

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Solid lipid nanoparticles as vehicles of drugs to the brain: Current state of the art

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/155381> since 2016-02-01T16:22:16Z

Published version:

DOI:10.1016/j.ejpb.2014.05.004

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS, , 2014, 10.1016/j.ejpb.2014.05.004.

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

- (1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.
- (2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.
- (3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>), 10.1016/j.ejpb.2014.05.004

The definitive version is available at:

<http://linkinghub.elsevier.com/retrieve/pii/S0939641114001532>

Solid lipid nanoparticles as vehicles of drugs to the brain: current state of the art

Lucia Gastaldi, Luigi Battaglia*, Elena Peira, Daniela Chirio, Elisabetta Muntoni, Ilaria Solazzi,
Marina Gallarate, Franco Dosio

* corresponding author: luigi.battaglia@unito.it

affiliation: Dipartimento di Scienza e Tecnologia del Farmaco – via Pietro Giuria 9 – Torino, Italy
+390116707668

Abstract

Central nervous system disorders are already prevalent and steadily increasing among populations worldwide. However, most of the pharmaceuticals present on world markets are ineffective in treating cerebral diseases, because they cannot effectively cross the blood brain barrier (BBB). Solid lipid nanoparticles (SLN) are nanospheres made from biocompatible solid lipids, with unique advantages among drug carriers: they can be used as vehicles to cross the BBB. This review examines the main aspects surrounding brain delivery with SLN, and illustrates the principal mechanisms used to enhance brain uptake of the delivered drug.

Keywords

Solid lipid nanoparticles, blood brain barrier, central nervous system, biodistribution

1. Introduction

Recent research efforts have been aimed to targeting drugs to the brain: many pharmaceuticals on the market are ineffective in treating cerebral diseases because they fail to reach the brain. Methods that may enhance drug delivery to the brain are thus of great interest. Despite much research in this field, patients suffering from fatal and/or debilitating central nervous system (CNS) diseases, such as brain tumors, HIV encephalopathy, epilepsy, cerebrovascular diseases, and neurodegenerative disorders, far outnumber those dying of all types of systemic cancer and heart disease. In many cases, the clinical failure of potentially-effective therapeutics is due to the difficulty of targeting the drugs to the site of action. Treating CNS diseases is particularly challenging, because significant obstacles hinder pharmaceutical access to the area: in particular, the so-called blood brain barrier (BBB) impedes drug delivery to the brain [1].

2. The Blood Brain Barrier (BBB)

The BBB acts as a physical barrier, regulating the passage of molecules from the bloodstream to the brain, and thus restricting the types of drug that can reach the brain upon systemic administration: more than 98% of potential new therapeutic molecules are unable to cross the BBB [2].

If there were not the BBB, the brain microvasculature would provide an extraordinary way to access the brain: this microvasculature has a total surface area of 20 m^2 and a total length of 640 km. This massive vascularization gives the brain the possibility to be permeated by small molecules (by simple diffusion) in half a second [3]. However, unlike peripheral capillaries, those of the brain present no fenestrae, few pinocytosis vesicles, and particular tight junctions, also known as zonula occludens, closely regulating the movement of molecules through the paracellular pathway, and comprising an almost impermeable barrier for drugs administered through the peripheral circulation [4]. A further contribution to the peculiar BBB functions is given by the periendothelial accessory structures, known as astrocytes, which have a multitude of functions important for brain

homeostasis (maintenance of potassium ion levels, inactivation of neurotransmitters, regulation and production of growth factors and cytokines) [4].

According to Patel et al. [5] the transport of solute molecules across the BBB membrane takes place by four different mechanisms:

- Paracellular diffusion: this is a non-saturable and non-competitive process occurring between cells. The presence of tight junctions in brain endothelial cells greatly limits paracellular diffusion. Only small water-soluble molecules can diffuse through the BBB, apparently by passing through the tight junctions.
- Transcellular diffusion: this takes place across cells, and is likewise non-saturable and non-competitive. The properties of the substances required for transcellular transport are high lipophilicity and low molecular weight: higher lipophilicity, and molecular weight < 450 Da facilitate transport of the molecule into the brain
- Carrier-mediated transport: the exchange of substances between the blood and the brain, including nutrients, occurs actively, by selective membrane-bound carrier systems. The process of carrier-mediated transport probably involves the development of transient narrow pores, induced by binding the particular substrate to the carrier, which in turn allows the passage of specific substrate molecules only. Several carrier systems have been described in brain capillaries: specific transporters (with activities regulated by the brain's metabolic needs, and by the concentration of various substrates in the plasma) facilitate the transfer of nutrients, including glucose, galactose, amino acids, nucleosides, lactates and pyruvates, adenine and guanine, choline, vitamins and hormones [6]. As glucose provides the main energy source for the brain, glucose transporters (such as GLUT1 and GLUT3) are of great significance [7]; equally important is the monocarboxylate (lactate; pyruvate) transporter system (MCT1) [8]. Moreover, specialized carriers exist for essential amino acids and vitamins [7].
- Receptor-mediated endocytosis: some large endogenous proteins and hormones are able to cross the BBB by endocytosis, mediated by certain receptors present on the luminal side of the

barrier; specific receptors have been identified for insulin, insulin-like growth factors, angiotensin II, folates and transferrin [9]. Lipids can also be internalized in the brain in the form of low-density lipoproteins (LDL), which are recognized and endocytosed by the endothelial cells, thanks to the expression of various apolipoproteins (especially ApoE) on the surface of the LDL [4]. Polycationic proteins (such as cationized albumins or immunoglobulins) can be transported across the BBB by means of absorptive transcytosis, without the involvement of specific plasma-membrane receptors. In this case, endocytosis is initiated through the association of polycationic substances with the negative charges present on the plasma membrane of endothelial cells [10].

From the drug delivery standpoint, these transporters may help the delivery of drugs to the brain, since drugs or pro-drugs can enter the brain through carrier-mediated transport in therapeutic concentrations, by mimicking nutrients or endogenous compounds [4].

Additionally, on the luminal side of endothelial cells the P-glycoprotein (P-gp) system is present; this is an ATP-dependent drug transport protein. It has been demonstrated, both *in vitro* and *in vivo*, that BBB P-gp can hinder the accumulation of many molecules in the brain, by expelling them from the cells. P-gp inhibition has been proposed as a possible strategy for enhancing brain drug penetration [11, 12].

The combination of these physical and metabolic mechanisms makes it extremely difficult to convey specific actives into the brain. It is now recognized that, for a drug molecule to cross the BBB, among other requirements it must have high lipid solubility and a molecular mass that is < 400 Dalton, and not be a substrate for active efflux transporters [13].

3. Methods to evaluate BBB permeation

Crossing the BBB is a very challenging goal in drug delivery: a number of different methods have been proposed to assess whether a drug or a formulation is suitable for brain delivery. Some of these methods involve the use of *in vitro* models, while others use *in vivo* models on rats or mice.

Among *in vitro* methods, the most important are artificial models of the BBB [14]. These generally comprise cultured brain microvasculature, which can be grown in the absence or in the presence of glial cells. Although primary cultures of brain endothelium alone may form tight intercellular junctions, co-culture with astrocytes generally leads to increased formation and complexity of endothelial tight junctions, and induces expression of specific BBB markers. Currently, the development of immortalized endothelial cell lines that preserve a stable BBB phenotype is of great interest. The tissue culture substrate most commonly used for culturing endothelial cells consists of a porous membrane support submerged in culture medium (Transwell apparatus). The Transwell system consists of cultured brain microvessel endothelial cell monolayers, grown on microporous membranes. Cultured cortical astrocytes are compartmentalized below the endothelial monolayer, and release soluble factors, which preserve the BBB properties. This system affords study of bidirectional transport across the BBB; it is an advantageous and well-established way of artificially evaluating the crossing of the BBB *in vitro*, without using *in vivo* models. Compared to *in vivo* models, *in vitro* models have the disadvantage of being static systems, in which the concentration of the molecules under study is always the same in the donor compartment. Conversely, *in vivo* the concentration of molecules is gradually reduced by the metabolism after administration. Thus in *in vivo* models the phenomenon of biodistribution occurs, which regulates the amount of drug present in the bloodstream, which can effectively reach the BBB and consequently be available for permeation.

Analysis of biodistribution after intravenous (i.v.) administration provides complete information concerning the eventual crossing of the BBB by a drug or a formulation: it can be performed by killing animals at different time points after i.v. administration, and quantitatively analyzing the drug content in various organs.

Apart from complete biodistribution studies, other animal models are available to evaluate the crossing of the BBB by a drug or formulation. These include the rat brain perfusion model: in this procedure, rats are anesthetized and a catheter is placed into the left common carotid artery after

ligation of the left external carotid, occipital, and common carotid arteries. Common carotid artery ligation is caudal to the catheter implantation site. The pterygopalatine artery is left open during the experiment [15]. The left common carotid artery is connected to a syringe containing the formulation to be administered, with an infusion pump for periods of 45 s at 10 ml/min. This perfusion rate is selected to maintain a carotid artery pressure of ~120 mm Hg [16]. The rats are then decapitated and cerebral samples are analyzed for drug content. Compared to i.v. administration, as used in biodistribution studies, the intra-carotid administration used in the rat brain perfusion model has the advantage of delivering the formulation directly toward the brain, excluding the liver first pass: this results in better uptake by the brain compared to i.v. administration, but has the disadvantage of being less typical of clinical situations than i.v. administration.

There are also a number of functional animal models that mimic particular brain diseases or conditions, and that may be useful to evaluate the effectiveness of a drug or a formulation that is supposed to cross the BBB. One of the most important and best known is the analgesia animal model, the so-called tail flick assay [17], which can be applied in the case of analgesic drugs, or formulations carrying them: the rat or mouse is given the drug or formulation, after which the animal's tail is placed on a heat source; the analgesia induced by the drug or formulation is measured by the interval of time before the tail flicks. If the analgesic drug penetrates the brain, the time will be increased compared to control animals.

Another important model is the rat or mouse glioma model, which can be used to study glioblastoma, the most common primary brain tumor [18]. Glioma can be induced in rats or mice by repetitive intravenous administration of nitrosurea compounds, or can be obtained through stereotactic implantation of tumor cells within the rat brain. The glioma model is a valid model to determine whether a cytotoxic drug penetrates the BBB and exerts its function on the glioma, since tumor growth can be monitored by magnetic resonance analysis. However, it must be taken into

account that, in the case of a developing glioma, the BBB is altered and consequently drug permeation is increased compared to healthy brain.

Numerous animal models have been developed for Parkinson's disease. One of the best known is the 6-hydroxydopamine rat model [19] [20] [21]: the toxin 6-hydroxydopamine is injected into one side of the rat brain, while the opposite side serves as an intra-animal control. This injection produces dopamine neuron loss on the injected side, causing a Parkinsonian akinesia on that side. If an anti-Parkinson drug or formulation works, the akinesia will be reduced after its administration.

Many different models have been reported for Alzheimer's disease, using transgenic or otherwise rats [22]. One of the most feasible models entails inducing the disease by prolonged oral administration of aluminum chloride to mice or rats. Brain ischemic injury can be induced in laboratory animals by the so-called ischemia- reperfusion injury model [23]: in this model the blood flow into the middle cerebral artery is blocked with an intraluminal suture introduced through the extracranial internal carotid artery. The reperfusion induced by treatments is tested under various experimental conditions.

4. Nanoparticles (NPs) as vehicles to cross the BBB

Crossing the BBB and overcoming the associated protective mechanisms may be achieved by using colloidal systems (i.e., micelles, liposomes, NPs) designed to deliver drugs to the CNS. Colloidal drug carriers usually have size ranging from 1 to 1000 nm and consist of molecular aggregates, within which the therapeutic drugs can be adsorbed, entrapped, or attached covalently. NPs in particular are very interesting for drug delivery purposes, thanks to their versatility. The potential mechanism of NPs-mediated drug delivery across the BBB is determined by the chemistry, architecture, and properties of the NPs [24].

Before describing the possible mechanisms through which NPs may overcome the BBB, a preliminary consideration is important: the major limiting factor upon the systemic use of NPs is their rapid clearance from the blood circulation by the reticulo-endothelial system (RES). Clearance

depends mainly on particle size, surface charge, and surface properties: colloidal particles, which have variable hydrophobic surface properties, are efficiently coated by specific plasma components (opsonins), including immunoglobulins (IgG) and albumin (elements of the complement system) and then cleared within minutes from the bloodstream by phagocytic cells [25]. Intravenously administered NPs are mainly distributed to the liver (60–90% of the injected dose), spleen (2–10%), lungs (3–20% or more), and bone marrow (1%) [26]. This accumulation in the RES organs severely limits the utility of NPs in targeting drugs to the CNS. Thus appropriate modification of the NPs' surface characteristics and size might be an effective strategy to alter their biodistribution and extend their circulation time in the bloodstream: NPs with a longer circulation time (“stealth NPs”) are in contact with the BBB for an increased time, and thus have a longer time to deliver their cargo to the brain [4].

The plasma half-life of NPs can be increased by different methods [27]. A method can be the decrease of particle size: the size and the deformability of particles play a critical role in their clearance by the sinusoidal spleens. Particles must be either small or sufficiently deformable to avoid the splenic filtration process at the interendothelial cell slits (IES) in the walls of venous sinuses [28]. The slit size rarely exceeds 200 to 500 nm in width: thus retention of blood cells and blood-borne particles at the IES depends on their bulk properties, such as size, sphericity, and deformability. The size of an engineered long-circulatory particle must ideally not exceed 200 nm. If larger, the particle must be sufficiently deformable to avoid IES filtration. However particle size should also not be too small in order to avoid the renal clearance. [29]

Another method consists on surface coating with hydrophilic polymers/surfactants. It is generally recognized that hydrophobic surfaces promote protein adsorption, and that negatively-charged surfaces activate the complement system and coagulation factors. Any shielding of the hydrophobic character of NPs will thus sterically stabilize them, reducing opsonization and phagocytosis, and increasing blood circulation time and bioavailability. Recognition by the RES can be prevented by coating particles with a hydrophilic or flexible polymer and/or a surfactant [25].

According to Kreuter et al., NPs can enhance drug transport to the CNS by a number of different mechanisms [30]:

- NPs are transcytosed or endocytosed through the endothelial cell layer, enabling their therapeutic cargo to be transported;
- NPs inhibit transmembrane efflux systems (i.e. P-gP);
- NPs open the tight junctions between endothelial cells and allow the drug to penetrate, either in its free form or together with the carrier;
- drug transport is enhanced through solubilization of the endothelial cell membrane lipids, by the surfactants associated with the NPs; this leads to membrane fluidization (surfactant effect);
- NPs induce local toxic effects on the brain vasculature, which leads to limited permeabilization of the brain endothelial cells.

The most recent brain targeting strategies for NPs, however, are based on surface engineering by functionalization or coating with specific ligands. These can facilitate endocytosis of the NPs by the endothelial cells, which remains the principal mechanism whereby drug transport to the CNS can be enhanced. Specific transport systems located at the BBB could be used as targets to enhance drug delivery to the CNS, through conjugation of a transporter directly to the carrier system [31]. Receptor-mediated carriers use both endogenous and chimeric ligands. However, owing to potential competition with plasma nutrients and other components of the host biological environment, endogenous ligands can be less effective as targeting systems. Chimeric ligands, such as monoclonal antibodies involved in receptor-mediated endocytosis, can be advantageous because their binding sites are different from the endogenous ligand binding sites [24]. Cationic NPs can also be used to enhance drug delivery through the BBB, since cationic substances can interact with the negative charges present on the plasma membrane of endothelial cells: cationic albumin has been conjugated onto NPs for drug delivery across the BBB. Cell-penetrating peptides (CPP), also known as protein transduction domains or membrane transduction domains, are also of interest, owing to their capacity to translocate across biological membranes and to aid the transport of

various substrates into and across the cell [32] [33]. However, the exact mechanism of cellular entry for CPP is yet to be elucidated.

Another interesting approach is based on modifying the NP surface with specific surfactants, that can preferentially absorb apolipoproteins (Apo) from the blood stream [34] [35]. Among these surfactants, the most important are the polysorbates (Tween[®]), especially Tween[®] 80. The Apo adsorbed on the surface, by interacting with specific receptors on the BBB luminal face, seems to be responsible for NP translocation into the brain [36]. This mechanism has recently been confirmed by the observation that Apo-E coated albumin NPs are delivered to the brain after i.v. injection [37]. Much research concerning delivery to the CNS is focused on the multi-drug efflux transporter P-gp, a member of the large ATP-binding cassette (ABC) superfamily of ATP-dependent transporters. P-gp is a 170-kDa membrane-associated glycoprotein that actively transports structurally different substrates; it is widely expressed in the BBB endothelial cells. Inhibition of the P-gp function can be obtained through down-regulation of its expression at transcriptional or translational levels, or by altering membrane targeting after synthesis of the P-gp protein, or through chemical inhibition of transporter functions. The first two approaches have as yet been little developed, whereas chemical inhibition of the transporter functions has been extensively studied. An alternative possibility is the use of NPs that could carry the drug into the endothelial cells by endocytosis, thereby bypassing the P-gp drug efflux mechanism [38].

5. Solid Lipid Nanoparticles (SLN)

SLN are nanospheres made from solid lipids; their mean photon correlation spectroscopy diameter is approximately 50 to 1000 nm. They consist of a solid lipid matrix, i.e. glycerides, fatty acids, or waxes, stabilized by physiologically-compatible emulsifiers such as phospholipids, bile salts, Tween[®], polyoxyethylene ethers, or polyvinyl alcohol. The lipids used in their production are solid at room temperature, and most of them have approved status, e.g. GRAS (Generally Recognized As Safe), due to their low toxicity [39] [40].

They are usually produced by the hot homogenization method, which generally entails using high pressure homogenization (HPH) [41]. However, this method involves some critical process parameters, e.g. high temperatures and high pressures (cavitation force), which can result in significant thermodynamic and mechanic stresses on the resulting product. For this reason, and in order to bypass patented methods, considerable work has addressed the development of suitable alternative and easy-handling production methods for SLN preparation. Among the best-known methods for SLN production there are microemulsion templates, solvent-based methods, coacervation, supercritical fluid technology, and the membrane contactor method [39] [40]. Further, a new generation of lipid NPs has recently been developed: they comprise nanostructured lipid carriers (NLC) and lipid-drug conjugates (LDC). NLC are characterized by a core consisting of a mixture of solid and liquid lipids: the resulting matrix of the lipid particles has a lower melting point than the original solid lipid, but the matrix is still solid at body temperature. Compared to SLN, they possess the advantage of offering increased drug loading and decreased drug leakage from the NPs. LDC NPs were developed to overcome the limitation upon SLN and NLC, namely their low drug loading capacity for hydrophilic drugs. In a typical LDC process, an insoluble drug-lipid conjugate is first prepared in bulk, either by salt formation (e.g. with a fatty acid) or by covalent linking (e.g. esters or ethers). The resulting LDC is then processed by HPH with an aqueous surfactant solution, to produce a NPs formulation [39] [40].

SLN offer a significant improvement in drug delivery by the topical, oral, and parenteral administration routes. In particular, encapsulation of drugs in SLN can help to:

1. overcome problems due to their low aqueous solubility,
2. protect them from chemical and physical processes of degradation and evaporation,
3. provide slow release over time to the surrounding environment, and
4. direct the entrapped substance to a specific target and/or cross several biological barriers.

6. SLN as drug delivery system to the CNS

Considerable attention has been focused on SLN as possible vehicles to cross the BBB, because they show several advantages as drug delivery systems [5]. In particular:

- being made of physiological lipids, SLN have good biocompatibility;
- SLN offer relevant drug loading capability;
- the production processes avoids the use of organic solvents;
- SLN can be produced easily on a large scale, and can be sterilized [41];
- SLN in the size range 120-200 nm and coated with an hydrophilic surface are not taken up readily by the cells of the RES and thus bypass liver and spleen filtration [42];
- controlled drug release can be made to last several weeks [43];
- the drug-targeting potential can be further enhanced by coating SLN or attaching ligands to them;
- SLN formulations are stable for as long as 3 years, a very significant advantage over the other colloidal carrier systems [44].

SLN are frequently proposed as drug delivery systems for various compounds targeted to the CNS. However, although a large bulk of experimental data has been published, there is as yet no univocal criterion to evaluate their real potential for delivering drugs to the CNS. From a detailed examination of published data, it emerges that many compounds, differing in their chemical and biological properties, have been encapsulated within SLN to enhance their penetration within the CNS. However, due to the wide range of different molecules encapsulated, and to the different models used to evaluate drug permeation through the BBB, data concerning the true efficacy of these nanoparticulate systems are often contradictory, especially regarding the extent of permeation. In order to clarify these controversial aspects, it has been attempted to classify the molecules delivered to the CNS employing SLN into different categories, according to the rationale underlying their encapsulation in SLN. Two preliminary considerations must be taken into account in

discussing this research. Firstly, SLN are employed to deliver drugs to the CNS for differing aims; in particular, SLN can be used to:

- 1- stabilize a molecule from the physico-chemical or biological standpoints (i.e. in the bloodstream);
- 2- improve the drug's pharmacokinetics and biodistribution, by obtaining a long-lasting formulation with an increased half-life in the bloodstream, consequently increasing accumulation in organs and tissues
- 3- trigger the endothelial cells of the BBB to enhance drug permeation, exploiting mechanisms discussed above.

Secondly, the documented extent of drug delivery to the CNS differs for different types of drugs and different SLN: any comparison among the resulting data can cause confusion, since different drug-loaded SLN models, with different efficacies, have been proposed to improve CNS delivery. For example, although almost 98% of drugs fail to cross the BBB, 2% of drugs can cross it, to varying extents and by different mechanisms. Many experimental works, aimed to deliver drugs to the CNS, are involved with this kind of drugs: consequently the encouraging results, obtained in terms of drug targeting to the CNS, are mainly due to the enhancement, by the encapsulation in SLN, of the biological profile of a molecule, which crosses the BBB by itself.

Numerous other studies deal with molecules that only cross the BBB to a very limited extent: consequently, the enhanced drug delivery to the CNS achieved by encapsulation in SLN, even if small in absolute terms, amounts to a significant success in the light of the poor penetration of the free drug; the effect in this case is entirely due to the vehicle.

Based on these considerations, a critical and analytical review of experimental research was made, dividing studies into three categories, by the aim of drug delivery in SLN, and the characteristics of the delivered molecule. In particular, SLN used to deliver drugs to the CNS may be subdivided into:

1. SLN used to stabilize molecules that suffer from physico-chemical or biological instability;
2. SLN used to improve the bioavailability of a drug that can cross the BBB:

3. SLN used to increase a drug's permeation through the BBB.

Obviously, this classification is not entirely rigorous, since some drugs cross the BBB to some extent but suffer from rapid clearance from the bloodstream: in this case, delivery in SLN can both improve the drug's bioavailability and help it to cross the BBB. Table 1 summarizes published studies subdivided by this classification.

6.1 SLN used to stabilize molecules with physico-chemical or biological instability

Many CNS drugs, despite their ability to penetrate or cross the BBB, show poor *in vivo* efficacy. In this context it is important to determine plasma stability, since these compounds may be rapidly degraded in the plasma. In many cases, plasma instability also results in misleading *in vitro* data, and complicates pharmacokinetic studies, since the compound continues to degrade even after blood samples have been taken. Compounds that are unstable in the plasma tend to have rapid clearance, short half-life, and poor *in vivo* performance [45].

To overcome these problems, various colloidal delivery systems have been formulated, including liposomes, microspheres, niosomes, polymeric NPs, and SLN. Important factors to be considered in designing a drug delivery system and making it effective are not only high drug loading and low toxicity, but also increased physical and chemical stability. For brain targeting, plasma stability is an important aspect of the stability profile; it includes microsomal stability and buffer stability. In targeting drugs to the brain, the stability of a candidate drug in the plasma is essential in order to maintain an acceptable drug concentration and biological half-life, without which the desirable pharmacological effects will not be achieved.

Compounds containing certain functional groups are more susceptible to hydrolysis by plasma enzymes than others. These include esters, amides, lactones, lactams, carbamides, sulfonamides, and peptide mimetics. An example is given by the camptothecin-based compounds. Camptothecin (Campt) and its derivatives have been shown to be highly efficient against a large variety of cancers, and numerous clinical and preclinical studies have confirmed the effectiveness of Campt in

glioblastoma therapy [46], since the drug readily crosses the BBB [47]. However, Campt is not used in clinical anticancer protocols because of the instability of its lactone ring in physiological conditions, its limited aqueous solubility, and its several toxic side effects. Consequently, there is great interest in developing effective drug delivery systems to target Campt to the brain in its active form, and overcome its pharmacokinetic limitations; this would enhance the drug's antitumor brain efficacy and clinical applications.

In the attempt to stabilize Campt, a number of advantages have been reported for SLN compared to other colloidal carriers. Yang et al. [48] [49] have shown that SLN increased Campt's stability against hydrolysis, and increased the retention time of the active lactone. In particular, Campt molecules loaded in SLN were found to be converted into the inactive carboxylate form only after the drug was released from the NPs into the neutral buffered medium. However, when the release medium was acidic, the released drug remained in the lactone form. This implies that SLN protected the drug from undergoing hydrolysis until it was released. *In vivo* studies in nude mice demonstrated an extended half-life of the active lactone drug form in the whole blood and in the main organs, when delivered in SLN: as a consequence, accumulation in the brain was also greatly increased.

Recently, Martins and co-workers [50, 51] tested SLN as a suitable platform to stabilize Campt for brain delivery, in a study in which formulations of Campt-loaded SLN made up of cetyl palmitate and Tween[®] 80 were prepared by hot HPH. Combining all the stability parameters, the developed formulations were considered stable in terms of size and charge, for up to a year of storage. *In vitro* release studies in plasma showed a prolonged release profile of Campt from SLN, confirming the physical stability of the particles under physiological pH. Interestingly, SLN altered Campt's biodistribution *in vivo*, prolonging retention time in the brain, exhibiting a brain targeting effect, and increasing the potential antitumor effect against glioma. At the same time, the side effects were also reduced. All these findings indicate that SLN are capable of protecting labile molecules known to be susceptible to hydrolysis.

6.2 SLN used to improve the bioavailability of drugs that cross the BBB

In order to develop a drug that can reach the brain, even if theoretically it can cross the BBB, some factors that could hinder its progress must be taken into account. Among others, there must be a sufficiently high concentration of the drug to overcome the weak permeability of the BBB. This permeability may be enhanced by separating the blood vessels from the cerebral parenchyma, or through inhibition of BBB-active drug efflux transporters, which are present in the cerebral endothelium in large numbers, and play an important role in the efflux mechanism of a wide variety of drugs. For example, many orally-administered drugs are absorbed into systemic circulation via the portal blood, and are subjected to first-pass metabolism; they therefore exhibit low oral bioavailability. In this context, Manjunath and Venkateswarlu [52] demonstrated that it is possible to improve the bioavailability and therapeutic efficacy of lipophilic drugs by suitably incorporating them into SLN. They used clozapine, an effective lipophilic atypical antipsychotic drug with poor oral bioavailability, due to the first pass effect. Positively-charged clozapine SLNs, made of different triglycerides, enhanced the bioavailability of the drug 3.1- to 4.5-fold, on intraduodenal administration.

Conversely, as described above, intravenously injected SLN are rapidly cleared from the systemic circulation by opsonization. As reported, stealth lipid NPs have longer circulation times, avoiding complement activation and preferential uptake by the RES. In this connection, in order to enhance SLN distribution to the brain, surface modification using Pluronic F-68, poly(ethylene glycol) (PEG) or Tween[®] 80 has been proposed. The modification causes a steric hindrance effect, decreasing adsorption of opsonin onto SLN in the plasma, and slowing removal of the particles by the RES. Wang and co-workers [53] employed SLN modified with Pluronic F-68, into which was incorporated 5-Fluoro-20-deoxyuridine (FUdR), a derivative of 5-fluorouracil with significant cytotoxic activity that crosses the BBB to a moderate extent; however, it is rapidly metabolized after administration, particularly by the liver. The study demonstrated that SLN incorporation

enhanced penetration and transport of the molecule into the brain. The study authors put forth two explanations: surface modification of the SLN with Pluronic F-68 could cause a steric hindrance effect that would decrease the adsorption of opsonin onto SLN in the plasma, reducing RES uptake and prolonging retention time in plasma. Secondly, increased retention of the SLN in the brain capillaries combined with adsorption onto the capillary walls might create a higher concentration gradient, which would enhance transport across the endothelial cell layer, and consequently delivery to the brain. The SLN might also be endocytosed by the endothelial cells, followed by drug release within these cells and delivery to the brain [54].

Another interesting example is given by noscapine (Nos), a drug that crosses BBB and inhibits proliferation of glioblastoma cells, but that has a short plasma half-life and undergoes rapid elimination. Madan et al. [55] constructed SLN and PEG conjugated SLN carrying Nos. The drug's plasma half-life was increased by as much as 11-fold and 5-fold, respectively, by Nos-PEG-SLN and Nos-SLN, versus drug in solution. This is the first report demonstrating a workable approach to regulating the administration of multiple injections of Nos, and warrants further studies to examine *in vivo* tumor regression. Cytotoxicity assays demonstrated that Nos-PEG-SLN possesses higher activity and a low IC₅₀, which might be attributed to the enhanced cell uptake of stealth NPs in glioblastoma cells compared to Nos-SLN and Nos as such. Interestingly, surface PEGylation was found to prevent RES uptake, explaining the prolonged circulation time of Nos-PEG-SLN and its increased bioavailability in non-RES organs. In particular, the higher brain concentration may be attributed to either endocytic uptake of Nos-PEG-SLN by brain-capillary endothelial cells, or to diffusion/convection through disrupted BBB, which further promotes the interaction of PEG with the brain endothelial cells. PEG-SLN increased both the plasma half-life and the mean retention time of Nos in the mouse brain, thereby reducing the need for multiple injections.

In a study aimed at enhancing brain-specific targeting distribution, employing surface-modified SLN, Yusuf et al. [56] prepared piperine SLN by an emulsification-solvent diffusion technique, with Tween[®] 80 coating. Piperine is a natural alkaloid having a potent antioxidant effect, with

potential applications in Alzheimer's disease, since it readily crosses the BBB. Due to intense first-pass metabolism, the administration of piperine for brain delivery is not straightforward. Piperine was successfully targeted to the brain, and was found to be effective at a low dose (2 mg/kg bodyweight) in a Tween[®] 80 solid lipid formulation; this proved to be successful in providing effective delivery across the BBB, with a generous payload and good delivery capabilities [57].

In a recent study, tristearin-tricaprin-based NLC were developed as carriers for bromocriptine, used in treating Parkinson's disease [58]. Bromocriptine, a dopamine agonist, marketed more than 30 years ago, possesses a slow onset of action (1–2 h) and prolonged half-life (3–5 h), which probably explain the lower dyskinesigenic potential compared to L-DOPA: using longer-acting dopamine agonists further reduces dyskinesia compared to L-DOPA. In this context, SLN encapsulation represents a novel strategy to obtain stable plasma levels and increase the drug's half-life. NLC were formulated and characterized and their potential in the symptomatic control of Parkinson's disease was evaluated in a rat model, after intraperitoneal administration. The results indicated attenuated akinesia in hemi-Parkinsonian rats, which was more evident for bromocriptine-loaded NLC than for free bromocriptine; this might be due to the different pharmacokinetic profiles of the two formulations.

In a recent study, Huang et al. [59] compared the pharmacokinetics and biodistribution of temozolomide (TMZ - a drug that readily crosses the BBB, and is currently used in therapy for glioblastoma) carried by SLN with conventional administration, using mice as animal model. After intravenous injection, SLN were mainly accumulated in the RES organs; however, TMZ-SLN increased significantly the drug brain targeting efficiency, which changed from 6.76% of the injected dose for free TMZ, to 13.25% for TMZ-SLN. This result was probably due to the increased concentration in the bloodstream achieved with SLN.

6.3 SLN used to improve drug bioavailability and cross the BBB

Some drugs cross the BBB to a moderate extent, but the dose diffused in the brain is not sufficient to achieve a therapeutic effect: this may be attributed to rapid elimination from the bloodstream and/or poor ability to cross the BBB. Delivery of these drugs in SLN can help to increase blood concentration, and may also enhance drug permeation through the BBB, by the mechanisms mentioned above. Although the contributions made by these two factors to increasing the brain concentration are undefined, it has been demonstrated that drugs can be delivered to the brain in therapeutically-active concentrations.

Manjunath and Venkateshwarlu made SLNs with the lipophilic drug nitrendipine (Ntd), which moderately crosses the BBB [60] [61]; their goal was to improve its bioavailability upon i.v. administration. Ntd-loaded SLNs were prepared using different triglycerides (tripalmitin, trimyristin, and tristearin), emulsifiers (soy lecithin, poloxamer 188) and charge modifiers (dicetyl phosphate, stearylamine). After i.v. administration, Ntd SLN were found to be taken up by the brain to a greater extent than was Ntd suspension. The C_{max} values in the brain (almost 5.5 mcg/G for stearylamine Ntd SLN) were respectively 3.2, 7.3, and 9.1 times higher than that of Ntd suspension, with tripalmitin SLN, tripalmitin dicetyl phosphate SLN, and tripalmitin stearylamine SLN. The study authors hypothesized that the higher Ntd brain concentrations of SLN formulations are due to entrapped drug, because free drug is immediately distributed to the fluids and eliminated, but also that the higher concentration of Ntd in the brain may be the consequence of transport of intact Ntd SLN through the BBB, by endocytosis [62].

The biodistribution patterns of orally-administered free edelfosine (a synthetic alkyllysophospholipid antineoplastic drug now in phase II trials for treating brain cancers) similarly revealed that the drug was widely scattered in many organs, while a smaller, though significant, amount was found in the brain [63] [64]. However, also in this case, when edelfosine was administered orally in SLN, the biodistribution pattern was altered, with more drug accumulating in the brain, thus supporting the hypothesis that intact SLN exist in the plasma and are subsequently

able to cross the BBB. Moreover, *in vitro* studies showed that edelfosine-loaded SLN were able to reverse the drug-resistance of C6 glioma cells, by inhibiting P-gp, thanks to the component Tween[®] 80. The efficacy of edelfosine-loaded SLN for treating glioblastoma was confirmed in a subcutaneous glioma model [65].

Dhawan et al. prepared quercetin-loaded SLN [66], using Tween[®] 80 as surfactant, for potential utilization as antioxidant in Alzheimer's disease. Although free quercetin has moderate biodistribution in the brain after systemic administration [67] [68], the results of this study on a rat Alzheimer's disease model indicate that chronic treatment with quercetin-loaded SLN can reverse the deleterious neurodegenerative effects of aluminum chloride, unlike free quercetin treatment. This was attributed to an increased uptake of quercetin in the brain with the SLN formulation.

The limited ability of many therapeutic molecules to cross the BBB can be improved by administering them through the intraventricular or intrathecal route. But since these invasive routes cause considerable patient non-compliance, specific carrier systems may be a valuable replacement. One example is intrathecal baclofen administration, the reference treatment for spasticity of spinal or cerebral origin, for which the risk of infection or catheter dysfunction are important limits. Baclofen's ability to cross the BBB is debatable: although it has been reported to cross the BBB [69], the failure of oral therapy has also frequently been attributed to its inability to cross the barrier [70]. To explore the possibility of alternative administration routes, Priano and co-workers [71] studied baclofen-loaded SLN, prepared from a multiple (w/o/w) warm microemulsion (water, stearic acid as oil phase, epikuron 200 as surfactant, with propionic acid, butyric acid, and sodium taurocholate as cosurfactants). After intraperitoneal injection, the effect of baclofen-loaded SLN in a rat model (h-reflex examination) was greater than that of baclofen solution; this was attributed to higher drug concentrations in the plasma, and especially in the CNS (nearly 700 ng/g C_{max} in the brain with SLN, compared to some 300 ng/g with solution).

6.4 SLN used to increase permeation of drugs through the BBB

SLN can be used as vehicles to interact with the BBB, enhancing the penetration of drugs that are unable to cross this barrier. In particular, two different approaches have been reported for brain delivery employing SLN: the first entails the use of plain SLN as drug delivery systems to the brain; the second explores a targeting approach to enhance SLN uptake by the endothelial cells of the BBB.

6.4.1 Plain SLN approach

Doxorubicin, a highly hydrophilic drug that does not cross the BBB, has shown significant activity against a wide range of human cancers; unfortunately, the clinical use of doxorubicin is limited by its unusual cardiomyopathy: this produces acute toxicity, which can be avoided if the drug is enabled to reach the site of action as efficiently as possible. In one such study, Zara et al. incorporated doxorubicin, as an ion-pair complex, in SLN prepared from a warm oil-in-water microemulsion containing stearic acid as internal phase, Epikuron 200 as surfactant, and taurocholate sodium salt as cosurfactant. The study showed that, after i.v. administration to rats, SLN can modify the pharmacokinetic distribution of doxorubicin, in comparison with a commercial doxorubicin solution. In particular, the drug concentration was lower in the liver, heart and kidneys, and an unusual presence of doxorubicin in the brain, which does not occur with the commercial doxorubicin solution, was noted. The lower uptake of doxorubicin SLN by the RES could increase the drug's bioavailability in non-RES tissue targeting [72].

The presence of a stealth agent may increase the plasma circulation of drugs delivered in SLN, and consequently help their brain uptake. The above experimental work was continued by Fundarò et al. [73], who prepared non-stealth and stealth SLN, containing doxorubicin as an ion-pair complex. Stearic acid-PEG 2000 was used as stealth agent. These SLN showed significantly higher drug concentration in the rat brain (nearly 10 mcg/g) compared to non-stealth SLN (2 mcg/g) and

doxorubicin solution, after i.v. administration. The Pegylated surface combined with the lipophilicity of the SLN may explain the presence of doxorubicin in the brain tissues.

These data were further confirmed by Zara et al. [74], who prepared non-stealth and stealth doxorubicin-loaded SLN. Stearic acid-PEG 2000 at three different concentrations (0.15, 0.30 and 0.45%) was used. Both non-stealth and stealth SLN enhanced the transport of doxorubicin through the BBB after i.v. administration in rabbits, in particular the amount of drug present in the brain after 30 min from injection was 27.5 ng/g for non-stealth-SLN, 75.0 ng/g for stealth-SLN-0.15, 225.0 ng/g for stealth-SLN-0.30 and 242.0 ng/g for stealth-SLN-0.45. According to the authors of the article, the increased quantity of doxorubicin reaching the brain could be explained by longer circulation time of non-stealth and stealth NPs, despite the fact that surface hydrophilicity of the stealth-SLN could hinder their passage through the BBB.

Bargoni et al. [75] demonstrated that plain SLN can cross the BBB, by studying the tissue distribution of tobramycin (tobra) loaded stearic acid SLN after duodenal and i.v administration to rats. Tobra was found in the brain when administered in SLN (5.1 $\mu\text{g/g}$ after i.v. administration), but was not detected after administration of drug solution. Zara et al. [76] compared the pharmacokinetics and tissue distribution profiles of idarubicin (ida)-loaded SLN and ida solution, administered to rats either by the duodenal route or intravenously. Ida SLN produced a fair brain concentration (11.2 ng/g 24 hours after administration), whereas none was detected after administration of the solution, indicating that SLN were able to pass the BBB.

In a study on Dalton's lymphoma bearing mice, Reddy et al. [77] evaluated the capability of etoposide loaded tripalmitin NPs (ETPL) to enhance tumor uptake of etoposide (an anticancer drug with low brain distribution), and investigated the influence of the administration route on biodistribution. ETPL NPs were prepared by melt-emulsification and HPH, followed by spray drying of the nanodispersion. Brain uptake of the ETPL NPs was significantly higher compared to free etoposide, after i.v., intraperitoneal, or subcutaneous administration, indicating the potential use of this formulation in treating brain malignancies. The brain distribution of etoposide reached 0.02

and 0.13% of the injected dose per gram of organ/tissue, for i.v. and intraperitoneal administration respectively, compared to 0.01 and 0.06 % with free etoposide.

Peira et al. prepared SLN loaded with supermagnetic iron oxide, by the microemulsion technique, and proposed this formulation as a new type of NMR contrast agent [78]. The study revealed that, after encapsulation in SLN, supermagnetic iron oxide acts as a contrast agent; it has increased brain uptake compared to supermagnetic iron oxide as such, which does not cross the BBB. This suggested that these SLN could be used as a MRI agent for CNS.

Several other studies have examined drug-loaded SLN. Bondi et al. prepared SLN loaded with riluzole (a drug used to treat amyotrophic lateral sclerosis) by a microemulsion technique [79]. The SLN formulation showed a better capability to deliver riluzole to the rat brain compared to riluzole aqueous dispersion. Gao et al. prepared SLN loaded with daidzein (one of the major soy isoflavones, used in China to prevent cardiovascular diseases) by a hot homogenization method, with PEGylated phospholipid as stabilizer [80]. Compared with free daidzein, these SLN enhanced the drug's accumulation in the brain 12 h after administration to rats. Moreover, daidzein-loaded SLN also acted as a protecting agent in a rat brain ischemia reperfusion injury model.

In a study by Koziara et al. [81], emulsifying wax SLN, stabilised with Brij 78 as surfactant (E78 SLN), and Brij 72 SLN, stabilised with Tween 80[®] as surfactant (E72 SLN), were engineered from microemulsion precursors. The SLN were radiolabeled, and their transport across the BBB was quantified *in vivo* using a rat brain perfusion method, after intra-carotid administration. Significant brain uptake of both SLN formulations compared to sucrose (a molecule which does not cross the BBB) suggested that the SLN were transported across the BBB, although the mechanism involved was not elucidated in this study.

Researchers from the same group prepared paclitaxel (a highly efficient agent against a large variety of cancers) loaded SLN, stabilized with Brij 78 [82]. The SLN formulation significantly increased the drug's distribution to the brain, compared to Taxol[®], as determined in a rat brain perfusion experiment after intra-carotid administration. In this case it was hypothesized that, by masking

paclitaxel's characteristics, the carrier limited its binding to P-gp, reducing its efflux from the brain. In addition, based on previous *in vitro* and *in vivo* studies, it was reported that the SLN formulations used had no significant effect on BBB baseline parameters, such as integrity, permeability, blood flow, and active transport of choline. Occludin and claudin-1 Western blot analyses were run to examine the molecular integrity of the tight junction, and showed no changes in protein expression [83].

Plain SLN have also been investigated for permeation *in vitro*, on artificial models of the BBB. Chattopadhyay et al. [84] proposed SLNs prepared by a thin film hydration technique for enhanced brain delivery of the potent HIV protease inhibitor atazanavir, using a well-characterized human brain microvessel endothelial cell line (hCMEC/D3) representative of the BBB. Delivery of atazanavir by SLNs led to a significantly higher accumulation by the endothelial cell monolayer, compared to the drug in aqueous solution. The results of cell viability studies, coupled with the higher drug cellular uptake that was found (nearly thrice) show that these SLNs effectively deliver atazanavir to the human brain endothelial cell line.

6.4.2 Targeting approaches

According to the above data, plain SLN can cross the BBB, but only lead to a limited increase in drug accumulation. In recent years, biology-based approaches for developing BBB drug-targeting strategies have been proposed. Delivering drugs to the brain by exploiting the different endogenous transport systems within the BBB requires drug delivery systems to be reformulated; surface engineering NPs with specific ligands can enable carrier systems to be diversified, and may possibly lead to target specificity. Modified SLN with an active targeting mechanism may have great potential, and represent a new challenge in SLN formulation.

The first study that utilized the principles of active targeting to deliver SLN was by Gupta et al. [85]; it investigated the ability of transferrin (Tf)-conjugated SLN to deliver quinine dihydrochloride to the brain, for the management of cerebral malaria. Tf receptors are reported to be

present on the surface of diverse cell types, and to mediate the internalization of iron-saturated Tf through receptor-mediated endocytosis. The receptor-mediated endocytosis of Tf from the blood into the brain is well documented [86] [87, 88]. Tf was coupled to the phosphatidylethanolamine present on the surface of SLN, by incubating Tf with quinine-loaded SLNs in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) hydrochloride in phosphate buffered saline (pH 7.4) as cross-linker. In *in vivo* studies, quinine plasma levels and tissue distribution after intravenous administration of drug-loaded Tf-SLN, unconjugated SLN, and free quinine, were investigated. Conjugation of SLN with Tf significantly enhanced brain uptake of quinine (12% of the administered dose) compared with unconjugated SLNs or drug solution. However, although the amount recovered in the brain was very high, it should be noted that the free drug solution lead to a recovery of 1.6% of the injected dose in the brain: this high level means that the drug itself crosses the BBB [89]. Clearly, though, active targeting by incorporating the drug into SLN enhances transportation [85].

In another interesting experimental study, Venishetty et al. [90] explored betahydroxybutyric acid (HBA) grafted docetaxel loaded SLN (HDSLN). Transportation of HDSLN relies on the transport of a novel ligand, HBA, by the monocarboxylic acid transporter (MCT1) which is expressed on brain endothelial cells. Surface modification of docetaxel-loaded SLN with HBA enhanced the brain uptake of docetaxel compared with unmodified docetaxel-loaded SLN (DSLN) or with Taxotere[®] (almost 1 mcg/g tissue for HDSLN, compared with 0.5 mcg/g for DSLN, and 0.2 mcg/g with Taxotere[®]). Later on Venishetty et al. [91] reported the successful delivery of docetaxel and ketoconazole using SLN surface-modified with folic acid. Docetaxel, like paclitaxel, is used in the treatment of many types of cancer, but its entry into the brain is restricted by the P-gp efflux. A potential drug–drug interaction exists between docetaxel and ketoconazole, because ketoconazole can inhibit the P-gp efflux of docetaxel at the BBB. The encapsulation of both drug and inhibitor in a colloidal drug-delivery system was found to be a suitable strategy to overcome the drug efflux mechanism. The brain permeation coefficient of folate-grafted docetaxel and ketoconazole-loaded

SLN (almost 1.5mcg/g tissue C_{max} in the brain) was 44 times higher than that of Taxotere[®]. The folic acid present on the SLN surface improved brain uptake by the folate receptor, in comparison with unmodified SLN.

In a recent study, Ren et al. prepared borneol-modified and non-borneol-modified ganciclovir (GCV)-loaded SLN, to determine whether borneol could enhance the transport of ganciclovir to the mouse brain after i.v. administration. The study demonstrated [92] that borneol markedly loosens the intercellular tight junctions in the BBB, and accelerates the transport of substances through the intercellular passages, by increasing the levels of histamine and 5-hydroxy-tryptamine in the hypothalamus, also significantly inhibiting P-gp activity. The SLN were prepared using a modified microemulsion method. Pharmacokinetic and biodistribution studies were performed in Kunming mice after i.v. administration of GCV solution (GCV-inj), GCV loaded SLN (GCV-SLN), and three types of GCV loaded SLN, containing different amounts of borneol (GCVb-SLN). The C_{max} in the brain ranged from 3.96 mcg/g for GCVinj to 4.63 mcg/g for GCV-SLN and to 10 mcg/g for GCVb-SLN. Enhancement of the penetration of GCV into the brain is probably due to a combined function of SLN and borneol [93].

Recently Kuo and Ko [94] employed 83-14 monoclonal antibody (MAb) modified SLN (83-14 MAb/SQV-SLN) to improve the brain-targeted delivery of saquinavir (SQV). 83-14 MAb, an insulin-like peptidomimetic MAb, has a strong affinity to the brain capillaries, and can strongly bind to a subunit of the human insulin receptor. Endocytosis of 83-14 MAb/SQV-SLN into human brain-microvascular endothelial cells (HBMECs) was studied by staining cell nuclei, insulin receptors, and drug carriers; increased concentrations of surface 83-14 MAb enhanced the ability to cross the BBB and uptake by HBMECs. In a different study, Allen et al. [95] prepared coated SLN from Brij 78 and emulsifying wax, with thiamine ligand (linked to 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE) via a PEG spacer). They performed rat brain perfusion experiments after intra-carotid administration, and biodistribution experiments after tail-vein administration. Although the NPs showed a long-circulating profile, the authors were unable to obtain a high NPs

concentration in the brain (<0.5% injected dose) and biodistribution studies failed to record significant differences in brain accumulation between uncoated SLN and thiamine-coated SLN; this failure was attributed to a number of factors, including insufficient thiamine ligand coating and preferential binding of the thiamine ligand to the blood thiamine transporters.

As discussed above, coating SLN with Tween[®] is a valid strategy to increase absorption of ApoE on the NPs surface, thus enhancing brain endocytosis of the SLN. Goppert and Muller made Tween[®]-stabilized SLNs for delivering drugs to the brain, using a hot HPH method [96]. As an incidental finding during this research, they discovered that apo Cy and apo CII inhibit the receptor-mediated binding of lipoproteins containing apoE, such as b-VLDL, to the LDL receptor. To achieve brain targeting it would thus be advantageous to have a high apoE/apoCII ratio adsorbed on the particles. It emerged that SLN stabilized with Tween[®] 80 showed low adsorption of apoCII, thus reducing recognition by macrophages. In the perspective of drug delivery to the brain, Blasi and co-workers [97] [98] likewise prepared and characterized Tween[®] 80 coated SLN: SLN were successfully formulated by HPH using 3 different lipid matrixes and the effect of the main preparation parameters, namely surfactant concentration and number of homogenization cycles, was investigated; freeze-drying and spray-drying of the SLN have also been performed in order to obtain a powdered form of the NPs.

Kakkar et al. [99] have also proposed SLN coated with Tween[®] 80, as vehicles to deliver curcumin to the brain via oral administration. Curcumin is an antioxidant molecule with various applications in the field of neurological and neurodegenerative diseases [100] [101]. The area under the curve values obtained in blood, after oral administration of curcumin-loaded SLN, were more than eight times higher than that for free drug; this confirms the prolonged circulation of the former. The concentration in the brain was 30 times higher for curcumin-loaded SLN than for free curcumin. The ability of curcumin-loaded SLN to reach the brain in substantial amounts is ascribed in the main to the small average particle size of curcumin-loaded SLN, which helps bypass the first-pass metabolism of curcumin in the liver. Nonionic surfactants, such as Tween[®] 80 and lecithin, used in

formulating SLNs also enhance their permeation through the intestine, owing to the high affinity between lipid particles and the intestinal membrane.

The use of cationic NPs is another important strategy to increase SLN endocytosis by the endothelial cells of the BBB [10]. As a drug delivery system, this nanosized biomaterial combines the advantages of lipid matrix and hydrophilic layer. The lipid core of cationic SLN serves as a reservoir into which to load hydrophobic drugs, and the cationic surface favors endocytosis. In addition, the positive surface charge can modify drug entrapment and release, producing an interesting pharmacokinetic distribution. It has been hypothesized that positively-charged NPs may escape the action of lysosomal enzymes, ensuring a high drug concentration around the cell nuclei.

Jin et al. [102] conjugated cationic SLN reconstituted from natural components of protein-free low-density lipoprotein, to PEGylated c-Met short interfering RNA (siRNA). RNA interference is a powerful strategy that inhibits gene expression through specific mRNA degradation; however, *in vivo* studies have shown that negatively-charged siRNA have extremely low cellular uptake and transfection efficiency; when administered intravenously they undergo rapid chemical degradation. The application of RNA interference is severely limited by instability and poor delivery into target cells and tissues, especially in the case of glioblastomas. The c-Met siRNA-PEG/SLN complex efficiently down-regulated c-Met expression levels, as well as decreasing cell proliferation in U-87MG cells *in vitro*. In an orthotopic U-87MG xenograft tumor model, intravenous administration of the complex significantly inhibited c-Met expression in the tumor tissue, and suppressed tumor growth, without showing any systemic toxicity in mice. Use of near infrared Cy5.5 conjugated SLN revealed enhanced accumulation of the siRNA-PEG/SLN complexes, specifically in the brain tumor, demonstrating the feasibility of using these complexes as a potential carrier of therapeutic siRNA for systemic treatment of glioblastoma.

Agarwal et al. [103] exploited Cationic Bovine Serum Albumin (CBSA) as a ligand for transporting methotrexate (MTX)-loaded SLN across the BBB. CBSA is a ligand with a good accumulation profile in the brain, having favorable pharmacokinetic properties and higher selectivity for brain

tissues than other organs, like the liver, heart, or lung. The ligand has been found to selectively promote transport of fluorescent probes across the BBB, which activity can be attributed to its ability to undergo adsorption-mediated transcytosis across the barrier. *In vitro*, transendothelial transport studies on brain capillary endothelial cells (BCs) found CBSA-conjugated SLNs to undergo transcytosis to a greater extent. These SLNs were preferentially taken up by BCs and by human neuroglial culture (HNGC)-1 tumor cells, compared with either unconjugated SLNs or plain MTX. The results indicate that CBSA-conjugated SLNs loaded with MTX have good prospects in brain cancer chemotherapy, due to their increased ability to cross the BBB.

In an *in vitro* study, Kuo and Wang [104] analyzed the biocompatibility of cationic SLNs upon HBMECs. They demonstrated that SQV-loaded cationic SLNs containing cholesterol can be effective in providing the controlled release of SQV, without inducing significant endothelial toxicity.

7. Conclusions

SLN have unique advantages as drug carriers. However, the mechanism translocating NPs into the brain remains incompletely understood. It has been suggested that NPs may open the tight junctions between endothelial cells in the brain microvasculature, thus creating a paracellular pathway for their translocation; other possible mechanisms include simple passive diffusion, active transport, and endocytosis. After intravenous administration, colloidal systems strongly interact with plasma proteins, through the opsonization process: hydrophobic colloidal particles are coated with plasma components known as opsonins, and rapidly removed from the circulation by the macrophages of the liver and the spleen, which possess specific opsonin receptors. Only small and hydrophilic colloidal particles can escape opsonization, and so remain in circulation for relatively prolonged periods of time, enabling them to be taken up by the brain.

SLN can be useful in the process of brain uptake at various levels: they can stabilize drugs against chemical degradation in biological fluids, increase the permanence in the bloodstream indirectly favoring translocation to the brain, or they can directly trigger the endothelial cells by inducing endocytosis, which may also be receptor-mediated through an active targeting mechanism. Although plain SLN have in the past been used as vehicles to enable drugs to cross the BBB, research is currently evolving towards the use of targeted SLN, to improve the selectivity of the interaction between NPs and endothelial cells. The ability to cross the BBB differs with the different molecules delivered, and also with the different models used to evaluate it. It must in any case be stressed that it is not as important to know precisely how SLN cross the BBB and remain within the brain, as it is to know that the drug concentration in the brain is effectively increased, compared to the corresponding free drug. It is also especially important to determine, case by case, whether the amount of drug that has reached the brain is enough for a therapeutic dose.

8. Acknowledgements

Thanks are due to Ms Frances Cooper for kindly revising the English version of the manuscript.

The authors wish to acknowledge Italian Ministry of Education - MIUR (Finanziamento Ricerca Locale – ex 60%) and Compagnia di San Paolo, under the research project “Development of solid lipid nanoparticles (SLN) as vehicles of antineoplastic drugs to improve the pharmacological glioblastoma therapy” (Ortowinst11).

9. References

- [1] A. Misra, S. Ganesh, A. Shahiwala, S. Shah, Drug delivery to the central nervous system: a review, *Journal of Pharmacy and Pharmaceutical Sciences*, 6 (2003) 252-273
- [2] W.M. Pardridge, Why is the global CNS pharmaceutical market so under-penetrated?, *Drug Discovery Today*, 7 (2002) 5–7.
- [3] W.M. Pardridge, Blood-brain barrier drug targeting: the future of brain drug development, *Molecular interventions*, 3 (2003) 90-105.
- [4] P. Blasi, S. Giovagnoli, A. Schoubben, M. Ricci, C. Rossi, Solid lipid nanoparticles for targeted brain drug delivery, *Advanced Drug Delivery Reviews*, 59 (2007) 454-477.
- [5] M. Patel, E.B. Souto, K.K. Singh, Advances in brain drug targeting and delivery: limitations and challenges of solid lipid nanoparticles, *Expert Opinion on Drug Delivery*, 10 (2013) 889-905.
- [6] M. Ricci, P. Blasi, S. Giovagnoli, C. Rossi, Delivering drugs to the central nervous system: A medicinal chemistry or a pharmaceutical technology issue?, *Current Medicinal Chemistry*, 13 (2006) 1757-1775.
- [7] B.V. Zlokovic, The blood-brain barrier in health and chronic neurodegenerative disorders, *Neuron*, 57 (2008) 178–201.
- [8] S. Ohtsuki, T. Terasaki, Contribution of carrier-mediated transport systems to the blood–brain barrier as a supporting and protecting interface for the brain; Importance for CNS drug discovery and development, *Pharmaceutical Research*, 24 (2007) 1745-1758.
- [9] T. Yoshikawa, W.M. Pardridge, Delivery of peptides and proteins through the blood–brain barrier, *Advanced Drug Delivery Reviews*, 46 (2001) 247–279.
- [10] M.W. Smith, M. Gumbleton, Endocytosis at the blood–brain barrier: From basic understanding to drug delivery strategies, *Journal of Drug Targeting*, 14 (2006) 191-214.
- [11] A. Tsuji, I. Tamai, Blood-brain barrier function of P-glycoprotein, *Advanced Drug Delivery Reviews*, 25 (1997) 287-298.
- [12] A.H. Schinkel, P-Glycoprotein, a gatekeeper in the blood–brain barrier, *Advanced Drug Delivery Reviews*, 36 (1999) 179-194.
- [13] W.M. Pardridge, Crossing the blood–brain barrier: are we getting it right?, *Drug Discovery Today*, 6 (2001) 1-2.
- [14] G.G. Grant, N.J. Abbott, D. Janigro, Understanding the physiology of the blood-brain barrier: *In vitro* models news, *News in physiological sciences*, 13 (1998) 287-293.
- [15] Q.R. Smith, Brain perfusion systems for studies of drug uptake and metabolism in the central nervous system, *Pharmaceutical biotechnology*, 8 (1996) 285–307.
- [16] Y. Takasato, S.I. Rapoport, Q.R. Smith, An in situ brain perfusion technique to study cerebrovascular transport in the rat, *The American journal of physiology*, 247 (1984) 484– 493.
- [17] F.E. D'Amour, S.D. L, A method for the determining loss of pain sensation, *Journal of Pharmacology and Experimental Therapeutics*, 72 (1941) 74-78.
- [18] L. Sun, Glioma - Exploring its biology and practical relevance, in: Intechopen (Ed.) *Animal Models of Glioma*, Anirban Ghosh, 2011.
- [19] R.K.W. Schwarting, J.P. Huston, The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments, *Progress in neurobiology*, 50 (1996) 275–331.
- [20] G.J.R. Delcroix, E. Elisa Garbayo, L. Sindji, O. Thomas, C. Vanpouille-Box, P.C. Schiller, C.N. Montero-MeneI, The therapeutic potential of human multipotent mesenchymal stromal cells

combined with pharmacologically active microcarriers transplanted in hemi-parkinsonian rats, *Biomaterials*, 32 (2011) 1560–1573.

[21] E. Garbayo, E. Ansorena, J.L. Lanciego, M.J. Blanco-Prieto, M.S. Aymerich, Long-term neuroprotection and neurorestoration by glial cell-derived neurotrophic factor microspheres for the treatment of Parkinson's disease, *Movement Disorders*, 26 (2011) 1943–1947.

[22] L. Lecanu, V. Papadopoulos, Modeling Alzheimer's disease with non-transgenic rat models, *Alzheimer's Research & Therapy*, 5 (2013).

[23] E.Z. Longa, P.R. Weinstein, S. Carlson, R.R. Cummins, eversible middle cerebral artery occlusion without craniectomy in the rats, *Stroke*, 20 (1989) 84–91.

[24] E. Barbu, E. Molnàr, J. Tsibouklis, D.C. Górecki, The potential for nanoparticle-based drug delivery to the brain: overcoming the blood–brain barrier, *Expert Opinion on Drug Delivery*, 6 (2009) 553–565.

[25] S.M. Moghimi, A.C. Hunter, J.C. Murray, Long-circulating and target-specific nanoparticles: Theory to practice, *Pharmacological Reviews*, 53 (2001) 283–318.

[26] M. Dekker, *Colloidal Drug Delivery Systems*, in, New York, 1994, pp. 219–342.

[27] I.P. Kaur, R. Bhandari, S. Bhandari, V. Kakkar, Potential of solid lipid nanoparticles in brain targeting, *Journal of Controlled Release*, 127 (2008) 97–109.

[28] S.M. Moghimi, C.J.H. Porter, I.S. Muir, L. Illum, S.S. Davis, Non-phagocytic uptake of intravenously injected microspheres in rat spleen: Influence of particle size and hydrophilic coating, *Biochemical and Biophysical Research Communications*, 177 (1991) 861–866.

[29] H.S. Choi, W. Liu, P. Misra, E. Tanaka, J.P. Zimmer, B.I.B. Ipe, M.G., J.V. Frangioni, Renal Clearance of Nanoparticles, *Nature Biotechnology* 25 (2007) 1165–1170.

[30] J. Kreuter, S. Gelperina, Use of nanoparticles for cerebral cancer, *Tumori*, 94 (2008) 271–277.

[31] L. Juillerat-Jeanneret, The targeted delivery of cancer drugs across the blood–brain barrier: chemical modifications of drugs or drug-nanoparticles?, *Drug Discovery Today*, 13 (2008) 1099–1106.

[32] M. Mazza, I.F. Uchegbu, A.G. Schätzlein, Cancer and the blood–brain barrier: ‘Trojan horses’ for courses?, *British Journal of Pharmacology*, 155 (2008) 149–151.

[33] D. Karkan, C. Pfeifer, T.Z. Vitalis, G. Arthur, M. Ujjiie, Q. Chen, S. Tsai, G. Koliatis, R. Gabathuler, W.L. Jefferies, A unique carrier for delivery of therapeutic compounds beyond the blood-brain barrier, *Plos one*, 3 (2008) 1–14.

[34] T.M. Göppert, R.H. Müller, Plasma protein adsorption of Tween 80-and poloxamer 188-stabilized solid lipid nanoparticles, *Journal of drug targeting*, 11 (2003) 225–231.

[35] S.D. Tröster, K.H. Wallis, R.H. Müller, J. Jörg Kreuter, Correlation of the surface hydrophobicity of ¹⁴C-poly(methyl methacrylate) nanoparticles to their body distribution, *Journal of Controlled Release*, 20 (1992) 247–260.

[36] J. Kreuter, Application of nanoparticles for the delivery of drugs to the brain, *International Congress Series*, 1277 (2005) 85–94.

[37] A. Zensi, D. Begley, C. Pontikis, C. Legros, L. Mihoreanu, S. Wagner, C. Büchel, H. von Briesen, J. Kreuter, Albumin nanoparticles targeted with Apo E enter the CNS by transcytosis and are delivered to neurones, *Journal of Controlled Release*, 137 (2009) 78–86.

[38] D.S. Miller, B. Bauer, A.M.S. Hartz, Modulation of P-Glycoprotein at the blood-brain barrier: Opportunities to improve central nervous system pharmacotherapy, *Pharmacological Reviews* 60 (2008) 196–209.

[39] L. Battaglia, M. Gallarate, Lipid nanoparticles: state of the art, new preparation methods and challenges in drug delivery, *Expert Opinion on Drug Delivery*, 9 (2012) 497–508.

[40] B. Lasa-Saracibar, A. Estella-Hermoso de Mendoza, M. Guada, C. Dios-Vieitez, M.J. Blanco-Prieto, Lipid nanoparticles for cancer therapy: state of the art and future prospects, 2012, 9 (2012) 1245–1261.

- [41] R.H. Müller, K. Karsten Mäder, S. Gohla, Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art, *European Journal of Pharmaceutics and Biopharmaceutics*, 50 (2000) 161–177.
- [42] R.H. Muller, S. Maassen, H. Weyhers, W. Mehnert, Phagocytic uptake and cytotoxicity of solid lipid nanoparticles (SLN) sterically stabilized with poloxamine 908 and poloxamer 407, *Journal of Drug Targeting*, 4 (1996) 161-170
- [43] A. zur Mühlen, C. Schwarz, W. Mehner, Solid lipid nanoparticles (SLN) for controlled drug delivery – Drug release and release mechanism, *European Journal of Pharmaceutics and Biopharmaceutics*, 45 (1998) 149–155.
- [44] C. Freitas, R.H. Müller, Effect of light and temperature on zeta potential and physical stability in solid lipid nanoparticle (SLNTM) dispersions, *International Journal of Pharmaceutics*, 168 (1998) 221–229.
- [45] L. Di, E.H. Kerns, Y. Hong, H. Chen, Development and application of high throughput plasma stability assay for drug discovery, *International Journal of Pharmaceutics*, 297 (2005) 110–119.
- [46] C.S. Djuzenova, T. Güttler, S. Berger, A.F. Katzer, M. Differential response of human glioblastoma cell lines to combined camptothecin and ionizing radiation treatment, *Cancer Biology & Therapy*, 7 (2008) 364-373.
- [47] T.H. Tsai, C.H. Lee, P.H. Yeh, Effect of P-glycoprotein modulators on the pharmacokinetics of camptothecin using microdialysis, *British Journal of Pharmacology*, 134 (2001) 1245–1252.
- [48] S. Yang, J. Zhu, Y. Lu, B. Liang, C. Yang, Body distribution of camptothecin solid lipid nanoparticles after oral administration, *Pharmaceutical Research*, 16 (1999) 751-757.
- [49] S.C. Yang, L.I. Lu, Y. Caic, J.B. Zhu, B.W. Liang, C.Z. Yang, Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain, *Journal of Controlled Release*, 59 (1999) 299–307.
- [50] S.M. Martins, B. Sarmiento, C. Nunes, M. Lúcio, S. Reis, D.C. Ferreira, Brain targeting effect of camptothecin-loaded solid lipid nanoparticles in rat after intravenous administration, *European Journal of Pharmaceutics and Biopharmaceutics*, 85 (2013) 488–502.
- [51] S. Martins, I. Tho, I. Reimold, G. Fricker, E. Souto, D. Ferreira, M. Brandl, Brain delivery of camptothecin by means of solid lipid nanoparticles: Formulation design, in vitro and in vivo studies, *International Journal of Pharmaceutics*, 439 (2012) 49–62.
- [52] K. Manjunath, V. Venkateswarlu, Pharmacokinetics, tissue distribution and bioavailability of clozapine solid lipid nanoparticles after intravenous and intraduodenal administration, *Journal of Controlled Release*, 107 (2005) 215–228.
- [53] G.R. Spratto , A.L. Woods, *Delmar nurse's drug handbook*, in, 2009.
- [54] J.-X. Wang, X. Sun, Z.-R. Zhang, Enhanced brain targeting by synthesis of 3',5'-dioctanoyl-5-fluoro-2'-deoxyuridine and incorporation into solid lipid nanoparticles, *European Journal of Pharmaceutics and Biopharmaceutics*, 54 (2002) 285–290.
- [55] J. Madan, R.S. Pandey, V. Jain, O.M. Katare, R. Chandra, A. Katyal, Poly (ethylene)-glycol conjugated solid lipid nanoparticles of noscipine improve biological half-life, brain delivery and efficacy in glioblastoma cells *Nanomedicine: Nanotechnology, Biology and Medicine*, 9 (2013) 492–503.
- [56] S.H. Buhner, *Herbal antibiotics: Natural alternatives for treating drug-resistant bacteria*, in, Storey Publishing, 2012.
- [57] M. Yusuf, M. Khan, R.A. Khan, B. Ahmed, Preparation, characterization, in vivo and biochemical evaluation of brain targeted Piperine solid lipid nanoparticles in an experimentally induced Alzheimer's disease model, *Journal of Drug Targeting*, 21 (2013) 300–311.
- [58] E. Esposito, M. Fantin, M. Marti, M. Drechsler, L. Paccamiccio, P. Mariani, E. Sivieri, F. Lain, E. Menegatti, M. Morari, R. Cortesi, Solid lipid nanoparticles as delivery systems for bromocriptine, *Pharmaceutical Research*, 25 (2008) 1521-1530.

- [59] G. Huang, N. Zhanga, X. Bia, M. Doub, Solid lipid nanoparticles of temozolomide: Potential reduction of cardiac and nephric toxicity, *International Journal of Pharmaceutics*, 355 (2008) 314–320.
- [60] C.J. Maxwell, D.B. Hogan, E.M. Ebly, Calcium-channel blockers and cognitive function in elderly people: results from the Canadian study of health and aging, *Canadian Medical Association Journal*, 161 (1999) 501-506.
- [61] S. Uchida, S. Yamada, K. Nagai, Y. Deguchi, R. Kimura, Brain pharmacokinetics and in vivo receptor binding of 1,4-dihydropyridine calcium channel antagonists, *Life Sciences*, 61 (1997) 2083-2090.
- [62] K. Manjunath, V. Venkateshwarlu, Pharmacokinetics, tissue distribution and bioavailability of nitrendipine solid lipid nanoparticles after intravenous and intraduodenal administration, *Journal of Drug Targeting*, 14 (2006) 632–645.
- [63] A.E.-H. de Mendoza, M.A. Campanero, J. de la Iglesia-Vicente, C. Gajate, F. Mollinedo, M.J. Blanco-Prieto, Antitumor alkyl ether lipid edelfosine: Tissue distribution and pharmacokinetic behavior in healthy and tumor-bearing immunosuppressed mice, *Clinical Cancer Research* 15 (2009) 858-864.
- [64] A. Nagler, Edelfosine for the treatment of brain tumours, U.S. Patent 6514519 B1 (2003).
- [65] E.H.A. de Mendoza, V.M. Pr at, F., M.J. Blanco-Prieto, In vitro and in vivo efficacy of edelfosine-loaded lipid nanoparticles against glioma, *Journal of Controlled Release*, 156 (2011) 421–426.
- [66] S. Dhawan, R. Kapil, B. Singh, Formulation development and systematic optimization of solid lipid nanoparticles of quercetin for improved brain delivery, *Journal of Pharmacy and Pharmacology*, 63 (2011) 342–351.
- [67] L. Liu, Y. Tang, C. Gao, Y. Li, S. Chen, T. Xiong, J. Li, M. Du, Z. Gong, H. Chen, L. Liu, P. Yao, Characterization and biodistribution in vivo of quercetin-loaded cationic nanostructured lipid carriers, *Colloids and Surfaces B: Biointerfaces*, 115 (2014) 125-131.
- [68] W. Gang, W.J. Jie, Z.L. Ping, D.S. Ming, L.J. Ying, W. Lei, Y. Fang, Liposomal quercetin: evaluating drug delivery in vitro and biodistribution in vivo, *Expert Opinion on Drug Delivery*, 9 (2012) 599-613.
- [69] J.B. van Bree, C.D. Heijligers-Feijen, A.G. de Boer, M. Danhof, D.D. Breimer, Stereoselective transport of baclofen across the blood-brain barrier in rats as determined by the unit impulse response methodology, *Pharmaceutical Research*, 8 (1991) 259-262.
- [70] M.K. Shellenberger, L. Groves, J. Shah, G.D. Novack, A controlled pharmacokinetic evaluation of tizanidine and baclofen at steady state., *Drug Metabolism and Disposition*, 27 (1999) 201–204.
- [71] L. Priano, G.P. Zara, N. El-Assawy, S. Cattaldo, E. Muntoni, E.S. Milano, L., C. Musicanti, C. P erot, M.R. Gasco, G. Miscio, A. Mauro, Baclofen-loaded solid lipid nanoparticles: Preparation, electrophysiological assessment of efficacy, pharmacokinetic and tissue distribution in rats after intraperitoneal administration, *European Journal of Pharmaceutics and Biopharmaceutics*, 79 (2011) 135–141.
- [72] G.P. Zara, R. Cavalli, A. Fundar , A. Bargoni, O. Caputo, M.R. Gasco, Pharmacokinetics of doxorubicin incorporated in solid lipid nanospheres pharmacological research, *Pharmacological Research*, 40 (1999) 281–286.
- [73] A. Fundar , R. Cavalli, A. Bargoni, D. Vighetto, G.P. Zara, M.R. Gasco, Non-stealth and stealth solid lipid nanoparticles (SLN) carrying doxorubicin: pharmacokinetics and tissue distribution after I.V. administration to rats, *Pharmacological Research*, 42 (2000) 337–343.
- [74] G.P. Zara, R. Cavalli, A. Bargoni, Intravenous administration to rabbits of non-stealth and stealth doxorubicin-loaded solid lipid nanoparticles at increasing concentrations of stealth agent: pharmacokinetics and distribution of doxorubicin in brain and other tissues, *Journal of Drug Targeting*, 10 (2002) 327-335.

- [75] A. Bargoni, R. Cavalli, G.P. Zara, A. Fundarò, O. Caputo, M.R. Gasco, Transmucosal Transport of Tobramycin incorporated in Solid Lipid Nanoparticles (SLN) after Duodenal Administration to Rats. Part II – Tissue Distribution, *Pharmacological Research*, 43 (2001) 497–502.
- [76] G.P. Zara, A. Bargoni, R. Cavalli, A. Fundarò, D. Vighetto, M.R. Gasco, Pharmacokinetics and tissue distribution of idarubicin-loaded solid lipid nanoparticles after duodenal administration to rats, *Journal of Pharmaceutical Sciences*, 91 (2002) 1324–1333.
- [77] L.H. Reddy, R.K. Sharmab, K. Chuttanib, A.K. Mishrab, R.S.R. Murthya, Influence of administration route on tumor uptake and biodistribution of etoposide loaded solid lipid nanoparticles in Dalton's lymphoma tumor bearing mice, *Journal of Controlled Release*, 105 (2005) 185–198.
- [78] E. Peira, P. Marzola, V. Podio, S. Aime, A. Sbarbati, M.R. Gasco, In Vitro and In Vivo Study of Solid Lipid Nanoparticles Loaded with Superparamagnetic Iron Oxide, *Journal of Drug Targeting*, 11 (2003) 19-24.
- [79] M.L. Bondi, E.F. Craparo, G. Giammona, F. Drago, Brain-targeted solid lipid nanoparticles containing riluzole: preparation, characterization and biodistribution, *Nanomedicine: Nanotechnology, Biology and Medicine*, 5 (2010) 25-32.
- [80] Y. Gao, W. Gu, L. Chen, Z. Xu, Y. Li, The role of daidzein-loaded sterically stabilized solid lipid nanoparticles in therapy for cardio-cerebrovascular diseases, *Biomaterials*, 29 (2008) 4129-4136.
- [81] J.M. Koziara, P.R. Lockman, D.D. Allen, R.J. Mumper, In situ blood-brain barrier transport of nanoparticles, *Pharmaceutical Research*, 20 (2003) 1772-1778.
- [82] J.M. Koziara, P.R. Lockman, D.D. Allen, R.J. Mumper, Paclitaxel nanoparticles for the potential treatment of brain tumors, *Journal of Controlled Release*, 99 (2004) 259-269.
- [83] P.R. Lockman, J. Koziara, K.E. Roder, J. Paulson, T.J. Abbruscato, R.J. Mumper, D.D. Allen, *In vivo* and *in vitro* assessment of baseline blood-brain barrier parameters in the presence of novel nanoparticles, *Pharmaceutical Research*, 20 (2003) 705-713.
- [84] N. Chattopadhyay, J. Zastre, H.L. Wong, X.W. Wu, R. Bendayan, Solid lipid nanoparticles enhance the delivery of the HIV protease inhibitor, atazanavir, by a human brain endothelial cell line, *Pharmaceutical Research*, 25 (2008) 2262-2271.
- [85] Y. Gupta, A. Jain, S.K. Jain, Transferrin-conjugated solid lipid nanoparticles for enhanced delivery of quinine dihydrochloride to the brain, *Journal of pharmacy and pharmacology* 59 (2007) 935–940.
- [86] H. Hatakeyama, H. Akita, K. Maruyama, T. Suhara, H. H, Factors governing the in vivo tissue uptake of transferrin-coupled polyethylene glycol liposomes *in vivo*, *International Journal of Pharmaceutics*, 281 (2004) 25–33.
- [87] C.C. Visser, S. Stevanović, L.H. Voorwinden, P.J. Gaillard, D.J.A. Crommelin, M. Danhof, A.G. de Boer, Validation of the transferrin receptor for drug targeting to brain capillary endothelial cells *in vitro*, *Journal of drug targeting*, 12 (2004) 145-150.
- [88] V. Soni, D.V. Kohli, S.K. Jain, Transferrin coupled liposomes as drug delivery carriers for brain targeting of 5-florouracil, *Journal of drug targeting*, 13 (2005) 245-250.
- [89] J. Franco-Pérez, C. Paz, Quinine, a selective gap junction blocker, decreases REM sleep in rats, *Pharmacology Biochemistry and Behavior*, 94 (2009) 250–254.
- [90] V. K. Venishetty, R. Samala, R. Komuravelli, M. Kuncha, R. Sistla, P.V. Diwan, β -Hydroxybutyric acid grafted solid lipid nanoparticles: A novel strategy to improve drug delivery to brain, *Nanomedicine: Nanotechnology, Biology, and Medicine*, 9 (2013) 388–397.
- [91] V. K. Venishetty, R. Komuravelli, M. Kuncha, R. Sistla, P.V. Diwan, Increased brain uptake of docetaxel and ketoconazole loaded folate-grafted solid lipid nanoparticles, *Nanomedicine: Nanotechnology, Biology, and Medicine*, 9 (2013) 111–121.

- [92] C. Gao, X. Li, Y. Li, L. Wang, M. Xue, Pharmacokinetic interaction between puerarin and edaravone, and effect of borneol on the brain distribution kinetics of puerarin in rats, *Journal of pharmacy and pharmacology*, 62 (2010) 360–367.
- [93] J. Ren, M. Zou, P. Gao, Y. Wang, G. Cheng, Tissue distribution of borneol-modified ganciclovir-loaded solid lipid nanoparticles in mice after intravenous administration, *European Journal of Pharmaceutics and Biopharmaceutics*, 83 (2013) 141-148.
- [94] Y.-C. Kuo, H.-F. Ko, Targeting delivery of saquinavir to the brain using 83-14 monoclonal antibody-grafted solid lipid nanoparticles, *Biomaterials*, 34 (2013) 4818-4830.
- [95] D.D. Allen, P.R. Lockman, M.O. Oyewumi, J.M. Koziara, K.E. Roder, R.J. Mumper, Brain uptake of thiamine-coated nanoparticles, *Journal of controlled release*, 93 (2003) 271–282.
- [96] T.M. Goppert, R.H. Muller, Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: comparison of plasma protein adsorption patterns, *Journal of drug targeting*, 13 (2005) 179-187.
- [97] P. Blasi, S. Giovagnoli, A. Schoubben, C. Puglia, F. Bonina, C. Rossi, M. Ricci, Lipid nanoparticles for brain targeting I. Formulation optimization, *International Journal of Pharmaceutics*, 419 (2011) 287– 295.
- [98] P. Blasi, A. Schoubben, G.V. Romano, S. Giovagnoli, A. Di Michele, M. Ricci, Lipid nanoparticles for brain targeting II. Technological characterization, *Colloids and Surfaces B: Biointerfaces* 110 (2013) 130– 137.
- [99] V. Kakkar, A.K. Mishra, K. Chuttani, I.P. Kaur, Proof of concept studies to confirm the delivery of curcumin loaded solid lipid nanoparticles (C-SLNs) to brain, *International Journal of Pharmaceutics*, 448 (2013) 354– 359.
- [100] V. Kakkar, I.P. Kaur, Evaluating potential of curcumin loaded solid lipid nanoparticles in aluminium induced behavioural, biochemical and histopathological alterations in mice brain, *Food and chemical toxicology*, 49 (2011) 2906–2913.
- [101] V. Kakkar, I.P. Kaur, Antidepressant activity of curcumin loaded solid lipid nanoparticles (C-SLNs) in mice, *American journal of pharmtech research*, 2 (2012) 729–736.
- [102] J. Jin, K.H. Bae, H. Yang, S.J. Lee, H. Kim, Y. Kim, K.M. Joo, S.W. Seo, T.G. Park, D.-H. Nam, *In vivo* specific delivery of c-Met siRNA to glioblastoma using cationic solid lipid nanoparticles, *Bioconjugate Chemistry*, 22 (2011) 2568–2572.
- [103] A. Agarwal, S. Majumder, H. Agrawal, S. Majumdar, G.P. Agrawal, Cationized albumin conjugated solid lipid nanoparticles as vectors for brain delivery of an anti-cancer drug *Current Nanoscience*, 7 (2011) 71-80.
- [104] Y.C. Kuo, C.C. Wang, Cationic solid lipid nanoparticles with primary and quaternary amines for release of saquinavir and biocompatibility with endothelia, *Colloids and Surfaces B: Biointerfaces*, 101 (2013) 101– 105.

Drug	SLN functionalization	Particle size (nm)	Study addressing the BBB	References
SLN used to stabilize molecules with physico-chemical or biological instability				
Camptothecin	plain SLN	196.8	biodistribution	[48]
Camptothecin	plain SLN	196.8	biodistribution	[49]
		< 200	biodistribution, uptake by brain capillary endothelial cells (BCEC)	
Camptothecin	Tween 80 coated SLN			[51]
Camptothecin	Tween 80 coated SLN	130-160	biodistribution	[50]
SLN used to improve the bioavailability of drugs that cross the BBB				
Clozapine	plain SLN	96.7-163.3	biodistribution	[52]
Floxuridine	plain SLN	76	biodistribution	[54]
Noscapine	PEG conjugated SLN	80.5	biodistribution	[55]
		312.0	biodistribution, Alzheimer's disease model	
Piperine	Tween 80 coated SLN			[57]
Bromocriptine	plain SLN	154.3-216.8	Parkinson's disease model	[58]
Temozolomide	plain SLN	65.9	biodistribution	[59]
SLN used to improve bioavailability of drugs and to cross the BBB				
Nitrendipine	plain SLN	101.9-123.5	Biodistribution	[62]
		105.4-111.2	biodistribution, glioma model	
Edelfosine	Tween 80 coated SLN			[65]
Quercetin	Tween 80 coated SLN	152	Alzheimer's disease model	[66]
		161.4	biodistribution, H-reflex examination	
Baclofen	plain SLN			[71]
SLN used to increase drug permeation through the BBB				
Doxorubicin	plain SLN	80	biodistribution	[72]
Doxorubicin	PEG 2000 coated SLN	90	biodistribution	[73]
Doxorubicin	PEG 2000 coated SLN	58-95.5	biodistribution	[74]
Tobramycin	plain SLN	85	biodistribution	[75]
Idarubicin	plain SLN	80	biodistribution	[76]
Etoposide	plain SLN	387	biodistribution	[77]
iron oxide	plain SLN	159-233	biodistribution	[78]
Riluzole	plain SLN	88	biodistribution	[79]
		126	biodistribution, ischemia-reperfusion injury model	
daidzein	PEG 2000 coated SLN			[80]
	Brij 72 and Brij 78 coated SLN	<100 (58, 98)		
-	SLN		rat brain perfusion model	[81]
Paclitaxel	Brij 78 coated SLN	<100	rat brain perfusion model	[82]
	transferrin conjugated SLN	108-126		
Quinine	SLN		biodistribution	[85]
	betreliesoxybutyric acid grafted SLN	76.4		
Docetaxel			biodistribution	[90]
docetaxel, ketoconazole	folic acid grafted SLN	95.9		
Ganciclovir	borneol-modified SLN	113.7-142.5	biodistribution	[93]
	monoclonal antibody (MAb) modified SLN	100-500	human brain-microvascular endothelial cells permeation	[94]
Saquinavir				
-	thiamine coated SLN	67	rat brain perfusion model,	[95]

curcumin	Tween 80 coated SLN	N.D. 117.4	biodistribution biodistribution	[99]
c-Met siRNA	cationic SLN		biodistribution, glioma model	[102]
Methotrexate	cationic bovine serum albumin coated SLN	94.5	BCs permeation	[103]
Saquinavir	cationic SLN	85.4-94.5	human brain-microvascular endothelial cells permeation	[104]