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TeaserInnovative strategies to design carriers made up of biocompatible materials and structured as supramolecular aggregates are discussed to propose novel approaches for brain drug delivery.

Supramolecular devices to improve the treatment of brain diseases

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The blood–brain barrier (BBB) hinders the accumulation of active compounds in the central nervous system, thus decreasing their therapeutic effectiveness. To overcome this obstacle, interesting supramolecular nanodevices are herein considered. These systems have many advantages over the conventional formulations, such as having structures made up of biocompatible and biodegradable materials, the possibility of bypassing the BBB in a non-invasive manner (without structural modifications) and the possibility of being structurally modified to modulate the biopharmaceutical properties of the encapsulated compounds. Polymolecular (liposomes, niosomes, nanogels) and oligomolecular (cyclodextrins) devices have potential clinical applications in brain drug delivery, being capable of active targeting that can concentrate bioactives in the brain.

The development of systems that deliver drugs to the central nervous system (CNS) represents one of the main fields of interest in modern pharmaceutical research. The treatment of CNS diseases is very difficult because the brain is not directly accessible to intravenously (i.v.) administered drugs owing to the presence of the blood–brain barrier (BBB) [1]. Nearly 100% of high molecular weight drugs, such as peptides, Mabs, RNAi-based drugs and 98% of small-molecule drugs, cannot cross the BBB [2]. It is a specialized physiological structure made up of highly differentiated endothelial cells, characterized by the presence of intercellular tight junctions, which hinder the access of ions, charged molecules and high molecular weight molecules into the brain parenchyma.

Anatomical compartments and physiological mechanisms are also involved in the passage of substances through the BBB. Passive diffusion, which generally occurs in other body compartments as a function of the molecular weights and lipophilic characteristics of various compounds, is regulated in the CNS by the presence of highly efficient efflux pumps (ATP-binding cassette transporters) such as P-glycoprotein (P-gp) involved in multidrug resistance (MDR) [3]. These mechanisms protect the brain from the external environment and exogenous xenobiotics,

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TABLE 1

Invasive and non-invasive strategies proposed for brain drug delivery

Strategies	Outcome	Methods	References
Invasive strategies			
Surgical implantation of polymeric matrices	Controlled release of drug by an intracranial implanted-biodegradable polymeric system	Intracranial implantation of wafers composed of doxorubicin and a biodegradable polymer	[23,24]
Neurosurgical disruption of the BBB	Opening of the tight junction for a few hours as an effect of the variation in the osmotic pressure	Intracarotid administration of a hyperosmotic solution of mannitol	[25]
Non-invasive strategies			
Modification of the surface properties of carriers to obtain active targeting of the BBB	Site-specific delivery of targeted drugs by means of the decoration of carrier surface with an appropriate compound that recognizes a specific transporter system (AME, RME or CMT systems)	Direct conjugation of ligands on the surface of carriers by means of covalent [34,36] or non-covalent [64] synthetic approaches	[34,36,64]
		Indirect coating of nanovector surface with hydrophilic surfactants	[30,31,33]

preserving its physiological functions. Nevertheless, nutrients and oxygen must be delivered here because of the complex structure of neurons. This is why transporters and ion channels are involved in the delivery of nutrients and bioactive compounds to the CNS. In this overview, invasive and non-invasive strategies are discussed (Table 1) [4–7].

In particular, invasive strategies include mainly transcranial drug delivery and BBB disruption or modification techniques, whereas non-invasive strategies include the conjugation of small molecules with lipid anchors and/or macromolecular compounds, as well as the preparation of innovative devices [2,8] to assure the delivery.

Invasive methods imply structural modifications of the BBB that allow the entry of various endogenous substances such as plasma proteins into the brain, thus inducing neurotoxicity [9]. The lipidization of small molecules is a method used to increase the lipophilicity of drugs that do not otherwise cross the BBB because of their hydrophilicity. However, this method causes an increase in molecular weight and a decrease of the plasma area under the concentration curve, features that are inversely related to brain uptake. Moreover, the degree of solubility in the cerebral interstitial fluids is reduced and this could explain their inefficiency [2,10].

The use of drug delivery systems, for example colloidal vesicles (liposomes [11,12] and niosomes [13]), macromolecular carriers (cyclodextrins) [14,15] and nanogels [16], represents an innovative strategy that enables the physiological crossing of the BBB, thus minimizing systemic drug levels and, consequently, their side effects. In this scenario, the combination of these carriers, made up of macromolecular and lipidic materials as native and/or biological molecules (i.e. Mabs, endogenous ligands), has led to the advancement of the supramolecular devices. The use of these innovative carriers has provided encouraging results in the experimental treatment of brain tumors and other cerebral diseases.

Recently, an innovative perspective in pharmaceutical fields has been gained by the design of supramolecular devices for the delivery of bioactive compounds [17,18]. The conjugation strategies and preparation procedures employed in the use of these systems are extensively used in the modification of the physicochemical characteristics of carriers to give them the necessary features for brain delivery. Although various innovative carriers have been proposed in recent years, the best strategy is the modification of conventional carriers. The modification of the carrier surface through the use of macromolecules (i.e. polymers, phospholipids, amino acids and peptides) has enabled us to bring about long-circulating properties and active-targeting characteristics. In general, this effect has been obtained by coating liposomal, niosomal or nanogel surfaces with polyethylene glycol (PEG) and/or other copolymer moieties, which prevents the opsonization phenomena and reticuloendothelial system (RES) uptake, and also by conjugating targeting agents (such as Mabs or small selective ligands) to supramolecular devices [1,19–22].

In this overview, our purpose is to furnish a clear vision of the supramolecular devices used for brain drug delivery and to describe the strategies that ameliorate brain accumulation of bioactive molecules.

Active targeting for brain drug delivery

In the treatment of brain diseases, the failure of the chosen therapy is often caused by the inability of i.v. drugs to overcome the BBB and to act on the brain parenchyma. As previously reported, various invasive strategies such as neurosurgical disruption of the BBB and the surgical implantation of polymeric matrices, such as microspheres loaded with an anticancer drug, have been used to enhance drug penetration into the brain [23,24]. However, the intracranial implantation of the device is a highly invasive and dangerous method, owing to the possibility of provoking various side effects, such as infections and cerebral edemas.

Non-surgical strategies can also be invasive, and have been considered in our investigation. These approaches consist of the intracarotid administration of a hyperosmotic solution of various sugar macromolecules, such as mannitol, leading to a rapid diffusion of the injected fluid across the cerebral endothelium, from the endothelial cells into the vascular lumen, thus causing the opening of the tight junctions for a few hours, as a result of the variation in osmotic pressure [25,26].

According to a recent revision of the available literature on the use of colloidal carriers for the delivery of drug compounds to the brain [4], the active targeting of the BBB represents the best and most utilized non-invasive approach. It is generally mediated by the presence of specific molecules on the outer surfaces of the carriers, which allows the targeting of encapsulated substances to inner CNS compartments by endocytosis. Normally, these mechanisms are involved in the internalization of nutrients and include carrier-mediated transport (CMT), receptor-mediated endocytosis (RME) and adsorptive-mediated endocytosis (AME)

systems. The CMT and AME systems are involved in the transport of small molecules between blood and brain, whereas RME systems are involved in the delivery of various endogenous high-molecular-weight molecules [2]. Transporters for sugar and amino acids, such as the carriers for D-glucose (GLUT-1), monocarboxylic acids (MCT1), large neutral aminoacids (LAT1), excitatory aminoacid (EAAT), cationic amino acids and organic cations, are generally distributed on the cerebral endothelium vessels of the BBB [27] and are involved in BBB delivery, as are the macromolecular transporters, such as specific receptors for low density lipoprotein (LDL), insulin, insulin-like growth factor, interleukin-1 (IL-1), folic acid and transferrin (Tf) [28]. Many drugs were designed to interact specifically with AME, RME and CMT systems. In particular, AME systems were one of the most investigated mechanisms for active targeting of brain tumors [29]. Unlike receptor-mediated transcytosis, AME systems do not require specific binding sites on cell surfaces and the endocytotic process takes place after the interaction between positively charged ligands and negatively charged membranes of the BBB. This is why an important strategy has been using positively charged compounds conjugated on the surfaces of drug-loaded supramolecular devices to promote interaction with the BBB. The modification of the surface charges of devices can thus be directly obtained by conjugating ligands on the surfaces of carriers by covalent or non-covalent synthetic approaches, or indirectly obtained by coating a nanovector surface with hydrophilic surfactants, such as polysorbate 80 (Tween®80), which are able to adsorb endogenous ligands after i.v. administration, thus transforming themselves into *in vivo* supramolecular devices. In fact, it is well known that Tween®80-coated carriers are internalized to the brain, acting as 'Trojan horses', as a consequence of the strong interaction of the surfactant with apolipoprotein E (ApoE), an essential factor involved in the transport of lipoproteins through the BBB by means of the LDL receptors [27,30,31].

The mechanism of drug transport into the brain by Tween®80-decorated carriers is still not fully understood. Moreover, some studies, such as that of Olivier *et al.* [32], demonstrated the presence of a toxic effect of Tween®80 on the BBB as a result of its nonspecific opening, caused by the modification or disruption of the tight junctions between brain microvessel endothelial cells. However, further studies have demonstrated that increased brain uptake is not caused by toxicity-related disruption or the opening of the BBB, as demonstrated by the evaluation of inulin and sucrose fluxes [33]. In fact, the BBB permeability of the radioactively labeled extracellular markers [^{14}C] sucrose and [^{3}H] inulin did not change significantly in comparison to the untreated control cells. The greater cellular uptake was probably caused by: (i) a recruitment of cerebral capillaries; (ii) stimulation of endocytosis in the endothelium; (iii) modulation rather than disruption of tight-junction permeability; or (iv) a combination of these factors. So, a new strategy was recently conceived based on the conjugation of apolipoproteins on a nanoparticle surface [30,34–36].

CMT systems are highly expressed on the cerebral endothelium. They play an important part because they mediate the internalization of nutrients such as glucose, choline, aminoacids and nucleotides to the brain parenchyma [37]. These carriers, which include GLUT-1 and choline transporter, can be used with the aim of targeting and enhancing drug delivery to the CNS.

RME systems require the binding of a ligand to a specific receptor located on the luminal membrane of the BBB. The interaction between ligand and receptor promotes the internalization process through endocytosis of the ligand–receptor complex. There are two types of ligands used in this approach: endogenous and chimeric. Endogenous ligands, such as insulin and Tf, the receptors of which are expressed in human cerebral capillaries, are widely used for CNS drug delivery [38,39]; whereas chimeric ligands, for example modified Mabs, bind extracellular receptors in a different site to that used by endogenous ligands, thus avoiding interference phenomena.

Colloidal carriers for brain drug delivery

Colloidal drug delivery systems include micelles, emulsions, liposomes, niosomes, nanoparticles (lipidic and polymeric), cyclodextrins and nanogels. Supramolecular devices are generally used to increase cellular or tissue targeting, to improve bioavailability of molecules, to modulate physicochemical parameters and to protect drugs from metabolic modification. All these factors lead to an increase in the amount of drugs that reach the CNS, as a result of the modulation of the properties of the supramolecular devices (Table 2).

PEGylation strategies

After i.v. administration, the 'opsonization phenomenon' can occur. This is based on the interaction of devices with elements of complement and, successively, with the immunoglobulins (Igs). Opsonization removes opsonin-coated supramolecular carriers from blood circulation and transports them to the liver and spleen by a macrophage-mediated process, where they are metabolized [40]. One way of overcoming the opsonization phenomenon is coating the surface of devices with hydrophilic moieties, such as PEG and/or other co-polymers that are able to prevent the phenomenon by masking the delivery systems from macrophage uptake and allowing the carriers to remain in blood circulation for a prolonged time [41,42].

However, various research teams [43,44] observed that repeated injections of PEGylated liposomes modify the pharmacokinetic properties of the colloidal carriers, causing them to be cleared rapidly from the bloodstream through liver accumulation, a phenomenon known as accelerated blood clearance (ABC) [45]. This phenomenon has a great impact in clinical settings, because an altered biodistribution of drug-loaded PEG-coated liposomes can cause toxicity or side effects [46]. In particular, Ishida and Kiwada [47] demonstrated that the ABC phenomenon is based on an accelerated splenic synthesis of anti-PEG IgM, a biological response that is triggered by the injection of PEGylated liposomes [47]. Anti-PEG IgM selectively binds the polymer after the successive administrations of the vesicular devices activating, subsequently, the complement system that leads to an enhanced uptake by Kupfer cells of the liver decreasing the colloidal blood circulation time [47]. The evidence that the ABC phenomenon does not occur in splenectomized rats indicates that the spleen plays an important part in promoting the formation of anti-PEG IgM. Moreover, the immune reaction in the spleen lasts for at least two to three days following the first injection [47].

To overcome this phenomenon, various strategies have been tried such as: (i) coating the carrier surface with a hydrophilic

TABLE 2

Colloidal drug delivery systems proposed for brain drug delivery

Strategies	Outcome	Methods	References
Vesicular carriers			
Liposomes	Enhanced BBB permeation of encapsulated drug with respect to the free form	The enhanced penetration of drugs across the BBB was obtained by different approaches:	
		(i) Pegylated liposomes conjugated with the OX26 antibody are able to efficiently transport daunomycin across the BBB	[67,68]
		(ii) The presence of fusogenic phospholipids that is DPPC, and of ganglioside G_{M1} in the lipid composition ensures a suitable penetration of liposomes through the BBB	[78]
		(iii) Convection enhanced permeation technique: the administration of a large volume of small colloidal devices allowed a sustained and increased diffusion of drugs into the brain compartments	[81,82]
Niosomes		The decoration of niosomal surface with a targeting agent, such as *N*-palmitoylglucosamine, permits an enhanced drug delivery to brain parenchyma	[13]
Molecular carriers			
Cyclodextrins (CDs)	Increase of drug penetration due to the BBB permeabilizing effect of CDs	CDs are able to extract membrane cholesterol and phospholipids. The permeabilizing effect of CDs could involve: (i) the temporary opening of the BBB tight junctions or;	[97]
		(ii) the decreased activity of the P-gp efflux multidrug complex	[103]
Innovative carriers			
Nanogels	The use of nanogels as colloidal supramolecular devices comes from their favorable features,	Nanogel structural modifications promote long circulation times and immuno-targeting systems.	[109–111]
	such as their mean size of $\leq$80 nm, a narrow size distribution, a high degree of entrapment efficiency of active compounds and their safety	Surface decoration of nanogels with a 1% (w/v) solution of Tween®80 allowed the achievement of the maximum accumulation of these supramolecular nanocarriers in the brain.	[1,16]

polymer apart from PEG, such as poly(*N*-vinyl-2-pyrrolidone) (PVP), poly(4-acryloylmorpholine) or poly(*N*,*N*-dimethylacrylamide) [48] and; (ii) the use of cleavable PEG–lipid derivatives that can efficiently prolong the circulation time of liposomes or vesicles while avoiding the ABC phenomenon [49].

In addition to the PEGylation, the conjugation of specific ligands to phospholipids, polymeric materials or PEG moieties can give supramolecular devices selective targeting properties. This selectivity is a noteworthy advantage for those systems for brain drug delivery. In fact, Mabs or small peptides can recognize specific receptors located on the luminal membranes of brain capillary endothelial cells [42]. These systems can be administered intravenously on a weekly basis without toxic effects and have the benefit of improving drug efficiency [40].

The influence of colloidal physicochemical properties on brain drug delivery

The efficiency of carriers is also correlated to their ability to remain stable in plasma and to the amount of drug released in the microenvironment of the brain after passage across the BBB. The stability of the supramolecular devices depends on their physicochemical properties and compositions as well as the chemical characteristics of the various drug compounds used. Different investigations have reported that size and zeta potential are important physicochemical parameters for the achievement of suitable devices. The modification of zeta potential values leads to a decrease of the negative charges that surround the nanoparticles in the nanosuspensions and also to a modification of the repulsion phenomenon between particles. The change in the equilibrium between the forces of attraction and repulsion in the devices gives rise to the opportunity of promoting or inhibiting aggregation events implicated in the colloid stability.

Another important parameter to be taken into account in the use of drug carriers for brain delivery is the release rate of the active compounds from the supramolecular devices. The importance of drug leakage depends on the necessity of achieving an ideal kinetic release profile. The modification of the drug release profile is done to optimize the bioavailability and blood concentration of molecules, their pharmacokinetic profiles and patient compliance [50]. The mechanism of drug release from nanocarriers can occur as a consequence of the desorption of drugs from colloidal surfaces and/or their diffusion through the components of the carrier envelope (lipids or polymers). Drug release also depends on interaction with biological membranes [51,52].

The advantages in using supramolecular devices as CNS drug delivery systems are strictly related to their biocompatibility. This property depends mainly on the material components. Colloidal carriers are prepared using substances that present low levels of toxicity and immunogenicity, and are degraded intracellularly by means of macrophage uptake without producing intracellular residues [53]. In particular, liposomes, as supramolecular devices, can be made up by using lipids and other biocompatible materials similar to those forming biological membranes. These safety features contribute to making colloidal carriers suitable candidates for brain delivery.

Basic concepts in the formulation of supramolecular devices

To date, many strategies in the formulation of supramolecular devices have consisted of the discovery of innovative drug delivery systems for therapeutic treatment of diseases. The technological parameters, such as controlled release, therapeutic dosages, plasma drug concentrations, tissue distribution, body clearance and multiple drug administrations, were widely investigated by pharmaceutical scientists.

These efforts were aimed at preparing lipid and polymeric devices, simple- and/or self-assembled into complex structures and having targeting and long-circulating properties that can modify the *in vivo* biodistribution features of these devices. In fact, these devices – having been subject to surface and formulation modifications – improved blood circulation time, decreased RES uptake [54] and increased active targeting in the body compartments compared with conventional colloidal carriers [41]. All these modifications can be used efficaciously for the treatment of various diseases and supramolecular devices have been proposed as therapeutic formulations for biomedical applications [40,55].

Vesicular carriers for brain drug delivery

The design of supramolecular vesicles involved the self-assembly of phospholipids and/or polymeric materials without modifying the native physicochemical properties of these macromolecules [18]. Generally, the lipid cores of these devices are maintained and covered and/or decorated with polymers and co-polymers. The integrity of this compartment was found to be an important prerequisite in the formulation of supramolecular devices and, in fact, vesicles were subsequently designed using various phospholipids [18] that did not modify this structure. These lipid materials are safe, biodegradable and biocompatible and can self-assemble into different kinds of colloidal aggregates. In particular, the structure of these vesicles, which forms the nucleus of the system, is characterized by the presence of different compartments (i.e. hydrophilic, hydrophobic and amphipathic regions), which allows these supramolecular devices to maintain versatile properties for drug entrapment. Vesicular nuclei can be liposomes, niosomes, ultradeformable liposomes and ethosomes [56–58]; however, only liposomes and niosomes have been used as carriers and/or nuclei for the design of supramolecular structures for brain drug delivery.

Liposomes

Liposomes are small carriers characterized by unilamellar or multilamellar vesicles surrounding aqueous compartments. They are a milestone in the scenario of colloidal drug delivery systems and they were first proposed as membrane models by Bangham [59]. Liposomes consist of various phospholipids, which can be neutral or charged (negatively or positively), cholesterol, gangliosides, polymers or co-polymers conjugated to lipid compounds [60]. Biocompatible and biodegradable lipids generally influence physicochemical (i.e. size, size distribution and surface charge) and technological (i.e. drug release, encapsulation efficiency, tissue uptake and distribution) parameters [61].

The presence of cholesterol in the bilayer hampered the rapid destruction of liposomal membranes by circulating high density lipoproteins (HDLs), thus allowing the liposomes to resist the attack of blood enzymes for a prolonged period of time [62]. In a manner similar to that of cholesterol, some phospholipids, such as 1,2-distearoyl-3-sn-phosphatidylcholine (DSPC) and sphingomyelins (SMs), can modulate the *in vivo* mechanical properties of the bilayer, thus increasing its stability towards phospholipid extraction that is mediated by HDL proteins.

Charged phospholipids are also involved in the modification of colloidal devices. The use of negatively charged lipids, such as phosphatic acid (PA), phosphatidylserine (PS), and phosphatidylglycerol (PG), elicited rapid liposome clearance from blood circulation [63]. By contrast, as previously described, the introduction of hydrophilic polymeric materials (i.e. PEG-750, PEG-2000 or PEG-5000) to phosphatidylethanolamine (PE) or distearoylphosphatidylethanolamine (DSPE) in liposomal formulation improves their pharmaceutical properties.

Active targeting strategies

An important strategy involved the preparation of liposomes acting as active targeting devices by the chemical conjugation of Mabs, small peptides and proteins to phospholipids or other lipid materials previously reported [64–66]. An interesting approach consisted of the conjugation of the OX26 antibody to PEG derivates. The experimental findings of Huwyler and co-workers showed that pegylated liposomes conjugated with the OX26 antibody can efficiently transport daunomycin across the BBB [67]. Similarly, digoxin encapsulated in pegylated OX26–liposomes is more permeable through brain endothelial cells when compared with the free drug [40]. These effects seemed to suggest that the presence of the OX26 antibody is involved in the BBB delivery of bioactive compounds [68–70] and the presence of this molecular antibody on the liposomal surface did not disrupt the integrity of the BBB and did not cause toxic side effects following repeated weekly i.v. administration, as was also demonstrated by Zhang and co-workers [71]. A significant increase of brain uptake also occurs when antibodies for Tf receptors are conjugated to cationic phospholipids. In this case, it is worth mentioning that the obtained cationic liposomes improved the luciferase gene expression activity of enzymes in glioma primary hippocampal cells and cortical neurons. This effect seemed to suggest that the positively charged site of interaction in the Tf receptors increased brain uptake of carriers and could be used selectively as an innovative strategy in brain delivery [72].

There has been some criticism, however, regarding the use of OX26 as a targeting agent to the CNS [73]. It turns out that OX26 mainly accumulates in the brain capillary endothelial cells and not in the brain parenchyma. Thus, although the total amount of drug delivered following i.v. injection is great, most of it remains in the brain capillary endothelial cells [74]. Moreover, the overexpression of Tf and OX26 receptors in peripheral organs compromises the effectiveness of this approach [73].

The choice of the lipid used to prepare liposomes is important when the delivery target is the brain. It has been demonstrated that phospholipid mixtures composed of 1,2-dipalmitoyl-sn-glycero-3-phosphocoline, dipalmitoyl-DL-phosphatidyl-L-serine and cholesterol provided not only the best parameters in terms of encapsulation efficacy and dimensions obtained but also the most suitable biological response in the therapeutic treatment in the case of rats with cerebral damage caused by ischemic reperfusion [75]. These systems are characterized by the presence of ganglioside G_{M1} on their surfaces, which ensured a suitable penetration of vesicles through the BBB [76].

The effect of BBB penetration, promoted by the presence of gangliosides, is greatly increased by using small unilamellar liposomes with a mean size <100 nm because of their ability to penetrate the BBB endothelial fenestrations elicited by ischemic events [77]. These data show that liposomes can represent a suitable brain delivery system for the therapeutic treatment of

ischemic injury disease. The reduced sizes of liposomes (50 nm), as well as the presence of the BBB fenestrations, caused an increase in the survival rate of ischemized and reperfused rats treated with CDP-choline-loaded liposomes [77]. The significant improvement in therapeutic response obtained by using CDP-choline-loaded liposomes as compared with the native drug depended, in the case of ischemic damage, on the increase in the amount of CDP-choline delivered to the CNS, and was detected in the ischemic area after systemic administration. This amount is generally 20% of the administered dose for CDP-choline-loaded small unilamellar liposomes versus 5% for the free drug.

A liposomal formulation made up of DPPC-DPPS-Chol-ganglioside G_{M1} (7:4:7:2 molar ratio) could be the best compromise for BBB delivery of liposomes; this formulation also showed a good level of efficacy in the treatment of the post-ischemic maturation phenomenon [78]. In particular, Fresta and co-workers showed that Wistar rats ischemized by bilateral clamping of common carotid arteries for differing times (5, 15 and 30 min) presented a noticeable increase in survival rate when treated with CDP-choline-loaded liposomes as opposed to those dosed with the free drug (45%, 53% and 100% increases, respectively for the incubation times above) [75].

In particular, the successful application of small unilamellar liposomes in the therapeutic treatment of damage triggered by post-ischemic reperfusion is caused by the long-circulating properties of the colloidal liposomal carriers, which can ensure the presence of a reservoir system in the bloodstream, thus allowing the penetration of the CDP-choline into the brain across time. At the same time, the fusogenic properties of liposomes, because of the presence of DPPS phospholipids in the bilayer, allowed a specific interaction between vesicles and BBB lipids as well as a biological effect on the BBB membranes injured by post-ischemic reperfusion. This effect seemed to be promoted by the entrapment of CDP-choline in the colloidal vesicles [78]. All this evidence suggests that fusogenic phospholipids (e.g. DPPS) should be widely used to design vesicular carriers for brain delivery and only the surface modification of these devices with G_{M1} gangliosides or PEG derivates can increase the circulation time in the bloodstream, thus obtaining a reservoir system for brain drug delivery.

Convection-enhanced delivery (CED)

A significant problem in brain drug delivery is the distribution of therapeutic agents in the cerebral parenchyma following their intracranial infusion. The administration of a small volume of infused drugs is not followed by rapid delivery to the CNS from the infusion site and the rate of diffusion depends on the drug gradient concentration. This parameter is directly correlated to the coefficient of diffusion, according to Fick's law, and shows that only a large volume of infused drug provides a high concentration of the drug in the brain compartments [79]. To overcome this problem and to provide an optimal therapeutic solution when a large volume of drug is to be administered by intracranial infusion, the convection-enhanced delivery (CED) technique has been developed [80]. This innovative approach, introduced by Bobo *et al.* [80], is based on the possibility of administering (by using bulk flow) small colloidal devices such as magnetic nanoparticles, liposomes and non-viral DNA complexes to obtain a sustained and increased diffusion of drugs into the brain compartments [81]. During experimentation, the CED technique generally delivered significant volumes of molecules, of high and low molecular weights alike, increasing the drug in the brain compartment – in contrast to the results seen using the conventional injection method.

The efficacy of the CED technique in brain delivery was recently investigated [82]. In particular, it was shown that cationic liposomes, made up using 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), pH-sensitive PEG diorthoester (POD) lipid, FITC-dextran-lysine, fluorescent lipid tracers DiI (D-282), iodinated benzamidine (^{125}I-BPE) phospholipids and tritiated cholesteryl hexadecyl ether (^{3}H-CHE), are rapidly accumulated in the brain cells after CED infusion (Fig. 1).

Biodistribution profiles of various colloidal vesicles showed that different molecular probes provided a similar incidence of organ accumulation after one hour infusion. Liposomes containing the non-degradable probe remain in the brain for two days, whereas ^{125}I-BPE-liposomes are no longer detected in the brain after two hour and probes are accumulated in the brain cells with no consequential toxic effects [82]. Moreover, it is interesting to note that, in the case of liposomes containing ^{3}H-CHE, the emission intensity did not diminish over a period of 48 hours, thus showing an insignificant elimination of liposomes from the brain parenchyma during this time. These experimental findings highlighted the fact that CED infusion increased the delivery of drugs when a small volume was intracranially administered and colloidal devices remained in the brain compartment for a protracted period without generating any toxic injury.

The spatial distribution of liposomes in the brain after CED application was also investigated by using fluorescent liposomes. The colloidal vesicles containing different fluorescent emission markers were co-infused in the animal model. The results obtained by using a fluorescent scanner and confocal laser scanning microscopy (CLSM) showed that liposomes could diffuse in different compartments of grey matter (Fig. 1a). CLSM analysis (Fig. 1c) highlighted the fact that liposomes coated with dextran macromolecules diffused into a larger area of the brain [83] when compared with liposomes that did not contain dextrans. For these formulations, the use of a red fluorescent probe as the molecular agent for brain detection showed that devices remained concentrated in a specific compartment and were accumulated in a subspecific population of brain cells (Fig. 1b). The results shown in Fig. 1g–i confirmed this ability of dextran-coated liposomes to allow a rapid and complete diffusion of probes into the brain. The administration of supramolecular devices using the CED procedure is also influenced by the physicochemical parameters of the formulations used. In fact, it has been demonstrated that specialized brain cells clear liposomes from the brain parenchyma into the bloodstream and particle sizes, phospholipid charges and polymer and co-polymer derivatives play an important part in the determination of this process. It was shown [82] that low amounts of positively charged phospholipids in the liposomal formulation decreased the brain distribution even if colloidal carriers are shielded by the presence of PEG moieties on the surface. This feature was demonstrated by preparing labeled neutral and positive liposomal vesicles and by monitoring their brain distribution. As shown in Fig. 2, positively charged liposomes

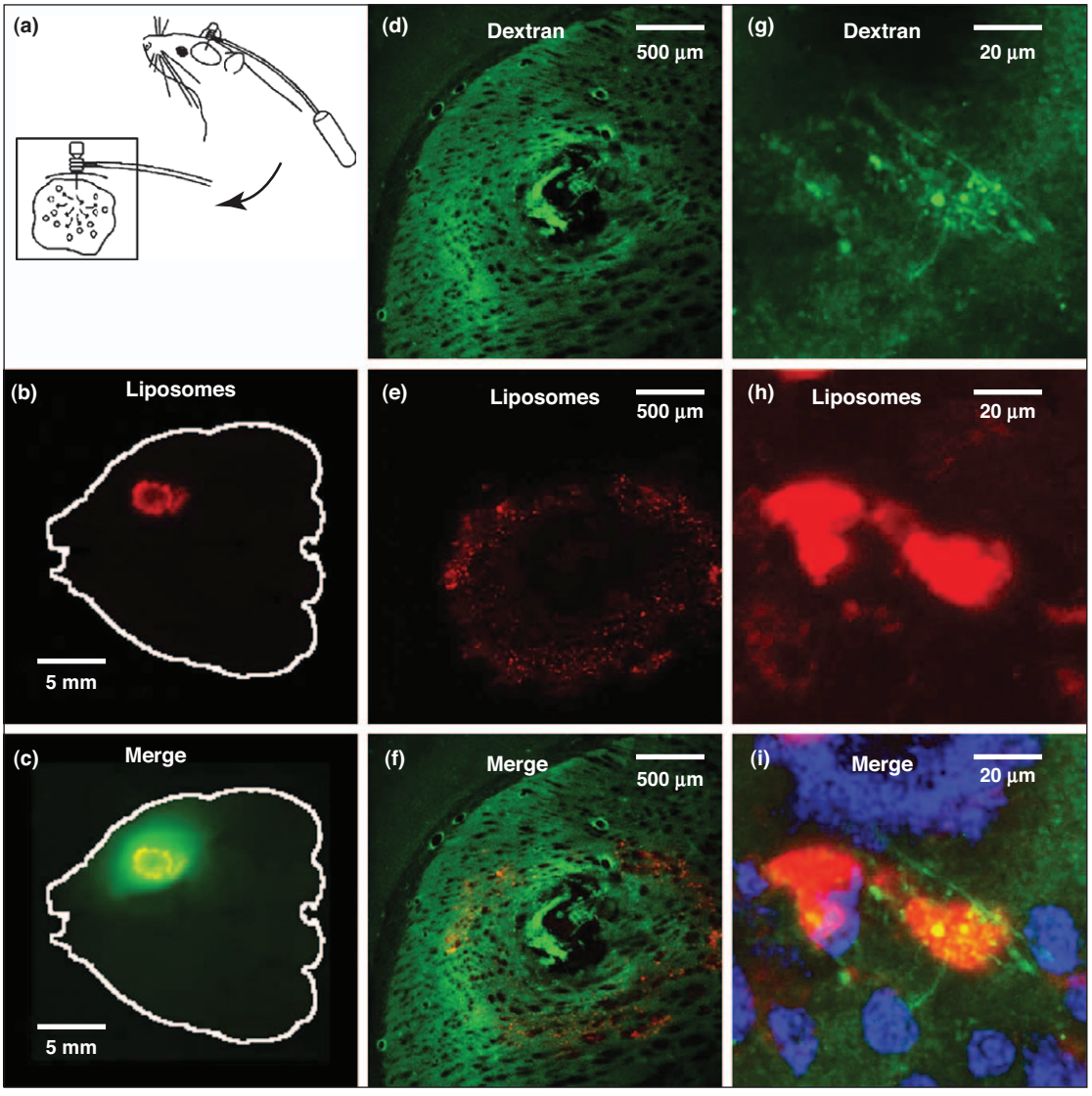

FIGURE 1

Comparison of the distribution of fluorescent liposomes to fluorescent 10 kDa dextran after co-infusion using convection enhanced delivery (CED). All images are taken from axial brain sections with either neutral 80 nm PEG liposomes (red) or 10 kDa dextran (green). **(a)** Animals are implanted with a brain infusion cannula attached to a subcutaneous osmotic pump as delivered in panels **(b)–(i)**. (b)–(c) A low-resolution image of the brain slice imaged in panels (d)–(i). (b) Fluorescent liposomes. (c) Merged image of liposomes and dextran. (d)–(f) Images taken using confocal microscopy with a 4× objective. Panel (d) centres on the defect left by the tip of the infusion cannula. (e) Cells containing liposomes appear as small punctuate objects surrounding the infusion site. (f) Merged image of (d) and (e) confirming that dextran penetrated beyond liposomes. (g)–(i) A *z*-projection through confocal images taken with a 40× objective. (g) Dextran primarily stained the interstitial space; however, the centre of this panel shows that dextran also stained a branching capillary. (h) Two cells containing red liposomes. (i) A merged image of panels (g) and (h) showing that liposome-containing cells are perivascular. The blue color depicts cells that excluded dextran. Two perivascular cells (red) in panel (i) also show internalized dextran within vacuoles (yellow) suggesting active phagocytosis [82].

were retained at the site of infusion with respect to neutral ones, thus restricting the diffusion of the colloidal carrier in other brain compartments. By contrast, the inclusion of negatively charged phospholipids in the liposome formulation enabled a degree of diffusion in the brain similar to that of neutral liposomes, thus showing that negatively charged phospholipids do not influence brain distribution after CED application [82].

Concerning particle size of colloidal vesicles: experimental investigations [75,82] have shown that vesicles with average diameters between 40 and 80 nm can deeply penetrate the brain parenchyma without presenting relevant accumulation phenomena; whereas liposomes of 200 nm are deposited locally around the site of injection. These data show that the distribution of liposomes after CED administration in the brain compartment is strictly correlated to their sizes and surface properties. For this reason the presence of PEG moieties increases their penetration into the brain and influences the interaction between liposomes and brain tissues when colloids leave the infusion system [84].

Another technique used for evaluating the distribution of liposomes in the brain parenchyma after CED administration was magnetic resonance real-time imaging (MIR). This procedure represented a powerful tool for monitoring real-time distribution

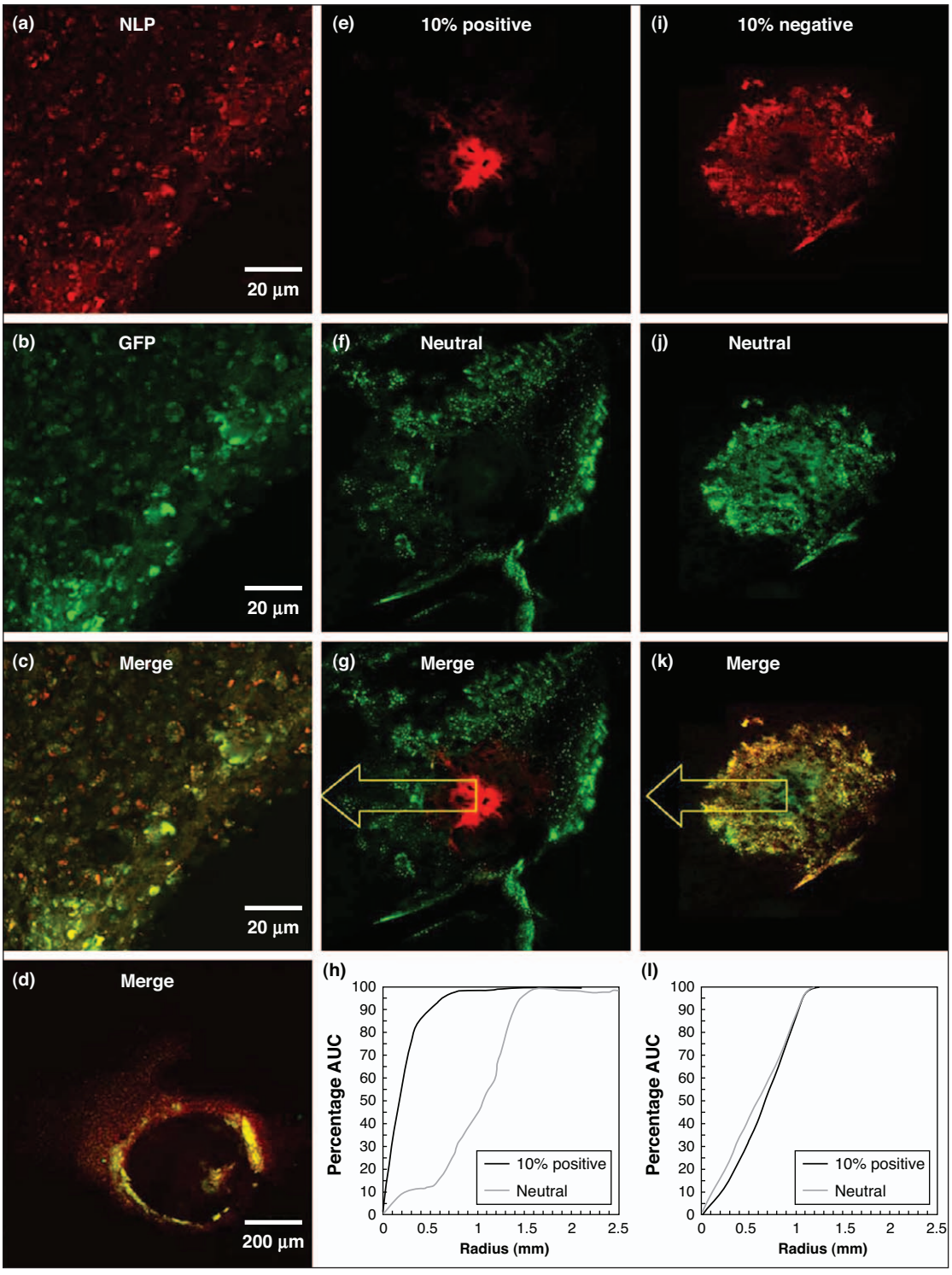

FIGURE 2

Effect of surface charge on liposome distribution after CED. All images are axial brain sections taken by confocal microscopy. **(a)–(d)** A pH-sensitive nanolipoparticle (NLP) containing green fluorescent protein (GFP) plasmid infused into a U251 (day 26) intracranial tumor following sacrifice (day 28). (a) DiI-labeled NLP (red). (b) GFP transfected cells (green). (c) Merged high-resolution image of panels (a) and (b) showing transfected cells (yellow) and untransfected cells with NLP entrapped in vacuoles (red). (d) Low-resolution merged image showing that NLP traveled only a short distance from the cannula tip; furthermore, extensive transfection was limited by poor particle penetration. **(e)–(l)** 4 mm × 4 mm images centred upon the tip of the infusion cannula. (e)–(h) Co-infusion of positive (red) and neutral (green) liposomes. (e) Liposomes with 10% positive surface charge under a 10% PEG coat. (f) Neutral liposomes. (g) Merged image of panels (e) and (f). (h) The AUC as a function of radius, calculated along the arrow in panel (g), shows that a positive charge restricts liposome penetration. (i)–(l) Co-infusion of negative (red) and neutral (green) liposomes. (i) Liposomes with a 10% negative surface charge under a 10% PEG coat. (j) Neutral liposomes. (k) Merged image of panels (i) and (j). (l) AUC as a function of radius, along the arrow in panel (k), shows no major difference between the liposomes [82].

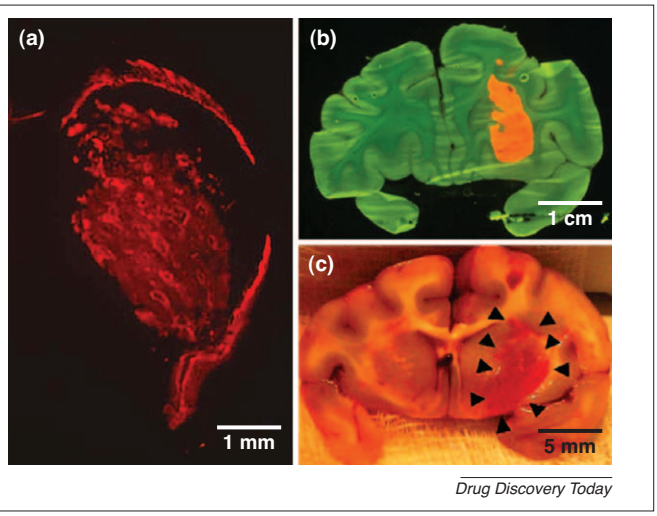

FIGURE 3

Extensive distribution of fluorescent liposomes in CNS. **(a)** Almost complete coverage of rodent striatum by a 20–2l liposome infusion; **(b)** distribution visualized by UV light in primate putamen after 66–2l infusion; **(c)** view of liposome distribution in putamen immediately after infusion procedure [116].

and, consequently, for quantifying the cerebral volume distribution of nanocarriers. Gadoteridol (GDL), a complex of gadolinium molecules, was encapsulated into liposomes comprising neutral phospholipids, cholesterol and PEG conjugated to phospholipids and used as a contrast agent. In *in vivo* experiments, the distribution of GDL-loaded liposomes in rat brain tissue is immediately obtained after their administration and the liposomes diffused in the *corona radiata*, the putamen and both hemispheres (Figs 3,4).

Niosomes

Niosomes are vesicular drug delivery systems made up of non-ionic surfactants that present a structure similar to that of liposomes. Initially, niosomes were proposed as delivery systems only for cosmetic applications. Over time, the similarity between niosomes and liposomes caused these colloidal carriers to be considered as an alternative to liposomes in drug delivery [85,86]. The main components of niosomal formulations are non-ionic surfactants. The Span®, Tween® and Brij® series of surfactants have been used to prepare the most common niosomal formulations. The physicochemical properties of surfactants were a vital factor that influences the fluidity and the stability of the system. Another important component of niosomes is cholesterol, which is used as an additive to promote the self-aggregation of non-ionic surfactants in bilayer structures [87]. In addition, the presence of cholesterol in niosomes reduced the gel to liquid–crystal-phase transition temperature as well as the HLB value of non-ionic surfactants [85].

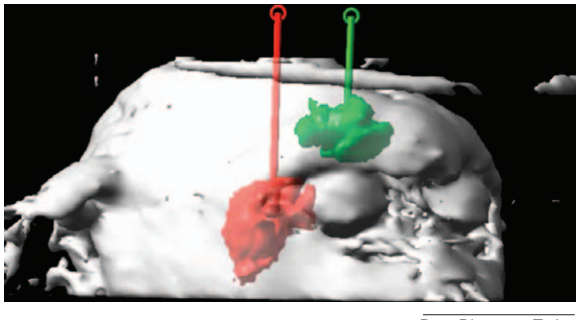

FIGURE 4

3D reconstruction of primate gadoteridol (GDL) infusion. 3D reconstruction of Vd demonstrates the relationship between GDL infusion and brain structures, and also allows volumetric measurements. Future applications are likely to use this feature for planning purposes of distribution *in vivo* [116].

The toxicological profile is an important aspect to be considered for the application of niosomes as drug delivery systems. This aspect depends on the thermotropic behavior of niosomes and alkyl chain lengths of non-ionic surfactants that can modify the transition temperature from the gel to the liquid phase [88].

In the case of parenteral administration, the toxicity of niosomes depends on the compatibility of the various systems with hematic components. The incubation of hexadecyl diglycerol ether (C16G2)-Span®60 niosomes with rat erythrocytes showed a value of hemolysis <5% after five-hour incubation. However, these findings are not relevant regarding the toxicological profile of niosomes, because <2% of C16G2-Span®60 niosomes are still present in the blood circulation after five hours [89]. The toxicity profile is important in the case of soluble surfactants, which are characterized by a dose-dependent effect, because only lower dosages of these components are completely incorporated into the niosomal bilayers, whereas high dosages of soluble surfactants elicited the formation of micelles that can exert their cytotoxic effect [89].

Although their usage in different pharmaceutical fields is widespread [57,90–93], the recent publication by Dufes and co-workers proposed the use of modified niosomes as colloidal carriers for drug delivery to the brain [13]. Niosomes were prepared using *N*-palmitoylglucosamine, Span®60, cholesterol, and solulan C24 and glucose derivates. The rationale of this approach was based on the ability of the β-D-glucose transporter (GLUT-1), which is generally located on brain capillary endothelial cells, to modulate the transport of large amounts of molecules across the BBB. These glucose-targeted niosomes were used to deliver the vasoactive intestinal peptide (VIP) to the brain parenchyma. ^{125}I-VIP encapsulated in glucose-bearing niosomes was used to measure the brain uptake of this compound, and the radioactivity level was used to detect the compound in the brain. The increase of brain radioactivity evidenced the passage of ^{125}I-VIP from the blood into the brain parenchyma. It was possible to observe that the maximum brain uptake was obtained 15 min after its administration and gradually decreased over the next 30 min. This effect could be caused by physiological clearance or the degradation of the VIP within the brain [13].

Experimental findings showed that glucose-bearing niosomes could represent an efficient strategy for the selective delivery of therapeutic molecules to the brain. In fact, ^{125}I-VIP is detected in the posterior and anterior parts of the brain, whereas its distribution is homogeneously disseminated in the different brain compartment after its administration. The biodistribution of VIP-loaded glucose-bearing niosomes is correlated to GLUT-1 receptors in the brain that are located at high density levels in the anterior and posterior compartments of cortical area sections 2 and 4 (retrosplenial, visual and auditory cortices, hippocampus, midbrain, frontal, orbital and motor cortical areas). By contrast, the poor distribution of GLUT-1 in sections 1 and 3 (striatum, thalamus, hypothalamus, pons and

medulla) showed a drastic reduction of radioactivity in these cerebral areas [94].

Molecular carriers for brain drug delivery

Molecular carriers include cryptands, calixarenes [95], cyclophanes [96], spherands, cyclodextrins (CDs) and crown ethers, which can form intermolecular interactions without covalent bonds with several compounds. The reactions between molecular carriers and the therapeutic agents are generally of the host–guest type. In the pharmaceutical field, CDs are the most important and representative molecular carriers. An interesting application of CDs is their use in drug delivery to the brain [97].

Cyclodextrins

CDs are cyclic oligosaccharides composed of 6, 7 and 8 glucose units; namely α-, β- and γ-CDs, respectively. This molecular system is largely used to modify the physicochemical characteristics of lipophilic drugs – for instance their solubility, dissolution rate, bioavailability and chemical stability [52,98,99].

The most important characteristic of CDs is their ability to form inclusion complexes together with hydrophobic drugs in solution as well as in the solid state. The obtained supramolecular inclusion complex can modify the physicochemical and biological features of guest molecules, energetically stabilizing both components of the molecular system and improving the aqueous solubility of drug compounds. The complex formation is a dimensional fit between the host cavity and the guest molecule, which creates a microenvironment where hydrophobic moieties can form a stable inclusion complex [100]. The interactions between molecules and carriers in the CDs do not involve the formation of stable covalent bonds and the force of complexion is driven by non-polar associations that promote the replacement of enthalpy-rich water molecules contained in the cavity with hydrophobic guest molecules present in the solution [101].

The advantages of CDs are their low cost and easy production. Furthermore, the presence of equal reactive hydrophilic residues in the molecular carrier allows several functional groups in the macrocyclic ring to be introduced. The success of CD modification depends on the possibility of obtaining modified devices for improving permeation through different biological membranes.

Recently, several studies have reported the use of CDs to transport drugs across the BBB. Nevertheless, the results obtained are contradictory. Some investigations demonstrated that the δ-opioid receptor peptide complexed with β-CDs was a successful strategy in increasing the BBB delivery of drugs after i.v. administration [97]. Concurrently, other studies showed that probucol complexed with hydroxypropyl-β-CD (HP-β-CD) – producing only a slight effect regarding the treatment of Niemann–Pick type C disease, probably owing to the reduced permeation of CDs across the BBB [102]. Similar findings were obtained by another research group in the case of testosterone complexed with HP-β-CDs. This supramolecular complex was rapidly cleared by the cerebrospinal fluid after intracerebral injection in rat models.

The importance of CDs as carriers for cerebral drug delivery has recently gained intense popularity as a consequence of the experimental investigations of Tilloy and colleagues [97,103]. The possibility of using natural and modified CDs as supramolecular drug delivery systems for the treatment of brain disorders was investigated by evaluating the therapeutic benefits and the potential toxicity of CDs on the BBB. Considering that the permeabilization effect of CDs can occur in the modification of the BBB structure, the integrity of the BBB and the potential toxicity of CDs were investigated by evaluating the permeability coefficiency of [^{14}C]-sucrose. This molecule diffuses very slowly across the BBB in physiological concentrations *in vitro* and *in vivo*, so it is used as an indicator to test the tight-junction integrity of brain microvessel endothelial cells (BMECs) [104]. Exposing the cerebral endothelial cell monolayer to natural or modified CDs increased the permeability coefficient of [^{14}C]-sucrose and a toxic effect resulted for α and β series at low concentrations (≤2.5 mM), whereas for γ-CDs a safety profile was observed up to 50 mM [105]. This behavior seemed to suggest that oligosaccharide units are involved in the toxicity of CDs in the brain compartment because of their permeabilization effect on the BBB structure. In fact, the increase of glucose permeability through the BBB is correlated to its breakdown due to phospholipids and cholesterol extraction mediated by CDs [106].

The evaluation of the cholesterol efflux from the brain endothelial cell monolayer showed that different concentrations of β-CDs could extract a greater amount of compound when compared with the α- and γ-series, probably because of the reduced aqueous solubility of β-CDs which increased the complex formation between this macromolecular carrier and lipids of the BBB. By contrast, in the case of phospholipids, particularly phosphatidylcholine and SM, α-CDs could extract both of them, whereas β-CDs were selective only for SM. γ-CDs and their derivates were generally safe and induced only a small efflux of lipids. To explain this phenomenon various authors claimed that the difference in terms of lipid extraction for various natural CDs depends on the molecular behavior of acyl chains of phospholipids, which fit tightly into the hydrophobic cavity of α-CDs and more loosely in the larger, inner space of β- and γ-CDs. In the case of cholesterol: its side moiety is preferentially included in the cavity of β-CDs, whereas γ-CDs showed the least selectivity for lipids [107].

These experimental findings also seemed to suggest that cholesterol- and phospholipid-extraction depended on the physicochemical nature of the CDs and natural and modified γ-CDs showed the best formulative profile for the BBB delivery of bioactive compounds. Interesting results were obtained when it was used in brain delivery. In this case, the toxicity was drastically decreased whereas therapeutic activity was increased.

To test this hypothesis, the safety profiles of γ-CDs and the supramolecular complex doxorubicin-hydroxypropyl-γ-CD were tested *in vitro* on the BBB membrane cell model. The improvement of doxorubicin transport through the BBB was related to the temporary opening of the BBB tight junctions and it was not a consequence of the P-gp inhibition effect elicited by the CDs. Also, the measurement of the permeability coefficient of inulin and the use of triazino-aminopiperidine derivative S9788 did not show significant modifications during experiments when the *in vitro* model of BBB was treated with the CD supramolecular complex of doxorubicin. These data highlighted the fact that the increase of the doxorubicin concentration in the brain compartment depended only on the CD permeabilization effect and was not related to a potential toxic effect of doxorubicin or the CD carriers [97].

The delivery of active compounds through the BBB was also evaluated as a function of CD derivates. In particular, the evalua-

tion of doxorubicin brain delivery was carried out for β-CDs and methyl-β-CDs in an *in vitro* model of BMECs. Experimental investigations showed that the transport of doxorubicin across the BMECs was increased by using methylated β-CD derivates (Rame-β-CDs and Crysme-β-CDs). This effect was not related to the disruption of the BBB but to the penetration enhancer effect of CDs. The addition of inulin during experimental investigation showed no significant modification of its permeability coefficient. Two possible mechanisms of action could explain the improvement of doxorubicin transport across the BBB: (i) the interaction between the brain capillary endothelial cells and CDs led to the weakening of the BBB without reducing its integrity or; (ii) the modification of membrane integrity caused a rearrangement of the BBB and reduced the activity of the P-gp efflux multidrug complex [103]. The latter hypothesis seems to be more plausible than the first one. In fact, the co-incubation of the brain capillary endothelial cells with Crysme-β-CDs, urea and vincristine showed that the transport of different compounds across the monolayer remained unvaried for urea and was doubled for vincristine. This means that in the case of Crysme-β-CDs drug delivery did not depend on the breakdown of the BBB and was a phenomenon strictly correlated to the P-gp efflux multidrug complex.

Nanogels

Nanogels are a new family in the field of drug delivery systems, and have been proposed by Vinogradov for the transport of drugs and biomacromolecules into the brain [108]. They are flexible hydrophilic polymer gels of nanoscale sizes, similar to hydrogels, synthesized from the co-polymerization of *N*-isopropylacrylamide (NIPAAM), *N*-vinylpyrrolidone (VP), ionic polyethylenimine (PEI) and non-ionic PEG chains (PEG-cl-PEI) by using the emulsification solvent evaporation method. The entrapment of the active compounds in these supramolecular colloidal aggregates occurs spontaneously and it is the result of electrostatic interactions between polymers and the decrease of the solvent volume that allow the modification of the polymeric matrix and the formation of dense nanoparticles. Nanogels, after drug complexation, normally present a mean size of 80 nm.

A typical advantage of nanogels over the classic nanoparticles is the possibility of obtaining an elevated degree of encapsulation of macromolecules. Nanogels seemed to be nontoxic and safe when examined in *in vitro* cell-based assays [108].

As with the other colloidal carriers, nanogels can be structurally modified to obtain prolonged circulation times and immunotargeting systems. These modifications generally concern the possibility of anchoring PEG moieties to the surface of nanogels by means of a chemical conjugation with polymeric materials or by the opportunity of introducing antibody fragments through a biotin–avidin coupling reaction [109]. The modification of the nanogel structure by using targeting agents, such as Tf or insulin, enables receptor-mediated transport of encapsulated molecules in the CNS [110]. The reduced size of nanogels with respect to other colloidal carriers seems to make this delivery device less susceptible to RES clearance and to improve cellular and tissue penetration [111].

Several investigations have shown that nanogels can be used efficiently as colloidal supramolecular devices for the delivery of oligonucleotides into the brain [108], because of their favorable physicochemical properties, such as their mean size of $\leq$80 nm and a narrow size distribution [16]. Past experimental investigation showed that the improvement of the oligonucleotide delivery through the BBB was the result of the high degree of efficiency in the entrapment of compounds in nanogels and also the fact that these supramolecular nanocarriers did not produce adverse toxic effects after *in vivo* administration [108]. A significant decrease of spleen and liver uptake was observed for nanogels, thus suggesting that this colloidal carrier can protect oligonucleotides from opsonization by the RES macrophages. This particular behavior of nanogels seems to be dependent on the physicochemical properties of nanogels as well as the composition of the various devices. As in the case of polymeric nanoparticles, the coating of colloidal devices with a 1% (w/v) Tween®80 solution could influence their biodistribution in the brain besides the pharmacokinetic properties of nanogels [1,16]. This strategy has been extensively investigated in the attempt to increase the BBB delivery of this supramolecular device and to demonstrate that only an optimal concentration, 1% (w/w), Tween®80 contained in the carrier surface allows the maximum accumulation of these supramolecular nanocarriers in the brain. This effect probably depends on the ApoE absorption obtained in the presence of surfactants. A paradoxical effect is obtained when Tween®80 is increased up to a critical concentration. In this case, the hydrophobicity of the nanogel was increased and ApoE reduced the cerebral uptake, thus showing a behavior similar to that observed for uncoated devices [16,112,113].

An estimation of the biodistribution of nanogels in the brain was carried out by measuring the permeability of the nanocarrier through BMEC models. In this case, the use of these systems labeled with molecular probes enabled the delivery of supramolecular nanodevices from the apical to the basolateral side of brain monolayers. This permeation effect was a function of the colloidal carrier composition and the degree of complexation with entrapped molecules (Fig. 5). These findings seem to suggest that the BBB structure is responsive to the polymeric material of nanogels. This effect is probably related to the nature of devices

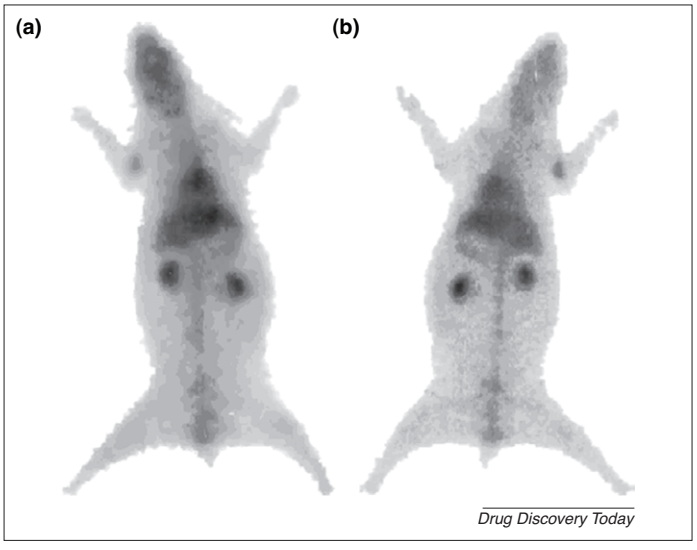

FIGURE 5

Gamma image of rabbit one hour post injection showing distribution of radiolabelled polysorbate 80 coated *N*-hexylcarbamoyl-5-fluorouracil (HCFU)-loaded nanogels **(a)**. Gamma image of rabbit one-hour postinjection showing distribution of radiolabelled uncoated HCFU-loaded nanogels **(b)** [16].

that showed a reduction in size and remained stable after *in vitro* and *in vivo* administration.

Conclusion and further perspectives

Supramolecular devices are a promising tool in the delivery of drugs to the CNS, owing to their ability to cross the BBB in a non-invasive manner. The use of these innovative drug delivery systems allows the improvement of the biopharmaceutical parameters of entrapped drugs. Furthermore, the use of active and passive targeting strategies allows us not only to obtain specific therapeutic action but also offers a series of clinical advantages in terms of drug dosage, reduction of side effects, the increase of cerebral distribution and improvement of patient compliance. These effects can be obtained by coating the surfaces of supramolecular carriers with PEG moieties, which confers a prolonged blood circulation time to colloidal devices and promotes their penetration into the CNS by interaction phenomena with the BBB. Moreover, to obtain a site-specific action the surface of supramolecular devices can be modified through the addition of targeting moieties, such as Mabs or small peptides.

Their supramolecular nature is the real advantage of these drug delivery devices, which can be designed in such a way as to obtain multifunctional activities, such as the facilitation of passage although the BBB, selective delivery, triggered therapeutic action, diagnosis and the fulfilment of the therapeutic requirements of various diseases affecting the CNS. Although not belonging to the category of supramolecular devices, a promising perspective to bypass the BBB is based on the use of hybrid proteins. In particular, Pardridge [114] realized different systems characterized by the conjugation of a synthetic molecule (ligand of a specific receptor of the BBB) with a therapeutic protein that provided excellent results in the treatment of neurodegenerative pathologies after i.v. administration. One of these, AGT-190, produced by ArmaGen® (a company founded by Pardridge) and made up of a Mab that latches onto part of the insulin receptor in the brain (without interfering with insulin binding, thus acting as a 'Trojan horse'), as well as the growth factor GDNF, generated interesting results in the treatment of Parkinson's disease [115]. AGT-190 is now under examination by the FDA to investigate its safety after multiple administrations in humans.

Further improvements and greater knowledge about supramolecular devices should be investigated to make these drug delivery systems suitable for their clinical use in brain diseases, to replace conventional methods and to overcome the disadvantages of traditional therapy. An interesting research field could be the use of these systems in gene therapy; and, in particular, the realization of a DNA-bearing device that can bypass the BBB – which could represent an important goal for modern research in the treatment of cerebral diseases.

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