endothelial membrane.<sup>19</sup>

Although the role of each component is not yet fully defined,<sup>20</sup> it is clear that the effectiveness shown by the NVU is the result of the intimacy and the close connection existing among all cell types composing it.<sup>2,21</sup>

### a. Neurons

The neuron may be considered as the “pacemaker” of the NVU;<sup>22,23</sup> in fact, due to its stringent metabolic needs, it is capable of reacting very quickly to the lack of nutrients and oxygen, converting these stimuli into chemical and electrical messages which modulate the activity of adjacent cells to restore physiological condition.<sup>12,24</sup>

In case of need neurons can communicate with vessels through astrocytes, affecting a change in vascular tone and blood supply in that area:<sup>16</sup> supporting this, there are anatomical evidence that show noradrenergic,<sup>25,26</sup> serotonergic,<sup>27</sup> cholinergic<sup>28</sup> and GABAergic neurons<sup>12,29</sup> directly innervating microvascular endothelial cells and/or astrocytic processes linked with.

### b. Astrocytes

In the past years it was believed that astrocytes played a secondary role only supporting endothelium activity, later it was discovered that they are in close communication with neurons and blood vessels being actively involved in the NVU.<sup>30,31</sup>

Anatomically this connection is due to the astrocyte end foot, which is a cell extension in contact with pericytes, myocytes and endothelium;<sup>32</sup> equally important is the syncytial organization of astrocytes as well as neurons. In this structure, cells are strictly united by gap junctions and functionally via calcium waves<sup>33,34</sup> and this also allows the propagation of electrical stimuli over long distances, recruiting other syncytia.<sup>35</sup>

Moreover, the astrocytes take part in chemical signaling producing substances such as NO, prostaglandins and ATP which occur in vasal tone modulation.<sup>36</sup>

### c. Pericytes

Pericytes are in close contact with endothelial cells and communicate with them promoting their maturation and development. Less is known about pericytes, and until recently they were considered as supporting cells, such as astrocytes.<sup>37,38</sup>

Evidences have shown that pericytes possess contractile proteins and it has been suggested that they may intervene in blood flow modulation given their proximity to brain capillaries.<sup>39</sup> In addition, pericytes seem to play key role in cerebral angiogenesis.<sup>38</sup>

### d. Extracellular matrix

The extracellular matrix contributes to the maintenance of what are the unique properties of the BBB along with neurons, astrocytes and pericytes. The most important function carried out by matrix is the anchoring of endothelial cells via interaction of laminin and other matrix proteins with endothelial integrin receptors,<sup>40</sup> although it influences the expression of tight junction proteins involved in the maintenance of BBB's integrity.<sup>41,42</sup>

(INSERT FIGURE 2)

## 1.2 BBB junctional complex

The junctional complex ensuring the functionality of the BBB is formed by tight junctions (TJs) and adherens junctions (AJs). Remarkably, there is an intimate connection between them in order to guarantee membrane integrity.<sup>43,44</sup>

Gap junctions also have been found in brain, although their function has not been clarified yet.<sup>45</sup>

### a. *Tight junctions*

The seal formed by TJs is due to a complex network of transmembrane proteins and cytoplasmic assessor proteins that allow to bind adjacent endothelial cells in depth linking actin-based cytoskeleton. The main transmembrane proteins making up TJs are claudins, occludins and junctional adhesional molecules (JAMs).<sup>43</sup>

Claudins are small proteins about 20-24 kDa, composed by four extramembrane domain and extracellular loops;<sup>46</sup> these proteins form the primary seal of tight junctions as they are able to form dimers with other claudins in adjacent cells.<sup>43,47</sup>

Occludin is an integral membrane protein bigger than claudin, about 60-65 kDa, and it has four transmembrane domains with both carboxy and amino terminus intracellularly; it has also two extracellular loops.<sup>48</sup> Likely occludin may increase the resistance across BBB and contribute to the formation of aqueous pore modulating the non-charged solutes flow.<sup>49</sup> In addition, the occludin cytoplasmic carboxy terminal domain is able to link cytoskeleton via guanylyl-kinase domains of accessory proteins such as zonula occludens-1, zonula occludens-2 and zonula occludens-3.<sup>50-52</sup>

At last, there are the JAMs, these proteins belonged to the immunoglobulin superfamily and have a single transmembrane domain with an extracellular part with two loops IgG-like. JAMs intervene in cell-cell adhesion and may regulate permeability and monocyte movement.<sup>53,54</sup>

Other cytoplasmic accessory proteins occur in TJ; among these, Zona occludens-1 (ZO-1), ZO-2 and ZO-3 belong to the MAGUK family (membrane-associated guanylate kinase proteins) and have been identified coupling transmembrane proteins to cytoskeleton actin, as mentioned above, and forming submembranous plaque of tight junctions.<sup>55</sup>

### b. *Adherens junctions*

AJs constitute a fundamental part of junctional complex in BBB; these are characterized by transmembrane glycoproteins that belong to the superfamily of cadherin, which are Ca<sup>2+</sup>-dependent receptors forming complexes with neighboring cells.<sup>56</sup> AJs intervene in forming a continuous "belt" that assures anchoring of adjacent cells.<sup>43</sup>

## 1.3 Enzymatic BBB

In addition, as mentioned above, the BBB not only acts as a physical passive barrier, but also performs a metabolic function; in fact, endothelial cells express a number of ectoenzymes, such as aminopeptidases, endopeptidases and cholinesterase, which work chemically modifying exogenous and endogenous substances, in order to prevent them reaching the sensitive brain compartment.<sup>57</sup>

## 2. HOW MOLECULES CROSS THE BBB UNDER PHYSIOLOGICAL CONDITIONS

The presence of the BBB prevents most molecules from reaching the brain, but there are transport mechanisms that allow the passage of substances required for nourishment and metabolism of nerve cells.<sup>58</sup>

The following is a brief outline of how trafficking of molecules occurs physiologically across the barrier. How some endogenous transporters might be exploited for CNS targeting will be explored next (Fig.3).

### 2.1 Passive diffusion

Small water-soluble molecules cross the membrane via paracellular pathway, while small lipid-soluble agent diffuse through transcellular routes.<sup>59</sup>

There are several factors influencing and limiting the spread of agents into CNS; in fact, a correlation has been found between the logP (octanol/aqueous buffer partition at pH 7.4), the logarithm of the partition coefficient (P) of a compound, and the permeability of the barrier:<sup>60</sup> for CNS permeation a logP about 1-3 is recommended.<sup>61</sup> Moreover, to permeate the brain it is important that Polar Surface Area (PSA) and Molecular Weight (MW) remain below definite limits, 60-70 Å<sup>2</sup> and 450 Da respectively.<sup>61,62</sup>

Compounds which carry a positive charge interact with the negatively charged glycocalyx and phospholipid head groups on the surfaces of cell membrane and this interaction assists their entry. On the other hand, the negatively charged bicarbonate ion diffuses poorly passively. At last, gaseous molecules such as O<sub>2</sub> and CO<sub>2</sub> are able to move passively according to their concentration gradients.<sup>5</sup>

### 2.2 Carrier-mediated transport

The presence of TJs protects the CNS from any kind of agent that could alter its sensitive microenvironment but, at the same time, TJs isolate brain cells from several polar solutes required for their sustenance. Thus, endothelial cells are provided with specific solute carriers to supply the brain with all the necessary compounds, such as glucose, amino acids, peptides, vitamins, nucleosides and others.<sup>5,59</sup>

These carriers can be bidirectional, and in this case the direction is guided by the concentration gradient of the substance, unidirectional transport can take place either inwards or outwards, or the direction can be reversible when the movement depend on the exchange of the substrate with another or with an ion.<sup>5,63</sup>

*a. Glucose.* Glucose is one of the main sources of energy for the CNS, so it is crucial that brain cells are supplied with it constantly. Its most important carrier is GLUT1, belonging to the family of sodium-independent glucose transporters, able to transport glucose and other hexoses across the BBB. GLUT1 is expressed both on the luminal and abluminal membrane of endothelial cells,<sup>64</sup> although its density is higher at the abluminal side. This asymmetrical arrangement allows homeostatic control of glucose flow and prevents an accumulation in the ISF higher than in blood.<sup>65,66</sup>

*b. Amino acids.* For amino acids, there are also specific carriers asymmetrically located on both the luminal and abluminal surface of the cells. It is essential that amino acids, despite being polar solutes, reach the CNS as several of these cannot be synthesized by tissues but they must be introduced by diet.<sup>64</sup> In addition, adequate amino acids supply is critical, as some are precursors to important neurotransmitters such as serotonin, dopamine, histamine.<sup>67,68</sup>

Several carriers deal with amino acids flux; for instance, system L transport large neutral amino acids with hydrophobic branched or aromatic side chains,<sup>69</sup> system y<sup>+</sup> carries cationic amino acids arginine, lysine and ornithine.<sup>70</sup> Both these systems are Na<sup>+</sup>-independent transporters expressed at high level in both the luminal and abluminal cell membranes.<sup>64</sup>

However, systems A, B<sup>0,+</sup>, ASC, X<sub>AG</sub><sup>-</sup> and β are Na<sup>+</sup>-dependent carriers;<sup>64</sup> both systems A and ACS transport neutral amino acids and are located predominantly on the abluminal membrane.<sup>71-75</sup> System B<sup>0,+</sup> deals with

neutral and basic amino acids,<sup>76</sup> while system  $\beta$  carries  $\beta$ -alanine and taurine,<sup>77</sup> both in the luminal and abluminal cell membranes.

At last, the anionic amino acids are transported by system  $X_{AG}^-$ , also in this case located on both sides of endothelial cells.<sup>78</sup>

*c. Nucleotides, nucleosides, nucleobases.* Adult mammalian brain cells do not have a significant capacity to produce nucleotides required for the synthesis of DNA and RNA, so there are specific carriers able to transport these precursors from blood to brain.<sup>79</sup> They are divided into two classes: CNT, concentrative nucleoside transporters, and ENT, equilibrative nucleoside transporters.<sup>80</sup>

*d. Monocarboxylic acids.* For monocarboxylic acids and ketone bodies, useful for the brain as metabolic substrates, there is a family of carriers named MCT; these transporters are proton coupled and bi-directional.<sup>81</sup>

*e. Organic ions.* Finally, the carriers specialized in organic ions transport, belonging to OAT family (organic anion transporters). All these carriers seem to carry para-aminohippuric acid.<sup>82</sup> In addition, there are specific transporters, called OATP, for a wide class of negatively charged molecules at physiological pH, including bile salts, thyroid hormones, steroid conjugates, small peptides and peptidomimetics.<sup>83-87</sup>

In the case of organic cations have been described two types of carriers, OCT (organic cation transporters)<sup>83</sup> and OCTN, in which the "N" means "novel".<sup>88-94</sup> A summary is given in Table1.

## 2.3 Vesicular transport

Large molecular weight solutes can cross the BBB via vesicular transcytosis; this process begins as an invagination of endothelial cell membrane assembling a caveola. The caveola detaches forming a free vesicle and migrates inside the cell to the membrane on the opposite side, with which it melts releasing its contents into the peri-endothelial basal lamina.<sup>64</sup>

The vesicular transports involve both RMT, Receptor-Mediated Transcytosis and AMT, Adsorptive-Mediated Transcytosis.<sup>5</sup>

*a. RMT.* Receptor-Mediated Transcytosis is triggered by the binding between a macromolecular ligand and its specific receptor situated on the cell surface; ligand-receptor complex is internalized into the endothelial cell and transported across the cytoplasm to the opposite pole. Likely ligand-receptor detachment occurs during transit or exocytosis.<sup>95</sup>

Several proteins required by the CNS use this transport mechanism, such as transferrin,<sup>96</sup> low-density lipoprotein,<sup>97</sup> insulin, insulin-like growth factor,<sup>57</sup> Immunoglobulin G.<sup>98</sup>

Two of the well-known processes are insulin- and transferrin-receptor-mediated transcytosis; both these mechanisms have been exploited to enhance drug permeation across the BBB,<sup>8</sup> and it will be discussed later.

*b. AMT.* Adsorptive-Mediated Transcytosis begins when a cationic macromolecule interacts with cell surface charged negatively and this interaction triggers the transcytosis process. For instance, albumin transcytosis is due to an electrostatic interaction generated between the cationized protein and sialic acid moieties on the luminal/ heparin sulfate groups on the abluminal surface of endothelial cells.<sup>99</sup>

Table2 provides a summary of mentioned mechanisms.<sup>100-107</sup>

(INSERT TABLE 1)



(INSERT TABLE 2)

## 2.4 ABC Transporters

Contrary to expectations several compounds with a logD appropriate to cross the BBB fail to reach a suitable concentration in the CNS due to the presence of efflux systems led by ABC (ATP-Binding-Cassette) transporters.<sup>108</sup>

In humans, 48 members belong to this family, which is divided into 7 subfamilies, A to G;<sup>109</sup> the ABC acronym derives from their function, in fact these transporters exploit the energy derived from ATP hydrolysis to move their substrates against concentration gradient.<sup>110</sup> The role of these transporters allows the removal of xenobiotics and neurotoxic endogenous substances from the CNS,<sup>111</sup> although many drugs undergo this efflux mechanism and thus are removed from the brain preventing them from performing their therapeutic activity.<sup>108</sup>

P-glycoprotein (P-gP, Multidrug Resistance Protein, ABCB1), Multidrug Resistance-associated Proteins (MRPs, ABCC1, 2,4,5) and Breast Cancer Resistance Protein (BRCP, ABCG2) are the main transporters playing a key role on the BBB.<sup>108,112</sup>

(INSERT FIGURE 3)

## 3. BBB IN CHILDHOOD AND AGING BRAIN: THE CONCEPT OF AGE-RELATED BRAIN TARGETING

Diseases affecting CNS are a major challenge for scientists around the world; in fact, in addition to related problems of overcoming the BBB, another point not to be underestimated deals with changes in the anatomic-physiological brain structure due to the pathology and/or aging process.

In the following section will be proposed an overview of the most common CNS-related diseases classifying them according to the age of incidence; this approach is functional to highlight the different therapeutic needs related to the type of pathology and/or the incidence time of the same.

### 3.1 Childhood acute and chronic brain diseases

#### 3.1.1 Traumatic Brain Injury.

TBI is a traumatic condition with a higher incidence in children and young adults, of which is one of the main causes of disability and/or death, although it may occur even in elderly individuals.<sup>113,114</sup> This lesion can be due to a non-penetrating blow that leads to the formation of an intracranial bruise, or to a lacerating blow that involves a physical destruction of the BBB.<sup>113</sup> TBI consists of a primary injury and a secondary injury mechanism;<sup>115</sup> primary injury is due to the mechanical trauma affecting brain structures, such as neurons, axons, blood vessels and glia, while secondary injury mechanism consists of several neurochemical events which stimulate the production of pro-inflammatory mediators such as prostaglandins, oxidative metabolites, chemokines, pro-inflammatory cytokines, resulting in the disruption of BBB.<sup>116</sup>

Thus, besides the primary injury mechanism, damage-associated molecular patterns (DAMPs) and biochemical derangements occur, leading cell death and the release of other DAMPs in a positive feedback mechanism that perpetrates over time;<sup>117,118</sup> the resulting neuroinflammation seems to be the predisposing factor to neurodegeneration.<sup>119</sup>

The BBB is massively affected by TBI; in fact, imaging studies have shown that immediately after traumatic brain injury there is the destruction of tight junctions, flattening of vascular smooth muscles, swelling of cells and subsequent narrowing of vasal lumen.<sup>120,121</sup> Subsequently, 3-7 days after the primary BBB's rupture, neuroinflammation occurs as secondary pathogenic mechanism which can last even for a long time;<sup>122</sup> trauma consequences diversify case to case and are related to the severity of the damage suffered.<sup>123</sup>

Interestingly, in animal models is possible to observe that there is a considerable increase in BBB permeability to albumin and other high-molecular weight molecules with a biphasic course that is about 4-6 hours and again 2-3 days after TBI.<sup>124-127</sup> This increase in endothelium permeability is due to both the mechanical damage suffered and the tight junction proteins change in expression and localization, within a higher pinocytic activity.<sup>128,129</sup>

However, the BBB breakdown provides a huge opportunity to passively direct modified delivery system to brain, in order to convey neuroprotective and restorative agents for the CNS itself.<sup>113</sup>

### 3.1.2 *Pediatric brain tumors.*

Pediatric brain tumors are the third most frequently occurring type of cancer in childhood and they represent a significant cause of death in children (besides traumatic injury).<sup>130</sup> Astrocytoma, medulloblastoma, ependymoma and brain stem glioma are the most frequent form of cancer in children,<sup>131,132</sup> although metastatic lesions are less common than in adults and approximately 50% of childhood brain tumors are benign.<sup>133</sup> Standard treatments for these diseases include surgery, radiotherapy and chemotherapy, even more the latter does not always lead to an improvement in the patient's condition.<sup>134-136</sup> Sometimes the therapy failure is related to the impossibility of chemotherapeutic agents to reach a proper concentration into the tumor tissue, due to the presence of the BBB which limits drugs enter into the CNS and prevents their accumulation due to the efflux systems.<sup>137,138</sup>

It has been shown that the BBB, in some cases, loses its integrity and this may allow the passage of drugs, such as large chemotherapeutic molecules or nanocarriers, normally excluded from the CNS. However, the issue appears to be more complex; in fact, it has been clarified that the cerebral capillary endothelium bearing tumor may be continuous and non-fenestrated, continuous and fenestrated, discontinuous and/or fenestrated/non-fenestrated depending on the type of brain lesion.<sup>130,139</sup> Therefore, the BBB could be formed by heterogeneous areas lacking integrity in close proximity to perfectly intact areas.<sup>138,140,141</sup> From this evidence, it is clear that childhood brain tumors represent an attractive field of application for innovative formulations: in parallel to the integrity loss of the BBB, which would enable the *in situ* passage of nanocarriers by simple passive diffusion, there is the possibility of functionalizing the nanosystems themselves by directing them towards a specific target. This type of therapeutic approach might allow to decrease the invasiveness of the treatment, leading to a significant improvement of the patient's condition.

### 3.1.3 *Epilepsy and seizures.*

Epilepsy is a serious neurological condition affecting with higher incidence in the first decade of life, both as genetic epilepsy and epilepsy associated with developmental disorders. Although childhood epilepsy is more likely to go into remission than in adults, it is very difficult to administer anti-epileptic drugs to children due to their faster clearance than in adults.<sup>142,143</sup>

One of the problems related with anti-epileptic therapy is the drug resistance; it afflicts about 30% of the patients and reduces the effectiveness of the therapy. Several studies have been carried out to highlight the BBB involvement in this phenomenon.

Drug resistance could be explained by two theories: the target hypothesis, according to which pharmacoresistance is due to a molecular target changes led by anti-epileptic drugs themselves, and the transporter hypothesis.<sup>144</sup>

According to this theory, it has been suggested that the drug resistance could be related to the changes in drug efflux transporters such as the overexpression of P-glycoprotein (MDR1).<sup>145-147</sup>

Moreover, it has been suggested that epilepsy and seizures could damage and weaken the BBB, and vice versa a compromised BBB could trigger seizures.<sup>144</sup> In addition, other studies have supposed that BBB metabolic defects, such as GLUT1 deficiency syndrome could induce a lack of brain glucose transport, and this could lead to seizures.<sup>148,149</sup>

### 3.1.4 *Childhood neurodegenerative disorders.*

Childhood neurodegenerative disorders represent a heterogeneous class of diseases, which can be classified as follow: disorders involving subcellular organelles, disorders of intermediary metabolism, disorders of metals metabolism, leukodystrophies and genetic inherited disorders.<sup>150-152</sup>

Under normal conditions, a healthy CNS undergoes an immune control by glia resting cells (innate immunity) and lymphocytes (adaptive immunity).<sup>153</sup> Both systems operate an immune surveillance that does not compromise BBB integrity; although in neurodegenerative conditions it has been shown that the localized glial activity triggers the neuroinflammation which may promote BBB alterations.<sup>154,155</sup>

## 3.2 Acute and chronic diseases in aging brain

### 3.2.1 *Brain tumor.*

Glioma is the most common brain tumor in adults, in fact it accounts for 80 % of the cancers affecting the brain;<sup>156</sup> according to the American Brain Tumor Association, glioma means any type of tumor that originated from glial cells.<sup>157</sup>

In addition, several malignant tumors such as lung, breast, and colon cancer lead to the formation of metastatic lesions in brain;<sup>158</sup> in the case of melanoma, brain metastases occur in 55% of patients.<sup>159</sup>

Interestingly, although it is widely known that brain tumors could alter the BBB organization, it should be clarified that these alterations vary depending on the type of brain neoplasm, not necessarily in relation to the tumor size, shape and localization.<sup>160</sup>

As in the case of pediatric brain tumors, even in adult age it has been underlined that BBB exhibits an area of increased permeability in the core of malignant lesion, whereas the surrounding brain tissue generally presents a good vascularity and a variable degree of integrity, allowing the passage of molecules and/or nanovectors passively.<sup>138,161</sup>

More specifically, several brain tumors are associated with the production of growth factors, vasoactive cytokines and pro-inflammatory mediators<sup>162,163</sup> which destroy the tight junctions between endothelial cells generating BBB disruption, vasogenic edema and hemorrhagic phenomena.<sup>164</sup>

Primary malignant tumors and brain metastases consist of blood vessels whose junctions are particularly weak and no longer expressing healthy BBB transport systems.<sup>165</sup>

In fact, it has been shown that post-mortem the expression of the glucose GLUT-1 carrier into brain tumor tissue microcirculation is inversely related to the malignancy of the tumor itself.<sup>166</sup>

Although Glut1 is normally expressed at vessels adjacent to the tumor and secondary lesions, it is missing into the endothelial cells inside tumor tissue and its metastasis; this evidence has highlighted that the lack of glucose transporters is a feature of the tumor tissue itself and this condition provides important information on the use of such carrier as a mean for an active drug delivery to the brain.<sup>166-168</sup>

### 3.2.2 *Stroke.*

Cerebral ischemia is an acute condition that occurs when the flow of blood, oxygen and nutrients to the brain is interrupted,<sup>169</sup> and it is associated with an increasing in microvascular permeability.<sup>162,170</sup>

Several studies have shown that ischemia/reperfusion phenomena lead to the loss of BBB integrity due to the TJs opening and this increases permeability.<sup>171-173</sup> Specifically, this process takes place with a double effect; first there is a rapid BBB opening, followed by a refractory period and a prolonged reopening that can remain hours or days. During this phase plasma substances have free access to CNS.<sup>174-178</sup>

### 3.2.3 *Neurodegenerative disorders.*

The neurodegenerative process, namely the progressive loss of neuronal structure and function leading to nerve cells death, is closely linked to age-related disease.<sup>179</sup>

*a. Alzheimer's disease.* Alzheimer's dementia is the most common type of dementia widespread in the world.<sup>180</sup> Both aging and Alzheimer's disease (AD) lead to several BBB changes in the neurovascular unit and endothelial cells. These alterations involve a decrease in endothelial mitochondria density, an increase in pinocytotic activity, loss of tight junctions, storage of extracellular matrix components into the basal lamina, thickening of vessel walls with decreasing elasticity and changes in astrocytic end feet.<sup>181,182</sup>

More specifically several BBB alterations could be related to AD onset; these changes affect cellular components of neurovascular unit and several transporters.

Evidences have shown a link between AD and TJs functioning; in fact, occludin seems to be susceptible to matrix metalloproteinases (MPP) attack which are related to disease development.<sup>183,184</sup> Moreover, the connection of AJs and TJs to actin cytoskeleton appears to be affected by tau protein.<sup>185</sup> Astrocytes and pericytes are also influenced by AD induced modifications, in fact it has been noted an abnormal astrocytic activity related to vascular instability,<sup>186</sup> while as far as pericytes are concerned, they are able to accumulate within themselves amyloid- $\beta$  peptides, and their resulting dysfunction would appear to be connected to the clearance of such deposits.<sup>187,188</sup> In addition, as mentioned above, some transporters undergo changes induced by AD; for instance, it has been observed that the brain transport of glucose is affected by this disease. In fact, the expression of GLUT1 transporter within brain capillaries in patients with AD is greatly reduced, although there is no alteration in protein mRNA structure<sup>189</sup> or transcription,<sup>190</sup> rather a reduction in BBB surface area available for glucose exchange.<sup>191</sup>

Among various transporters based on BBB, low density lipoprotein receptor-related protein 1 (LRP1) and transferrin receptor (TfR) have been identified as potential drug delivery targets.<sup>192</sup> LRP1's expression is compromised in AD,<sup>181</sup> while, intriguingly, it has been shown that both in aging process and development of Alzheimer's disease, the levels of TfR and the TfR-mediated uptake and internalization process are not compromised. The experimental evidence provided have shown that the mechanisms TfR-related do not undergo changes by AD neuropathology, highlighting TfR real potential in brain delivery innovative approach.<sup>193</sup>

*b. Parkinson's disease.* Possible dysfunction of the BBB and/or blood-CSF-barrier has also been noted for Parkinson's disease (PD) during its progression.<sup>194</sup> It appears to be an increase in BBB permeability in PD patients using histological markers of serum protein, iron and erythrocyte;<sup>195</sup> however, despite this, many aspects about Parkinson's disease on barrier remain to be clarified and the actual effect has not yet been fully explored.

*c. Multiple Sclerosis (MS) and Amyotrophic Lateral Sclerosis (ALS).* MS is a chronic disease that involves an abnormal attack performed by the immune system to several CNS components, such as CNS myelin, resulting in progressive loss of motor and sensitive functions.<sup>178</sup> Post-mortem histological analysis revealed a loss of TJs in brain areas affected by active lesions induced by MS, resulting in an increased barrier permeability with massive amounts of cell infiltration.<sup>196,197</sup>

ALS is a chronic neurodegenerative disease which causes loss of motor neurons in the spinal cord and motor cortex.<sup>178</sup> This disease, although not yet fully understood, also seems to induce BBB breakdown which was found to be due to the presence of albumin, cell infiltrate and IgG in CSF.<sup>198,199</sup>

*d. Huntington's disease.* Huntington's disease (HD) is an autosomal dominant disorder caused by an expansion of the CAG tract beyond 35 repeats in exon 1 of the huntingtin gene (HTT; formerly IT15), with longer repeat lengths that lead to earlier onset and greater disease severity.<sup>200</sup>

Individuals carrying the mutation develop motor defects, cognitive decline, and psychiatric disturbances.<sup>201</sup>

Although the cause of the disease is well established, it is still challenging to treat due to a number of pathological factors that have yet to be fully elucidated. Interestingly, Huntington's disease involves the occurrence of a number of functional anomalies of the BBB, including disruption of vascularization and increased permeability due to the opening of TJs.<sup>202</sup> This pathological phenomenon could represent the way by which nanosystems can cross the BBB and release the encapsulated drug.

#### **4. DRUG DELIVERY BRAIN TARGETING**

In recent years, drug delivery to the brain has become one of the most exciting challenges for academia, given the huge prevalence of diseases affecting CNS and the difficulty of treating them. As mentioned above, although the diseases afflicting the CNS are different in both etiology and clinical manifestations, all are characterized by difficulties in treatment, largely due to the presence of BBB. Indeed, it is the main obstacle that prevents most therapeutically active molecules from reaching adequate concentrations into the brain.<sup>203</sup>

One of the most widely explored approach to reach this goal is represented by the use of non-invasive pharmaceutical technology-based strategies. These are colloidal systems that, once administered intravenously, enter the circulatory stream and are transported by the blood flow into each district of the organism. For this reason, a fundamental requirement of nanovectors is their ability to remain for a long time into circulatory flow before interacting and being removed by the reticuloendothelial system (RES). The

nanosystems ability to escape the RES depends on certain factors, such as their size, their charge, and their surface features.<sup>8,204,205</sup>

Nanovectors can be made of natural or synthetic materials, which greatly influence the properties of the final formulation. For each pathology analyzed the nanocarrier must be carefully structured to meet the needs of the pathological tissue, in order to optimize the delivery of the drug while reducing the side effects associated as much as possible. With the proper formulation, it is also possible to add directional moieties on the surface of the nanosystems, thus obtaining that the drug is released only at the site of interest, decreasing the dose to be administered and the toxic effects associated with it. Among these new nanoformulations,<sup>206</sup> such as the well-studied liposomes,<sup>207</sup> dendrimers,<sup>208,209</sup> polymeric particles,<sup>210</sup> nanogels,<sup>211</sup> great attention has been focused on lipid-based nanosystems, SLNs and NLCs.<sup>3</sup>

The focus regarding these nanosystems is due to their ability to involve the same advantages of polymeric nanoparticles, fat emulsions and liposomes, also overcoming several limitations. In fact, various studies in literature suggest the high versatility of SLNs and NLCs as they show attractive characteristics that justify the great interest that has accompanied them in recent years.<sup>212,213</sup> Compared to polymeric nanoparticles and inorganic nanoparticles, these lipid-based carriers are less toxic, more biocompatible, biodegradable, non-immunogenic, flexible and safer, as their production can be carried out in absence of organic solvents.<sup>3,203,214</sup> SLNs and NLCs have demonstrated significant advantages over other lipid-based systems; in particular, compared to liposomes, they provide a longer shelf life, protection from enzymatic degradation, higher drug loading capacity, greater stability and they allow a prolonged release of the entrapped drug over time.<sup>3</sup>

They are capable to improve the pharmacokinetics and biodistribution of entrapped drugs, as SLN/NLCs are long-lasting formulations that increase the half-life of the delivered molecules, allowing their accumulation into organs and tissues (in addition to the possibility of being directed towards a specific target). These nanocarriers are useful to deliver a drug with poor physical-biological resistance into the bloodstream, or to increase the bioavailability of a molecule that can cross the BBB, or to facilitate the permeation of the molecule into the brain.<sup>215</sup>

More specifically, studies demonstrate that the use of such delivery systems improves the pharmacokinetics of the delivered drugs compared to the free drug. A specific example is doxorubicin; this drug is an important anticancer molecule that, however, shows significant cardiotoxic effects that limit its use. Zara et al. have investigated the improved effect of the use of SLNs as delivery system of this molecule; in this study, it was analyzed how the incorporation of the drug in SLNs led to an accumulation of doxorubicin into the brain, decreasing its distribution and therefore toxicity in other tissues compared to the commercial drug. The lower uptake of doxorubicin- loaded SLNs by the RES could increase the drug's bioavailability in non-RES tissue targeting.<sup>216</sup>

Moreover, their nanometric size enables them to cross the BBB via passive diffusion even without functionalization; this ability allows them to easily reach tissues with discontinuous capillary endothelium, as it happens in the liver, spleen, inflamed tissues and solid tumors, all areas in which endothelium is not characterized by the presence of TJ.<sup>204,205</sup>

SLNs/NLCs, similarly to other colloidal drug delivery systems, have special pharmacokinetic properties. In particular, their lipid composition provides these nanosystems with surface features that affect their biodistribution and interaction with biomembranes. When these naked nanovectors are administered intravenously, their hydrophobic surface is attacked by plasma opsonins. In order to avoid this process, it is useful to perform a PEGylation or use another surface moiety that allows the nanosystem to persist longer in the bloodstream by avoiding complement activation and uptake by the RES.<sup>215</sup>

Experimental evidence shows that the uptake of these lipid nanosystems is basically carried out by endothelial cells. In fact, the nanoparticles exploit various mechanisms to cross the BBB. According to Kreuter et al., it has been defined that NPs may have a local toxic effect on the endothelium, increasing its permeability; moreover, they may open tight junctions promoting the passage of the free drug or of the entire carrier.<sup>217</sup>

An interesting approach to increase the uptake of SLN/NLCs involves coating with some surfactants (polysorbates, including Tween 80) that can adsorb apolipoproteins (Apo). It has been shown that Apo adsorbed on the surface of the nanosystem are involved in the brain uptake of the NPs by the endothelium.<sup>218,219</sup> It has been noted that cationic NPs stimulate endocytosis by the endothelium, and it has

been hypothesized that the presence of the cationic surface may promote nanocarrier escape from lysosomal enzymes.<sup>215</sup>

Moreover, a study by Agarwal et al, demonstrated that using cationic bovine serum albumin (CBSA) as a ligand on the surface of methotrexate-loaded SLNs, it was possible to stimulate transcytosis by the endothelium across the BBB allowing the antitumor drug to reach the brain.<sup>220</sup>

This evidence suggests the large impact that these carriers could have in the treatment, for example, of tumoral diseases affecting the brain. As clarified previously, in this specific pathological state, both in children and adults, areas of increased permeability of the BBB develop through which SLNs and NLCs may achieve the target tissue merely by passive targeting. Instead, when occur pathologies in which there is no loss of integrity of the BBB, the possibility of decorating these nanocarriers with a directional moiety enables it to arrive at the target tissue merely, demonstrating once again the large versatility of these carriers according to the needs of the tissue to be treated.<sup>3</sup>

#### 4.1 Solid Lipid Nanoparticles

SLNs represent a promising colloidal system for delivering drugs into the brain. Structurally SLNs are solid lipid nanoparticles (nanometric size, approximately 50-100 nm) dispersed in water or in an aqueous surfactant phase.<sup>212,213</sup>

SLNs are prepared using lipids solid at room temperature, such as mono-, di- and triglycerides, fatty acids, waxes and steroids and various physiologically compatible emulsifiers, i.e. phospholipids, Poloxamers, and Polysorbates are added to stabilize nanoparticle formulations. The use of lipids FDA (Food and Drug Administration) and GRAS (Generally Recognized As Safe) approved minimize the risk toxicity-associated due to their good biocompatibility.<sup>214</sup> Moreover, SLNs enable to improve topical, oral and parenteral administration; encapsulating drugs into these nanoparticles provide a protection from chemical and physical degradation processes and overcome problems associated with drugs poor aqueous solubility.<sup>215</sup> It is possible to modify these nanovectors surface in order to avoid the so-called "burst effect";<sup>221</sup> in fact, SLNs drug release profile generally follows a biphasic pattern<sup>222</sup> in which it is possible recognize an initial burst effect followed by a controlled release phase that can last from days up to a few weeks (Fig.4).<sup>223</sup> This phenomenon has been explained considering that the first initial drug release may be due to a diffusion from the external particle surface or matrix erosion resulting by hydrolytic phenomena; the prolonged release, on the other hand, is probably associated with slow drug delivery via diffusion or dissolution from the lipidic core.<sup>224,225</sup>

The chemical structure and the nature of the components constituting SLNs are very significant technological parameters able to influence several aspects of these formulations, such as circulation time and stability.<sup>226</sup> They remain stable for over a year, overcoming other innovative formulation stability problems, if stored in refrigeration condition.<sup>227</sup> SLNs in size range 120-200 nm have the ability to avoid RES cells uptake and thus bypass liver and spleen filtration;<sup>228</sup> in addition, the surface coating with poly(ethylene) glycol (PEG) prolongs their circulation time into bloodstream.<sup>214</sup> Furthermore PEGylation seems to enhance SLNs permeation across epithelium.<sup>229</sup> In addition it is possible to avoid the use of organic solvents during the production process and sterilize the formulation.<sup>215,230</sup>

Depending both on the production technique used and drug solubility into lipid matrix, active substances may be entrapped into SLNs following three models: the drug can be distributed throughout the mass of the SLN (solid solution model), the drug can be localized on the outer shell around the inert lipid solid core (drug-enriched shell), or the core can be formed by the drug with the lipid layered around it (drug-enriched core) (Fig.5).<sup>231,232</sup>

Despite the remarkable characteristics of SLNs, they are also associated with some disadvantages, including the relatively low encapsulation efficiency (EE) due to the crystalline lipid structure that hinders the housing of the encapsulated drug, the tendency to gelate, the growth of lipid particles and polymorphic transition phenomena that may affect the lipid matrix which can lead to the expulsion of the embedded drug during the storage phase.<sup>231</sup>

(INSERT FIGURE 4)

(INSERT FIGURE 5)

## 4.2 Nanostructured Lipid Carriers

As for SLNs, it should be clarified that the use of only one type of lipid in the production of nanoparticles determines the formation of a perfect crystalline network in which there is no physical space for drug molecules, hence the low drug loading. Moreover, during the post-production storage phase, it may happen that transition processes of the crystalline network itself take place with consequent expulsion of the drug entrapped in the aqueous dispersion.

Since these processes represent a significant obstacle to the potential applicability of SLNs, Muller et al. have introduced structural modifications to SLNs in order to obtain a new generation of lipid-based nanoparticles, the NLCs.<sup>233</sup>

NLCs are modified SLNs in which the lipid phase consists of liquid lipids (oils) and solids at room temperature.<sup>231</sup>

This mix of solid and liquid lipids is able to overcome the problems mentioned above; in fact, to obtain a higher EE it is necessary to create spaces for the positioning of the drug within the nanosystems, and this is possible using long chain unsaturated lipids, therefore liquids at room temperature (oils). In this way an imperfect crystal is generated with the consequent increase in the amount of drug that can be entrapped inside. Generally, the solubility of a drug is higher in the liquid lipid than in the solid, and this means that the amount of drug that can be included during the preparation phase of the nanosystems is higher in the case of NLCs than in the SLNs.

Finally, using NLCs it is possible to avoid the phenomenon of expulsion of the entrapped drug because the use of different lipids leads to a solidification phase during cooling, but not to the formation of the crystalline network, so as to preserve the integrity of the formulation itself.<sup>233</sup>

It is possible to obtain three types of NLCs: NLCs of imperfect type, in which there is a mix of solid oils and lipids in the lipid phase, and the presence of unsaturated long chain fatty acids creates gaps within the system with increased drug loading; NLCs structureless type (non-crystalline matrix), in which the use of certain mixtures of lipids makes it impossible to crystallization and expulsion of drug during cooling; finally there is the multiple type O/F/W, in which the oily phase in which the solubility of the drug is maximum is surrounded by the solid lipid that determines the prolonged release of drug over time.<sup>231,233</sup>

## 4.3 SLNs and NLCs targeting the brain

Given the associated advantages, SLNs and NLCs have gained increasing attention as delivery system of active substances in recent years.

Interestingly, due to their nanometric size (< 100 nm), SLNs/NLCs have the innate ability to cross biological barriers, even the selective BBB;<sup>234,235</sup> besides this ability, it is also possible to functionalize the particle outer shell with several ligands. The addition of targeting moiety on the surface of these lipid nanovectors allows them to be directed towards a specific target and to interact with molecules on the target tissue. This modification is able to increase and improve the uptake of the nanosystems.

The following is an overview of explicative examples that illustrate how, in recent years, technological research has achieved remarkable improvements in studying and obtaining such attractive formulations (Table 3).

In 2018, Sistla et al. published a study in which Docetaxel-loaded SLNs decorated with a peptide were produced for the treatment of glioblastoma multiforme (GBM). This type of malignant glioma is very aggressive, in fact it is not possible to remove it surgically and its pharmacological treatment involves several difficulties, including the poor permeability through the BBB of existing drugs, the presence of efflux systems and the lack of specificity against tumor cells. In this case, the targeting moiety used is angiopep-2, an endogenous ligand of the LRP1 receptor, which is over-expressed both at the BBB and on the surface of glioma cells. The nanosystems thus obtained, loaded with Docetaxel (DTX), were tested to evaluate the effective anticancer activity. Cytotoxicity studies conducted on brain tumor cell lines (GL261 and U87MG) showed that the presence of the targeting molecule promoted internalization into tumor cells and the drug delivery system used has allowed the sustained release of the entrapped DTX, resulting in a superior toxic effect compared with the free drug. In addition, *in vivo* studies in C57BL/6 mice highlighted that the survival time of animals treated with the nanoformulation was significantly longer (39 days) than those treated with the commercially available drug Taxotere (24 days); probably the improved anti-glioma activity is the result of increased accumulation of drug in the glioma site due to the active targeting.<sup>236</sup>

In a recent work, Arduino et al. have prepared PEGylated-SLNs containing Pt(IV) prodrugs accurately designed to treat GBM. The obtained data revealed the formation of anionic Pt (IV)-Prodrug/PEG-SLNs with good stability in aqueous medium and characterized by average hydrodynamic diameters below 100 nm. It has been demonstrated that this formulation is able to permeate the *in vitro* BBB model based on a hCMEC/D3 monolayer, highlighting the innate SLNs ability to exceed such a selective barrier. Moreover, the anticancer activity of the prodrugs delivered by SLNs was investigated *in vitro* using human glioblastoma cell line (U87), showing increased cytotoxicity and uptake of the Pt (IV)-prodrugs when trapped into SLNs compared with the non-formulated prodrugs.<sup>235</sup>

Other research groups have explored the use of functionalized SLNs to increase drugs delivery to the brain; Liu et al. have prepared a brain delivery system based on OX26 antibody conjugation on PEGylated cationic solid lipid nanoparticles (OX26-PEG-CSLN); in this study PEGylated cationic SLN have been Baicalin-loaded and it was demonstrated an improvement in drug uptake across the brain due to the interaction between OX26 antibody and transferrin receptor on BBB surface. This interesting result indicated that conjugation with OX26 antibody could be a promising approach to reach the brain.<sup>237</sup> Furthermore, in a later study, the same research group have demonstrated that not only Baicalin-loaded OX26-PEG-CSLN have the ability to permeate BBB, but also drug released by SLN formulation had an elevated bioavailability in cerebral spinal fluid of rats, showing a higher ability than Sol group (the group of focal cerebral ischemia–reperfusion treated with Baicalin solution) in neuronal protection.<sup>238</sup>

Another attractive approach to direct nanoparticles and to enhance their brain permeation could be represented by the usage of peptide or peptidomimetics as SLN surface modifying agents, e.g., Kuo et al. showed that using SLNs decorated with monoclonal antibody, it is possible to increase brain delivery. They have exploited the interaction between 83-14 monoclonal antibody, which is an insulin-like peptidomimetic, with the  $\alpha$ -subunit of human insulin receptor, stimulating SLNs endocytosis into brain microvascular endothelial cells (BMECs) and the release *in situ* of the antiviral Saquinavir entrapped into nanoparticles.<sup>239</sup>

In addition, in another work Carmustine-SLN loaded have been functionalized with serotonergic receptor subtype antagonist (S1BRSA): in this case the binding between S1BRSA and its endogenous receptor based on brain endothelial cells promote the nanoparticles internalization. Resulting data showed that the Carmustine anticancer activity against the model of glioblastoma multiforme cells (U87) remains unchanged after SLNs release.<sup>240</sup>

Recently, Kuo et al. have produced SLNs based on various lipids, functionalized with transferrin on the surface, containing a series of molecules with antioxidant activity and stimulating nerve regeneration following neurodegenerative insults. The SLNs obtained contain a nerve growth factor (NGF), rosmarinic acid (ROA), curcumin (CURC) and quercetin (QU). The characteristic of the nanosystems thus produced (QU-CURC-ROA-NGF-DPSLNs) is that they have been manufactured by double emulsification, therefore they contain a water zone suitable for loading the hydrophilic molecules (ROA and NGF), and a lipid matrix for the hydrophobic molecules.<sup>241</sup>



Regarding NLCs, several results have been produced recently. Wu et al. have adopted these nanosystems to carry compounds in order to repair post-ischemia neuronal damage. In this study, NLCs carrying Salvianolic Acid and Baicalin were realized, and the nanovectors were functionalized with the transferrin receptor monoclonal antibody OX26 (OX26-BA/Sal BNLC). The *in vitro* results of the preliminary study have shown that this delivery system allows the release of entrapped compounds that have a restorative/improving effect on the condition of treated nerve cells.<sup>242</sup>

Recently, an interesting work has been published by Arduino et al. They have developed NLCs carrying a compound (MC111) capable of inducing the activity of two transporters expressed at the level of the cerebral endothelium, P-gP and BCRP, that are closely involved in the clearance of  $\beta$ -amyloid from the brain parenchyma. In this study the nanosystems have been functionalized using transferrin as a directing moiety. Intriguingly, the biological assays performed on hCMEC/D3 cells cultured in BBB-forming conditions, revealed that the treatment with NLC-MC111 led to an increase in the activity of the two transporters, which was however maximum in the case of Tf-NLC-MC111. This important result demonstrated that, in addition to achieving permeation of the barrier model, the functionalized nanosystems were able to deliver a greater amount of drug inside the cells, leading to an increase in the activity of P-gP and BCRP.<sup>243</sup>

Curcumin is a compound with multiple antioxidant and restorative properties, and a high potential of applicability for neurodegenerative diseases. Unfortunately, this compound shows a low bioavailability and considerable difficulties in crossing the BBB, so it appears to be an optimal candidate for trapping in innovative delivery systems. NLCs containing curcumin have been produced and this formulation has been tested in a rat model or AD. The results shown by this study have demonstrated how the appropriate delivery system allows the drug to reach the nerve cells and performs its therapeutic activity: in fact, the animals treated with Cur-NLC showed a decrease in oxidative stress parameters in hippocampal tissue and an improvement in spatial memory, in parallel with a decrease in amyloid beta deposits. These data suggest that indeed the formulation design adopted allows the drug to reach the target site, overcoming the problems associated with brain treatment.<sup>244</sup>

(INSERT TABLE 3)

## 5. CONCLUSION

Diseases affecting CNS are currently one of the greatest challenges facing scientific community, given the difficulty in their treatment due to the presence of selective barriers that actively prevent drugs from reaching therapeutic concentration *in situ*.

One of the pharmaceutical technology goals concerns the development of innovative safe non-invasive formulations capable of overcome the limitations associated with classical drugs.

From the wide interest and production in scientific literature, it is clearly shown that nanocarriers represent the future of drug delivery to the CNS. The BBB is selective to the entry of limited molecules, which helps prevent the intrusion of harmful molecules into the CNS. However, this protective feature of the BBB is also the biggest hurdle in the delivery of drugs for the treatment of brain diseases. Nanocarriers can cross the BBB and can thus be used as a tool for brain drug delivery. Inability of the drugs to permeate the BBB can be enhanced by their encapsulation inside the nanocarriers to facilitate their entry into the brain. This procedure is non-invasive, and drugs entrapped in nanocarriers can be administered in several ways.

In this context, this review focused the attention on solid lipid-based particles properties and their potential as brain drug delivery system. In this context, we explored the potential that solid lipid-based delivery systems offer. These delivery systems emerge as an improvement over past formulations. In fact, the use of polymeric nanoparticles and inorganic nanosystems is associated with vehicle toxicity issues that make them unusable for extended brain targeting. In addition, SLN/NLCs allow higher protection of the encapsulated drug in comparison with liposomes.<sup>226</sup>

In addition, the other key point of this work implies the need to carry out a deep study on the BBB; it is clear that the detailed knowledge of the structural and functional changes which may arise on BBB as a result of a pathology and/or a physiological aging process provides an essential starting point for the study of new therapeutic formulations reaching the brain.

Hence the need of age-related brain targeting; it represents a full of potential approach that could allow to obtain delivery systems designed to be more efficient as properly structured to meet the needs of their target, performing a promising step forward into personalized therapy.

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