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CNS DELIVERY OF DRUG VIA LOW-DENSITY LIPOPROTEIN RECEPTOR (LDLr) MEDIATED TRANSCYTOSIS

Anupam Sarma, Tapash Chakraborty* & Malay K Das

Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India

Abstract

Accessibility of therapeutic agents inside the brain is utmost important in the case of CNS diseases but in most of the cases, the conventional formulations are unable to fulfill this criterion. This hindrance is mainly due to the complex structure of the blood–brain barrier (BBB), the presence of efflux pumps, and the expression of metabolic enzymes. Although these barriers restrict the entry of many therapeutic agents into the brain but it selectively permeates the passage of many nutrients necessary for healthy brain function. To accomplish the task of nutrient transport, the brain endothelium possesses a diverse collection of molecular transport systems. One such class of transport system, known as a receptor-mediated transcytosis (RMT), employs the vesicular trafficking machinery of the endothelium to transport substrates between blood and brain. This review will mainly focus on various approaches that have been developed to target brain by utilizing Low-Density Lipoprotein receptor (LDLr) for the transcytosis of nanoformulations across BBB.

Key Words: Blood–Brain Barrier, Angiopep, apolipoproteins, Nanoparticles

Introduction

The brain is a very vital and delicate organ in human beings as it is the supreme house of major biochemical activities. The anatomy and physiology of Brain/Central Nervous System (CNS) make it very complex system; due to which it can provide a natural defense against harmful xenobiotics circulating in the blood. The integrity of the CNS is essential for its normal function (Peluffo et al 2015). Many existing pharmaceuticals are rendered ineffective in the treatment of CNS diseases due to inability to effectively deliver and sustain the therapeutic moieties within the brain. Despite extensive research, patients suffering from fatal and/or debilitating central nervous system (CNS) diseases, such as brain tumours, HIV encephalopathy, epilepsy, cerebrovascular diseases and neurodegenerative disorders, the number of deaths is increasing day by day. The clinical failure of much potentially effective therapeutics is often not due to a lack of drug potency but rather to shortcomings in the method by which the drug is delivered. The human brain is a flexible organ constantly gone through evolutionary processes

E-mail: tapash.mpharm@gmail.com

resulting in changes of the biological structure at the molecular and cellular level, thereby affecting information processing and flow (Grabrucker et al 2014). Drug delivery to the brain is very challenging as this tissue takes the advantage from a very efficient protective barrier. The way by which brain is protected from foreign substances; in that same way it restricts the entry of potentially active therapeutic moieties (Karanth & Murthy 2008). There is mainly three barrier systems that obstruct the drug transport to the brain parenchyma. They are the Blood Brain Barrier (BBB), localized in the capillaries in the brain; the blood-cerebrospinal fluid barrier (BCSFB), which is presented by the choroid plexus epithelium in the ventricles; and the ependymal, which is an epithelial layer of cells covering the brain tissue in the ventricles and limits the transport of compounds from the CSF to the brain tissue (De Boer & Gaillard 2007).

a. Blood–brain barrier

The existence of a barrier between blood and brain was first coined by Ehrlich in 1885(De Boer & Gaillard 2007). BBB is a unique barrier system that strongly separates the brain from the circulating blood. The blood capillaries found in CNS are structurally different from the blood capillaries in other tissues. These capillaries are structured in such a way that it can act as a permeability barrier between the blood within brain capillaries and the extracellular fluid in brain tissue. Capillaries of the vertebrate brain and spinal cord are of the continuous type with the lack of the small pores that allow rapid movement of nutrients and other molecules from circulation into other organs. These capillaries are coated with a layer of special endothelial cells with no fenestrations and are sealed with tight junctions (TJ) (Misra, Ganesh, & Shahiwala 2003). The TJ is constructed with three integral membrane proteins, viz. claudin, occluding, and junction adhesion molecules, and a number of accessory proteins like ZO-1, ZO-2, ZO-3, and cingulin, attach membrane proteins to actin, which is responsible for the maintenance of structural and functional integrity of the endothelium (Ballabh, Braun, & Nedergaard 2004). Peripheral capillaries allow the relatively free exchange of substances across cells, but the BBB strictly limits transport into the brain through both physical (tight junctions) and metabolic (enzymes) barriers. Thus, the BBB is the rate-limiting factor for permeation of therapeutic drugs into the brain (Kaur et al 2008). Beside this, a continuous uniform basement membrane surrounds the brain capillaries. This basal lamina encloses a special contractile cell called pericytes which form an intermittent layer and have the ability to play some role in phagocytosis and defense(Pathan et al 2009). Over 99 % of cerebral capillaries are covered by astrocyte,

which directly modulates and regulates BBB characteristics. Numerous studies have demonstrated that astrocytes play a critical role in maintenance, and induction, of BBB characteristics, thereby producing an electrical resistance of 1500–2000 Ωcm^2 much higher than that of the other systemic endothelia (3–33 Ωcm^2) (Sanchez-covarrubias et al 2014). Although it is well-established that small lipophilic molecule can easily diffuse the BBB. In fact, some of these small solutes unable to penetrate the brain as their lipid as per their lipid solubility suggest. This phenomenon is due to the presence of some active transporters in BBB, like P-gp, multidrug resistance protein (MRP), and breast cancer resistance protein (BCRP), which play crucial roles in active influx/efflux of the drugs regardless of the concentration gradient across the BBB (Mehdipour & Hamidi 2009). Some transporters like glucose transporter (GLUT1) or large neutral amino acids (LAT1) have been used as a transporter of some drugs and therefore enhance their uptake into the brain. The most widely used transporters for the delivery of large therapeutic compounds are transferrin receptor (TfR), LDL-related protein receptor (LPR), and insulin receptor (Abbott et al 2010).

b. Blood-CSF barrier

The blood-CSF (BCSF) barrier is framed by the choroid plexus (CP), which is the primary interface between the blood compartment and the CSFs. The choroid plexus is a vascular tissue found in all cerebral ventricles. The functional unit of the choroid plexus, composed of a capillary covered by a layer of differentiated ependymal epithelium. It is composed of fenestrated capillaries, which are joined together by TJs that connect adjacent choroid plexus epithelial cells and restrict paracellular diffusion of hydrophilic substances (Sanchez-covarrubias et al 2014). Considering the location of CP and the direction of the CSF flow, the choroidal epithelium at the CP is considered the most crucial part of the BCSFB located in the lateral ventricles and in the third and fourth ventricles. The CP act as a physical, enzymatic, and immunological barrier, and it plays a role in drug metabolism, drug transport, repair, and signaling (De Boer & Gaillard 2007). The resistance (150-200 Ωcm^2) offered by choroidal epithelial cells is lower as compared to capillary endothelial cells that form the BBB. So, various substances can migrate from the blood to the CSF depending upon the molecular weight and irrespective of their movement across the BBB. For example, the antiretroviral drug azidothymidine (AZT), used to treat HIV/AIDS, rapidly enters CSF across the choroid plexus epithelium but unable to cross the BBB (Grabrucker et al 2014). Therefore, the BBB can be considered as the primary barrier that prevents the passage of drugs into the CNS. Thus, the active percentage of drug in the CNS remains at zero level or far below

the therapeutically significant amount in most cases regardless of their permeability across the BBB.

1. Uptake mechanism of drug loaded nanoparticles by CNS

A number of possible ways exist for the mechanism of uptake of nanoparticles into the brain/CNS (Kreuter 2013; Kreuter, 2001).

a. An increase accumulation of nanoparticles in brain blood capillaries along with adsorption of nanoparticles to the capillary wall. This could create a higher concentration gradient that would increase the diffusion across the endothelial cell layer and as a result, enhance the delivery to the brain.

b. The coating of the nanoparticle by polysorbate 80 could inhibit the efflux system, especially P-glycoprotein (Pgp).

c. A general toxic effect on the brain vasculature leading to the permeabilization of the brain blood vessel endothelial cells.

d. A general surfactant effect characterized by a solubilization of the endothelial cell membrane lipids that would lead to membrane fluidization and an enhanced drug permeability across the BBB.

e. The nanoparticles could lead to an opening of the tight junctions between the endothelial cells. The drug could then permeate through the tight junctions in free form or together with the nanoparticles in bound form.

f. The nanoparticles may be endocytosed by the endothelial cells followed by the release of the drugs within these cells and delivery to the brain.

g. The nanoparticles with bound drugs could be transcytosis through the endothelial cell layer.

2. Receptor-mediated transcytosis

There are mainly two reasons for which the brain-targeted drug delivery system has its significance in disease control. First, the brain is the most delicate organ and is readily injured or impaired by diseases such as CNS disorders, cerebral microvascular disease, brain tumors, and bacterial or viral infections. Second, is the BBB, the limiting factor in the development of new drugs for CNS diseases (Jones & Shusta 2007; Brasnjevic et al 2009). Various strategies have been developed to enhance penetration of drugs into the

brain. Of these, the most frequently used are pharmacological technologies or comparable methods being developed for brain-targeted drug delivery system using receptor or absorptive or transporter-mediated transcytosis. Because of the various kinds of receptors existing in the brain capillary endothelium, receptor-mediated endocytosis is regarded as an efficient cellular uptake pathway for brain-targeted drug delivery system. Transferrin receptor (TfR), Insulin/Insulin-like growth factor receptor, Low-Density Lipoprotein Receptor (LDLr), Nicotinic Acetylcholine Receptor, Diphtheria toxin receptor, Scavenger Receptor (Class B Type I) etc. are available in brain capillary endothelium and have been studied for targeting drugs to the brain through receptor-mediated transcytosis (Jones & Shusta 2007; Guo, Ren, & Jiang 2012).

Low-Density Lipoprotein Receptor (LDLr): Michael S. Brown and Joseph L. Goldstein identified the Low-Density Lipoprotein receptor (LDLr) and its relation to cholesterol metabolism and familial hypercholesterolemia. For which they were awarded the Nobel Prize in Physiology or Medicine in1985.

Two classes of lipoprotein receptors have been identified:

- those that bind lipoproteins containing exogenous cholesterol absorbed from the intestine, i.e., chylomicron remnant receptors, and
- those that bind lipoproteins that carry endogenous cholesterol derived from the liver and other non-intestinal sources, i.e., LDL receptors.

The two classes of lipoprotein receptors are produced by different genes that are subject to different forms of metabolic regulation. In the body, most LDL receptors are expressed in the liver, where they supply cholesterol for secretion into bile, conversion to bile acids, and re-secretion into the plasma in newly synthesized lipoproteins. LDL receptors are also present in high concentrations in the adrenal cortex and the ovarian corpus luteum, where they function to provide cholesterol for steroid hormone formation. In CNS, neurons and glia cells express several lipoprotein receptors and ATP-binding cassette (ABC) transporters. Furthermore, LDLr is also over-expressed in a variety of tumor cells, including glioma cells but is sub-expressed in normal brain tissues. These lipoprotein receptors can bind to and take up CSF-HDL for cholesterol recycling (Miida & Hirayama 2009; Marzolo & Bu 2009; Goldstein & Brown 1987; Pfrieger & Ungerer 2011; Ladu et al 2000; Wang & Eckel 2014).

The Low-Density Lipoprotein (LDL) receptor protein consists of 839 amino acids which that mediates the endocytosis of cholesterol-rich LDL. It is a cell surface receptor

that recognizes the apoprotein B100, which is embedded in the outer phospholipid layer of LDL particles. The receptor also recognizes the Apolipoprotein-E found in chylomicron remnants and VLDL remnants (IDL). In simple words, LDL receptors sit on the outer surface of many types of cells, where they recognize and pick up low-density lipoproteins circulating in the bloodstream and transport them into the cell. Once inside the cell, the low-density lipoprotein is broken down to release cholesterol. The cholesterol is then used by the cell, stored, or removed from the body. After low-density lipoprotein receptors drop off their cargo, i.e. cholesterol, they are recycled back to the cell surface to pick up more low-density lipoproteins. In humans, the LDLr gene provides instructions for making the low-density lipoprotein receptor. LDLr belongs to the Low-density lipoprotein receptor gene family(Takahashi et al 2004; Jeon & Blacklow 2005; Mahley & Innerarity 1983).

Low-density lipoprotein receptors play a critical role in regulating the amount of cholesterol in the blood. They are particularly abundant in the liver, which is the organ responsible for removing most excess cholesterol from the body. The number of low-density lipoprotein receptors on the surface of liver cells determines how quickly cholesterol (in the form of low-density lipoproteins) is removed from the bloodstream.

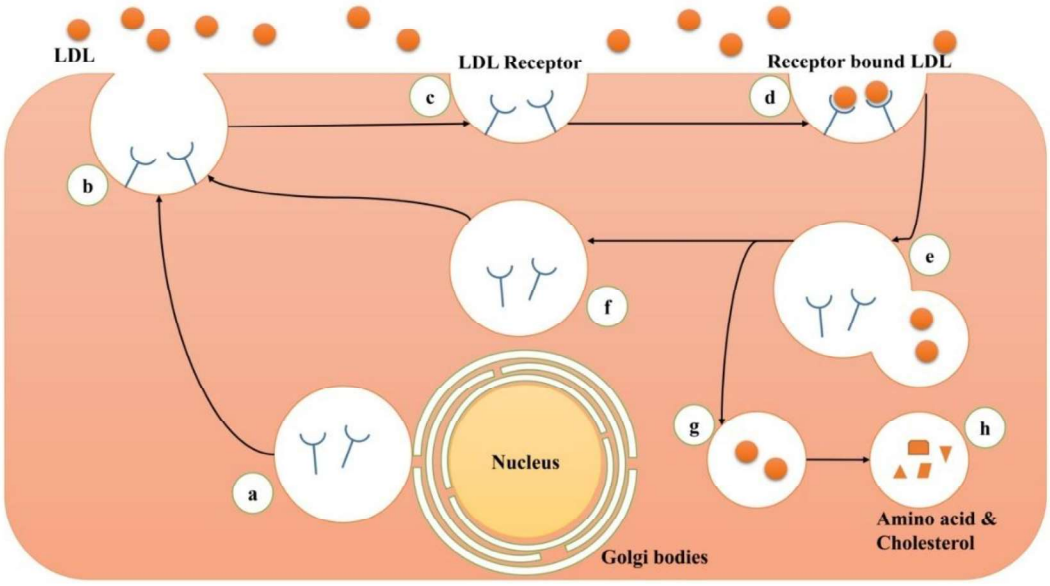

Fig 1: Mechanism of internalization of LDL by LDL receptor

3. Drug delivery via LDLr mediated transcytosis to CNS

LDLr targeted nanocarriers: Nanoparticles for pharmaceutical purposes are solid colloidal particles ranging in size from 1 to 1000 nm (1 μm) consisting of macromolecular materials in which the active principle (drug or biologically active material) is dissolved, entrapped, or encapsulated, or to which the active principle is adsorbed or attached. This definition deviates from the definition of physicists and material scientists who limit the upper size of nanoparticles to 100 nm. However, up to 1000 nm size appears to be of no important influence concerning uptake into cells of the reticuloendothelial system (RES), i.e. macrophages and endothelial cells, and most other parts of the body. It has been reported that different receptors are present on the luminal endothelial plasma membranes, including the transferrin receptor, the insulin receptor, and the low-density lipoprotein receptor-related protein (LRP) (Visser et al 2004; Pardridge 2005). Drug delivery systems based on the receptor-mediated mechanism have been explored to deliver drugs into the brain (Shi, Boado, & Pardridge 2001; Gabathuler et al 2005; Muruganandam et al 2002). So, brain targeting through LDLr have a great potential for delivery of drugs and biomolecules.The utilization of chylomicrons-like nanoparticles as drug delivery vehicles has been reported previously (Rensen et al 2001). Despite the fact that these preparations have been used to deliver a variety of agents to hepatocytes, cancer/glioma cell and atherosclerotic plaque (Rensen et al 1996; Rensen et al 1995; Maurer et al 2007; Tauchi et al 2000).

a) Endogenous LDL

The endogenous lipoproteins as part of nanoparticle delivery systems offer a significant number of advantages. Endogenous lipoproteins do not induce any immune reactions and are unable to recognize by the reticuloendothelial system (RES) (Rensen et al 2001). A number of reports showed loading of native LDL with photosensitizers, nucleosides, fluorescent imaging agents and efficacy of drug loaded LDL particles *in vivo* and *in-vitro* (Vitols et al 1990; Jori & Reddi 1993; Hammel, Laggner, & Prassl 2003; Li et al 2004). The yolk lipoproteins in the nematode are like human serum low-density lipoproteins (LDLs), serving as an intercellular transporter of fat molecules and cholesterol. Yolk lipoprotein has been investigated as targeting moiety to target LDLr. Nano diamonds coated with yolk lipoprotein have been studied for its targeting efficiency to LDLr in nematodes (*Caenorhabditis Elegans*) by Kuo Y. *et al* 2013. The study reveals that the yolk lipoprotein coated nondiamond secreted from the intestine to the pseudo coelomic space, followed by transporting into oocytes and subsequent accumulation in the multicellular embryos derived from the oocytes (Kuo et al 2013).

Low-density lipoproteins (LDLs) are a naturally occurring endogenous nano platform in mammalian systems. These nanoparticles (22 nm) specifically transport cholesterol to cells expressing the LDL receptor (LDLr). Corbin IR *et al* 2006, investigated isolated LDL Nanoparticles as magnetic resonance imaging contrast agent. They developed Amphiphilic gadolinium (Gd)-diethylenetriaminepentaacetic acid chelates incorporated into the LDL to produce a novel LDLr-targeted magnetic resonance imaging (MRI) contrast agent. Gd-labeled LDLs exhibited significant contrast enhancement 24 hours after administration in nude mice with human hepatoblastoma G2 xenografts (Corbin et al 2006). Apart from these advantages, the use of endogenous lipoproteins as a part of delivery nanoparticles is limited by a number of factors such as its natural aggregation during storage, difficulties in isolating procedures, loading of therapeutic agents and concerns with respect to safety (De Smidt & Van Berkel 1990; Masquelier, Vitols, & Peterson 1986; Almeida & Souto 2007). As a result, the focus of lipoprotein nanoparticles development has shifted towards the use of synthetic lipoproteins.

b) Synthetic reconstituted LDL

Reconstituted lipoproteins are the most widely studied class of synthetic lipoproteins. These are formed using isolated apolipoproteins combined with various lipids (Sabnis & Lacko 2012). Reconstituted lipoprotein offers several advantages over the native lipoprotein. First of all, since all components of the lipoprotein nanoparticle are known, they can be characterized individually to better understand the overall formulation. In addition, adjusting these individual components, such as lipid/protein stoichiometry, lipid type, and apolipoprotein choice can control the nanoparticles to exhibit uniform physiochemical properties such as uniform size, zeta potential, core and surface loading, etc. Finally, these reconstituted lipoproteins possess and take advantage of the known functions ascribed to native lipoproteins, which includes natural targeting, enzyme/pathway activation, cellular uptake and lipid transfer.

However, the development of reconstituted lipoprotein-based formulations has been hindered by the limited availability of apolipoproteins (Apo B-100, ApoA-I, and Apo E). This imposes significant challenges to the scalability of the manufacture of these particles (McConathy et al 2008). The development of recombinant apolipoproteins has been one approach that has been pursued to address this limitation. This approach has been undertaken based on the close similarity between recombinant molecules and their native counterparts (Rensen et al 2001). Apolipoprotein B (apoB) is the primary apolipoprotein of chylomicrons, VLDL, IDL, and LDL particles which are responsible for carrying fat molecules (lipids), including cholesterol, around the body to all

cells within all tissues. There are two apoB species: apoB100 and apoB48. ApoB100 is the ligand for the low-density lipoprotein (LDL) receptor (Olofsson & Borén 2012). Law SW *et al*. 1985 successfully cloned human ApoB-100 into an expression vector thus resulting in the 560 amino acid sequence for this protein (Law et al 1985). These and other efforts have utilized recombinant ApoB-100 to examine defining elements of its biology. For instance, Boren J *et al*. 1998 used recombinant wild-type and mutant ApoB-100 molecules to identify specific sequences that are relevant to its interaction with the LDL receptor (Borén et al 1998). Early work by Walsh MT *et al*. 1983 described a method for the reconstitution of LDL using ApoB solubilized from human plasma and its recombination with phospholipid using sodium deoxycholate (Walsh & Atkinson 1983). Subsequently, Ginsburg GS *et al* 1984 reported a process for solubilizing ApoB of LDL with phospholipid/cholesterol ester micro emulsions to generate reassembled LDL particles which exhibited many of the structural properties of native LDL (e.g. competition with human 125I-LDL for binding to LDL receptor) (Ginsburg et al 1984). Similar approaches have been described by Lundberg B *et al* 1987 which combined phosphatidylcholine, a hydrophobic drug (mustard carbamate) and Apo B-100 to successfully generate a 23-nm reconstituted LDL particle that was taken up by the LDL receptor of cultured fibroblasts (Lundberg 1987). More recent work has highlighted the viability of reconstituted LDL as a vehicle for delivery of photodynamic and imaging agents. For instance, reconstituted LDL which has been loaded with bacteriochlorin e6 bisoleate (Bchl-BOA) or napthalocyanine has shown efficacy as a photodynamic therapy agent delivery system (Marotta et al 2011; Song et al 2007). In the case of Marrotta DE *et al*. 2011, Bchl-BOA-LDL was able to mediate a delay in tumour regrowth in xenografts of human hepatoblastoma G2 (HepG2) tumours (Marotta et al 2011). Further work by Corbin IR 2006 *et al*. showed successful incorporation of amphiphilic gadolinium (Gd)-diethylenetriaminepentaacetic acid chelates into LDL thus generating a novel magnetic resonance imaging contrast agent that retained similar diameter/surface charge and selective uptake as the native LDL particle (Corbin et al 2006). Similarly, Allijn IE *et al 2013* showed that incorporation of fluorescently labeled gold nanocrystals into LDLs created a CT and optical contrast agent that maintained the biological functions of native LDL both *in vitro* and *in vivo* (Allijn et al 2013). As the LDL receptor also recognizes ApoE, an alternative approach has been the use of ApoE for the generation of LDL (Rensen et al 2001). Vogel T *et al*. 1985 showed that recombinant and natural plasma ApoE bind equally well to LDL receptor and that both were cleared at similar rates from circulation (Vogel et al 1985). The incorporation of recombinant Apo-E into nanoparticles may facilitate a more

specific uptake than that mediated by LDL (Rensen et al 2001). Recombinant ApoE has been used in the generation of ApoE enriched liposomes to mimic LDL targeting of tumors cells and delivery of biological agents (Rensen et al 1997). Neves AR *et al* 2016 successfully delivered Resveratrol loaded solid lipid nanoparticles functionalized with ApoE to the brain. Solid lipid nanoparticles of cetyl palmitate were prepared by the high shear homogenization technique of size lower than 200 nm and a zeta potential of around −13 mV. The permeability through hCMEC/D3 monolayers showed a significant increase (1.8-fold higher) for resveratrol-loaded solid lipid nanoparticles functionalized with ApoE when compared to non-functionalized ones (Neves, Queiroz, & Reis 2016).

Although synthetic reconstituted lipoproteins extended the feasibility of lipoprotein delivery vehicles, the use of recombinant ApoB-100 or Apo-AI is still limited by the significant effort and time required for manufacturing relevant amounts of apolipoproteins, including the removal of the tag from the recombinant protein and performance of additional steps for the purification of the secreted protein (e.g. proApoA-I). Another type of synthetic lipoproteins has been developed by employing peptides which mimic the functional properties of apolipoproteins to build lipoprotein-like nanoparticles. The major advantage of such synthetic lipoproteins is that they overcome several challenges faced by using apolipoproteins, such as their purity, quantity, the length of process time, as well as their safety (Sabnis & Lacko 2012; Nykiforuk et al 2011). Thus, the synthetic lipoproteins may significantly simplify the scaled-up manufacturing, as well as accelerate the clinical translation of the lipoprotein-based drug delivery.

c) Synthetic-peptide-mimetic LDL-like nanoparticles

As discussed earlier, the use of ApoB-100 protein in synthetic LDL has encountered several difficulties owing to the size and complexity of the ApoB-100 protein (Corbin & Zheng 2007). Therefore, an alternative strategy for synthetic LDL is the use of ApoB-100 mimetic peptides. Baillie G *et al* 2002 developed synthetic LDL (sLDL) formulations using lipid emulsions and four amphipathic peptides containing the ApoB receptor domain. *In vitro* cell proliferation assays using a lymphoma cell line demonstrated that the sLDLs behaved in a way that mimics the native LDL. It was observed that by utilizing different peptides, variable proliferation was achieved, which implied that the interaction with the LDLr can be controlled by varying peptide configurations (Baillie, Owens, & Halbert 2002). Nikanjam M *et al* 2007 studied the use of such synthetic LDLs as drug delivery vehicles. They developed a synthetic LDL

formulation, termed nano-LDL, from micro emulsions of phosphatidylcholine, triolein, and cholesteryl oleate, as well as a 29-amino acid bifunctional peptide, which contained a lipid binding motif and the LDLr binding domain of ApoB-100. This peptide is water soluble and readily binds to lipid emulsions in the manufacturing process. The nano-LDLs were sonicated and extruded to have a size of 10.5 nm. Through fluorescent labeling of the lipid and peptide moieties on the nano-LDL, it was shown through fluorescence microscopy that the nano-LDLs bound to the surface of glioblastoma multiform (GBM) cells in a similar pattern as the plasma-derived LDLs. This binding was inhibited after the introduction of an LDL inhibitor, suramin, further confirming that the nano-LDLs bound specifically to LDLr. In addition, the peptide and the lipid components of these nano-LDLs were co-localized within the lysosome following particle internalization, which further validated the specific receptor-mediated uptake of the nanoparticles (Nikanjam et al 2007). In a later study Nikanjam M, *et al* 2007a incorporated paclitaxel oleate (PO) into the core of this nano-LDLs (nLDL-PO). The optimal PO loading was found to be 6 % weight of nano-LDL. *In vitro* GBM cell survival assay demonstrated high cell killing by nLDL-PO (90% cell death after 72 hours compared to less than 10% in PO alone at 10 μM PO dose) and was time, concentration, and cell line dependent (Nikanjam et al 2007). Again, the use of LDLr inhibitor, suramin, significantly decreased the cell killing by nLDL-PO, confirming that the nanoparticles specifically utilized the LDL pathway.

Angiopep targeting to the low-density lipoprotein receptor-related protein-1 (LRP1) was identified to exhibit high transcytosis capacity and parenchymal accumulation. Ke W *et al* 2009 has studied angiopep as a ligand for effective brain-targeting gene delivery. Polyamidoamine dendrimers (PAMAM) were modified with angiopep through bifunctional PEG, then complexed with DNA, yielding PAMAM–PEG–Angiopep/DNA nanoparticles (NPs). The angiopep-modified NPs were observed to be internalized by brain capillary endothelial cells (BCECs) through a clathrin and caveolae-mediated energy depending endocytosis, also partly through macropinocytosis. Also, the cellular uptake of the angiopep-modified NPs were competed by angiopep-2, receptor-associated protein (RAP) and lactoferrin, indicating that LRP1-mediated endocytosis may be the main mechanism of cellular internalization of angiopep-modified NPs. And the angiopep-modified NPs showed higher efficiency in crossing blood–brain barrier (BBB) than unmodified NPs in an in vitro BBB model, and accumulated in the brain more in vivo (Ke et al 2009). Huang R *et al* 2013 prepared angiopep-conjugated nanoparticles for targeted long-term gene therapy of Parkinson's disease. Angiopep was applied as a ligand specifically binding to low-density lipoprotein receptor-related

protein(LRP) which is overexpressed on blood-brain barrier (BBB), and conjugated to biodegradable dendrigraft poly-L-lysine (DGL) via hydrophilic PEG, yielding DGL-PEG-angiopep (DPA). After encapsulating the therapeutic gene encoding human glial cell line-derived neurotrophic factor (hGDNF), DPA/hGDNF NPs showed a sphere-like shape with the size of 119±12 nm and zeta potential of 8.2±0.7 mV. It was seen that angiopep conjugated NPs exhibited higher cellular uptake and gene expression in brain cells compared to the unmodified counterpart (Huang et al 2013). Xin H *et al* 2011 developed Angiopep (ANG)-conjugated PEG-co-poly(e-caprolactone) nanoparticles as dual-targeting drug delivery system for brain glioma. Compared with non-targeting nanoparticles, a significantly higher amount of rhodamine isothiocyanate-labeled dual-targeting nanoparticles were endocytosed by U87 MG cells. The antiproliferative and cell apoptosis assay of paclitaxel-loaded ANG-NP (ANG-NP-PTX) demonstrated that ANG-NP-PTX resulted in enhanced inhibitory effects to U87 MG glioma cells. The particle size of ANG-NP-PTX was found 100 nm and zeta potential of -3.28 mV. The transport ratios across the BBB model in vitro were significantly increased and the cell viability of U87 MG glioma cells after crossing the BBB was obviously decreased by ANG-NP-PTX (Xin et al 2011). Huang S *et al* 2011 studied the dual targeting effect of Angiopep-2-modified, DNA-loaded nanoparticles for glioma. In this study, Angiopep-2 was conjugated to the nanoscopic high-branching dendrimer, polyamide amine (PAMAM), via bifunctional PEG and then complexed with the DNA, designated as PAMAM-PEG-Angiopep/DNA nanoparticles. The mean diameter and zeta potential of PAMAM-PEGAngiopep/DNA NPs were 114.1 ± 7.2 nm and 16.3 ± 1.6 mV, respectively. The in vivo biodistribution of PAMAM-PEG-Angiopep/DNA NPs in the brain especially the tumor site was found higher than that of PAMAM-PEG/DNA NPs and PAMAM/DNA NPs (Huang et al 2011). Ren J *et al* 2012 developed targeted delivery system of anticancer drugs to brain glioma by PEGylated oxidized multi-walled carbon nanotubes modified with angiopep-2 of particle size 202.23±3.43 nm and zeta potential -8.29±1.1 mV. The anti-glioma effect of DOX-loaded O-MWNTs-PEG-ANG (DOX-OMWNTs-PEG-ANG) was assessed by C6 cytotoxicity and median survival time of glioma-bearing mice, which showed a better anti-glioma effect than DOX (Ren et al 2012). Ruan S *et al* 2015 successfully developed doxorubicin loaded gold nanoparticle modified with angiopep-2 for glioma. The particle size of An-PEG-DOXAuNPs was 39.9 nm with a zeta potential of -19.3 mV, the release of DOX from DOX-AuNPs was pH-dependent. At lower pH values, especially 5.0 and 6.0, the release of DOX was much quicker than that at pH 6.8 and 7.4. After coating with PEG, the acid responsive release of DOX from PEG-DOX-AuNPs was almost the same as that from

DOX-AuNPs. Cellular uptake study showed the obviously higher intensity of intracellular An-PEG-DOX-AuNPs compared with PEG-DOX-AuNPs. In vivo, An-PEG-DOX-AuNPs could distribute into glioma at a higher intensity than that of PEG-DOX-AuNPs and free DOX (Ruan et al 2015). Gao X *et al* 2013 investigated the multivalent effect for up-regulating the intracerebral delivery of nanoparticles via receptor-mediated transcytosis. In this study, the nanoparticles were prepared from G5 dendrimer and labeled with near-infrared (NIR) fluorophore and different numbers of angiopep-2 peptides. The multimetric association between the angiopep-2 peptides labeled on the nanoparticle and the LRP receptors on the brain capillary endothelial cells significantly increased the intracerebral uptake of the nanoparticles. Nanoparticle Den- Angio4 labeled four angiopep-2 peptides achieved the highest BBB traverse efficacy. Den- Angio4 had the particle size of 7.4 nm and zeta potential of +6.4 mV. After penetrating the BBB, Den-Angio4 distributed heterogeneously and mainly located in hippocampus, striatum, and cerebellum in the brains (Gao et al 2013). Overall, although such synthetic LDL as delivery vehicles hold great promise, more in-depth studies regarding the physicochemical properties of the nanoparticles are still necessary. The key limiting issue of LDL aggregation with long term storage is likely to extend to synthetic LDLs.

Table 1: Angiopep modified nanocarriers for brain targeting through LDLr transcytosis

Carrier	**Active moiety**	**Particle size**	**Zeta potential**
Polyamidoamine dendrimers	DNA	-	-
dendrigraft poly-L-lysine	Neurotrophic factor (hGDNF)	119±12 nm	8.2±0.7 mV
PEG-co-poly(e-caprolactone) NP	Paclitaxel	100 nm	-3.28 mV
Dendrimer	DNA	114.1 ± 7.2 nm	16.3 ± 1.6 mV
Multi-walled carbon nanotubes	Doxorubicin	202.23±3.43	-8.29±1.1 mV
Gold nanoparticle	Doxorubicin	39.9 nm	-19.3 mV
Dendrimer	-	7.4 nm	6.4 mV

Conclusion

The application of synthetic lipoproteins as delivery vehicles for various molecules and agents has been diverse, ranging from delivering chemotherapeutics, antiviral agents, to siRNAs and imaging agents. These applications offer great potential in the translation of the synthetic lipoproteins into the clinical setting. Currently, the most widely used area for synthetic lipoproteins has been in oncology as well as for brain targeting, where the development of these nanoparticles has taken advantage of the overexpression of receptors for these lipoproteins in cancer cells. Currently, there is little research examining exchanges between artificial and endogenous lipoproteins for targeting the brain. As the development of lipoprotein nanoparticles becomes more prevalent, it will be important to determine the extent to this potential exchange could affect biodistribution and efficacy of these particles. One more challenge is that LDL receptors are also highly expressed in normal tissues such that delivery of cytotoxic agents by these nanoparticles may result in damage to these tissues. This undesired effect could be circumvented by the use of agents that specifically target relevant molecules in tumor cells. Alternatively, additional targeting moieties can be added to the synthetic lipoproteins, rerouting them to preferentially reach specific tumor cells or diseased sites, and thereby personalizing the treatment. Lastly, in order to increase the relevance of LDLr targeted nanoparticles as a tool for the treatment of cancer in the clinic, it is also essential to conduct an extensive preclinical examination of their efficacy against advanced metastatic disease, which is the typical presentation of patients in clinical trials.

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