Nanocarrier drug-delivery systems for temozolomide in the treatment of glioblastoma multiforme

A Review of Literature

Remco Lap

Department of Biomedical engineering

Rijksuniversiteit Groningen

Bachelor's Thesis

Under supervision of dr. I.S. Zuhorn

May 5, 2021

Abstract

Glioblastoma multiforme (GBM) is an aggressive brain cancer which is very hard to treat. The brain is a delicate tissue protected externally by the skull and internally by the blood brain barrier (BBB). Current standard treatment combines tissue resection, radiation therapy and administration of the chemotherapeutic agent temozolomide (TMZ). The effectivity of this treatment is limited, the non-specificity of TMZ administration gives rise to many side-effects and GBM-patient survival rates are particularly low. Nanocarrier drug-delivery systems offer perspective for the future effectivity of GBM treatment by TMZ delivery across the BBB to GBM tissue. This paper assesses the most extensively studied and promising nanocarrier systems, with the aim of designing a drug delivery system for the co-delivery of TMZ and a chemo-sensitizing drug, suited for transport to the brain, in order to improve the effectivity and efficiency of the treatment of GBM. The ideal carrier system is non-toxic, able to carry TMZ in combination with a chemo-sensitizer, able to penetrate GBM tissue, and able to perform controlled and sustained drug release. An extensive literature survey was conducted to TMZ-carrying nanoparticles (NPs), after which the NPs ability of the dual-loading of TMZ and the chemo-sensitizer curcumin (CUR) were assessed. The resulting, proposed nanocarrier system for GBM treatment is a reduction sensitive, pegylated, TMZ/CUR dual-loaded nanostructured lipid carrier (NLC), able to cross the BBB by targeting transferrin receptors (TfRs) using T7-peptides. The practical feasibility of this system is yet unknown and future research should point out whether this carrier could contribute to the continuous fight against cancer by increasing patient survival from GBM, while also increasing their quality of life during and after treatment.

Keywords: drug delivery, nanocarrier, glioblastoma multiforme, temozolomide, curcumin

Table of contents

1. Introduction

Glioblastoma multiforme (GBM) is the most common and most aggressive type of malignant tumor in the central nervous system (CNS) [1]. GBM is most diagnosed in adults between 45 and 65 years old and could be caused by exposure to ionizing radiation [2]. Complete surgical removal of GBM is very hard, due to the vulnerable area in which the tumor is located. The tumor often infiltrates into the eloquent cortex in the brain, which contains for example the primary motor complex and the visual and auditory cortex [3]. Resection of tumor tissue is therefore very tricky and tumor cells inevitably remain present in the brain after the procedure [3]. Treatment of brain diseases using drugs is also complicated, because of the human body's natural barrier, the blood brain barrier (BBB), between the bloodstream and the central nervous system.

To get from the bloodstream into the CNS, molecules need to cross the BBB. The blood brain barrier is a diffusion barrier, which complicates the non-selective penetration of solutes from the bloodstream into the extracellular fluid of the CNS [4]. Tight junctions, astrocytes and pericytes together make up the BBB (figure 1). The endothelial cells of the capillaries in the CNS are connected by tight junctions, which prevent solute diffusion in between endothelial cells, out of the blood stream [4]. Hydrophobic molecules can also diffuse through the cell membranes of endothelial cells, which is prevented by the cell's so called efflux pumps. Efflux pumps actively pump out recognized toxic compounds that diffuse into the cell [5]. The outside of the capillary walls is enclosed by astrocytic end-feet and pericytes, which ensure the structural integrity of the BBB [6]. Small hydrophobic molecules like O_2, hormones and small non-polar molecules are able to diffuse from the blood into the CNS. Larger, hydrophilic molecules are not able to pass the BBB without making use of a transport mechanism [7]. Therapeutic molecules often belong to the class of macromolecules, which makes it complicated to target brain tissues with drugs [8].

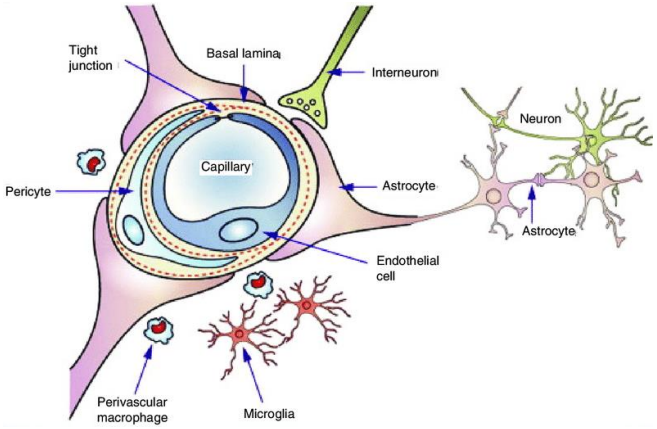

Figure 1, Schematic depiction of the Blood Brain Barrier. An endothelial cell connected with a tight junction, representing a capillary, supported by pericytes and astrocytes and surrounded by neuron networks present in the human brain. (Figure taken from: [9])

The standard treatment procedure of GBM starts with surgical resection of cancerous tissue, guided by preoperative MRI and CT images in order to enhance accuracy [10]. Following resection, the patient undergoes involved field radiation therapy (IFRT), in which high energy x-radiation is delivered locally to specific areas in the brain [11]. Radiotherapy is typically applied 4 to 5 days a week for a period of 3 to 6 weeks. In combination with radiotherapy, the patient orally receives the chemotherapeutic prodrug temozolomide (TMZ) for 5 consecutive days every month during treatment [12]. TMZ is a hydrophobic, electrophilic, cytotoxic, alkylating agent, and is the most used chemotherapeutic drug in GBM treatment [13][14]. After administration, TMZ molecules are taken up from the gut into the bloodstream of the patient and efficiently penetrate through the blood brain

barrier [15]. The TMZ concentration in the CNS is approximately 30% of the concentration present in the blood stream [15]. The penetration through the BBB is possible because of TMZ's relatively small molecule size and hydrophobic character [15][16]. In the CNS, TMZ is spontaneously converted to 3-methyl-(triazen-1-yl) imidazole-4-carboximide (MTIC) at a physiological pH of 7 [13]. TMZ penetrates the cell membrane of cancerous cells in the CNS and gets converted into MTIC, after which MTIC enters the cell's nucleus. In the nucleus, MTIC splits into 5-aminoimidazole-4-carboxamide (AIC) and monomethyl hydrazine (MMH), which mainly interacts with the nucleobase guanine in the DNA strands [17]. MMH interaction with guanine results in methylation, which inhibits the replication of the DNA strand and thus inhibits cell proliferation of the penetrated cells [14].

The treatment procedure combining radiotherapy with temozolomide administration is called the Stupp protocol [18]. This treatment yields a significant survival improvement compared to previous treatment protocols, but still only 26.5% of the GBM patients survive after two years, with a median survival time of 15 months [18][19]. The low overall survival time of GBM patients is due to the limited effect of treatment, because of low TMZ respondence of many patients [20]. At least 50% of the patients has shown to be resistant to TMZ, due to overexpression of methylguanine methyltransferase, which can reverse the methylation of guanine in the DNA of tumor cells, or the involvement of other DNA repair pathways in the GBM cells [20]. The cell membrane of GBM cells possess transport proteins, belonging to ATP-binding cassette (ABC) protein family, which are able to recognize foreign substances when diffusing over the cell membrane [21]. ABC proteins are efflux pumps, which use ATP to actively pump foreign substances out of the cell. In GBM cells, this leads to the extrusion of TMZ-molecules and a decrease in treatment effectivity [21]. The limited access of TMZ through the BBB, despite its hydrophobic characteristics, is due to the hydrolyzation of TMZ to MTIC [20]. After hydrolyzation, the compound is not able to cross the BBB anymore, which limits the amount of the therapeutic molecules that arrive at the target site in the tumor. To still get a therapeutic concentration of TMZ into the brain, the administered dose must be increased, intensifying the side effects of the drug [16]. Side-effects observed in patients after use of TMZ are bone-marrow suppression, cerebral hemorrhage, fatigue, nausea, and liver problems [18]. These are mainly due to the fact that TMZ is taken up in the bloodstream, causing the agent to be taken up by tissues everywhere in the body [20]. The relatively small gain in survival time of the patient, combined with the damaging side-effects give rise to the call for more effective treatment methods for the treatment of GBM [22].

Molecules that are unable to diffuse across the blood brain barrier could be transported to the CNS by temporally disrupting the tight junctions between the endothelial cells of the capillaries in the CNS to make diffusion of solutes easier [23]. Tight junction disruption can be done by for example osmotic-, or focused-ultrasound impulses, but this is hard to accurately control spatiotemporally and significantly enhances the risk of medical complications [24]. Another way for molecules to cross the BBB is by making use of carrier molecules. Carrier molecules comprise of organic nanoparticles, like polymer nanoparticles, liposomes, dendrimers and micelles, and inorganic nanoparticles, like gold-, silica-, or carbon nanoparticles [25]. Therapeutic agents with a target in the CNS can be conjugated to, or enclosed by, a nanoparticle, enabling them to travel across the blood brain barrier, while at the same time making the drug insusceptible to ABC proteins and enhancing the spatial accuracy of the drug delivery [25][26].

Multiple studies have already been conducted into the transport of temozolomide across the blood brain barrier, using nanocarrier systems, to increase the effectivity and efficiency of GBM treatment. Trials with the loading of drugs into polymer nanoparticles or liposomes show promising results with respect to drug transport over the BBB and treatment of GBM [27][28]. Combining TMZ with a chemosensitizer is suggested to increase the effectivity of GBM treatment [29]. Many different nanocarriers, transport pathways and drug-release mechanisms have been proposed, but only a very small percentage of the studies progress into clinical development. The aim of this paper is to assess from literature which nanocarrier system would be best suited for the transport of temozolomide and the chemo-sensitizer curcumin to the brain, in order to improve the effectivity and efficiency of the treatment of glioblastoma multiforme.

2. Nanocarrier systems

Many nanocarrier systems with the purpose of encapsulating, transporting and releasing drugs to specific target sites in the human body have been investigated over the past decades [27][28][30]. Different carrier materials, encapsulation strategies, transport pathways and release mechanisms can be used, which gives rise to a wide variety of drug-nanocarrier systems. Not all systems, however, are suited for blood-brain transport, which is inevitable in brain-disease treatment. A second criterium for a nanocarrier used in GBM treatment is the ability to carry hydrophobic loads, because of the hydrophobic nature of TMZ. The majority of the systems meeting these two conditions can be divided into three major categories: liposomes, polymer nanoparticles and nanostructured lipid carriers (NLCs) [31]. Important parameters to determine the effectivity of carrier systems are the blood circulation time, which indirectly relates to the BBB crossing properties of the drug-carrier system, the specificity of the transport mechanism to the BBB and the loading capacity of the carrier [32].

2.1. Liposome nanocarriers

Liposomes are spherical vesicles composed of a lipid bilayer. The liposomal core is suited for dissolving hydrophilic molecules. The hydrophobic bilayer interspace is suited to encapsulate and hold small hydrophobic molecules (figure 2). Encapsulation and transport of TMZ using liposomes is a well-studied treatment approach for GBM treatment [28][33]. The efficiency of liposome carriers depends on the exact properties of the liposome [28]. Liposome transport of TMZ reduces all risks and side-effects induced by the free drug concentration in the blood and has also shown to be able to increase drug accumulation in the cancerous tissue [28]. Besides this, liposomes show low toxicity, good biocompatibility and biodegradability and the ability of controlled drug release [26]. A big disadvantage of liposomal use is the high plasma clearance rate of intravenously administered liposomes. Modifying the surface of the liposome by the inclusion of polymers like polyethylene glycol (PEG) enhances the carriers blood circulation properties, which enhances transport to the brain [26][28].

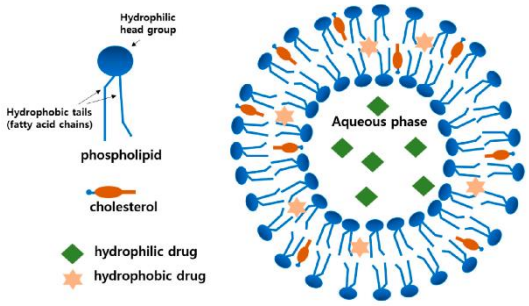

Figure 2, Structure of a liposome nanocarrier. The liposome consists of phospholipid and cholesterol molecules and encapsulates both hydrophilic and hydrophobic drugs. (Figure taken from: [34])

Another downside of liposome carriers is their limited transport volume. Endocytosis of the carrier by the tumor cells is complicated when the carriers exceed a diameter of 80 nm. Using smaller sized liposomes increases transvascular permeability, which improves transport efficiency across the blood brain barrier, but decreases the drug loading-capacity [35].

2.2. Polymer nanoparticles

Polymer nanoparticles (NPs) are another carrier system option for the transport of TMZ to the brain [27]. Polymeric nanoparticles that can be used in this application are for example nanocarriers made of polylactic-co-glycolic acid (PLGA), or chitosan [36][37][38]. PLGA is a biodegradable polymer that hydrolyzes into lactic acid and glycolic acid, which can be processed by the human body into carbon dioxide and water [39]. The polymer is made into nanoparticles and in this process, the drug that must be carried is loaded into the interior environment of the particles [39]. Uncoated PLGA nanoparticles are not suited for BBB drug-transport. The negative charge of the NPs along with the hydrophobic nature of the material result in a short blood circulation time and the targeting of non-target tissues [40]. Therefore, the surfaces of the nanoparticles are functionalized to increase the specificity of the particles [39]. PEG is for example often used to enhance the NPs blood circulation properties and ligands are added to the surface to enhance transport over the blood brain barrier (figure 3) [39]. Another polymer often used in the formation of nanoparticles is chitosan. Chitosan is a cationic biopolymer which can be made into nanoparticles. Chitosan nanoparticles (NPs) are proposed as potential drug-carrier, because of their biodegradability, biocompatibility, non-toxicity and easy manufacture and functionalization process [37]. Chitosan is a polysaccharide consisting of a backbone of OH and NH_2 groups. These groups can be easily modified by additional functional groups, which enhance the effectivity of drug delivery [41]. Properties that can be tuned by adding functional groups are the drug-release profile, mechanical resistance and blood-solubility [41].

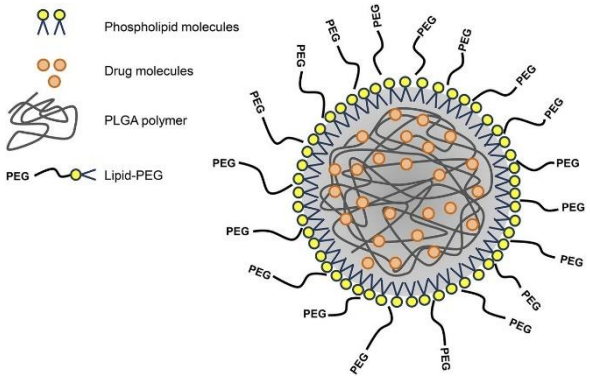

Figure 3, Structure of a drug-loaded PLGA nanoparticle. The drug-loaded PLGA polymers are enclosed by a phospholipid layer conjugated with PEG molecules to increase circulation time. (Figure taken from: [42])

2.3. Nanostructured lipid carriers

Nanostructured lipid carriers (NLCs) are nanocarriers comprised of a combination of both liquid and solid lipids and liquid oils [30]. The liquid and solid lipids together make up the inner core of the NLC. The liquid oils used are preferably natural oils that enable digestion by humans [30]. NLCs can carry drugs by dissolving or incorporating them into the solid lipids in the core of the carrier [43]. The ratio between solid and liquid lipids can be adjusted for different loads [43]. The liquid lipids serve the purpose of preventing the solid lipids from forming a perfect crystal lattice structure, which is what happens in solid lipid nanocarriers (SLNs). SLNs were the progenitor carriers of NLCs and had as major disadvantage that they could carry only small volumes of drugs because of the crystal lattice structure in the core [44]. Three NLC types, type I, II and III, are currently known. The first type is basically a SLN in which a small fraction of the solid lipids is replaced by liquid lipids, which results in a core with an imperfect crystal lattice structure (figure 4.a.). This increases the drug-loading capacity of the carrier

along with a reduced occurrence of unwanted expelling of drugs from the core [43]. In the second NLC type, the solid lipids in the core convert into an amorphous form instead of a crystal structure (figure 4.b.) [43]. The third type is called the multiple type, because of the presence of multiple hydrophobic nano-compartments in the NLC. The multiple type is developed to enhance the loading capabilities of hydrophobic drugs into the NLC core (figure 4.c.) [45]. Since hydrophobic drugs dissolve better in liquid lipids than in solid lipids, these carriers use higher concentrations of liquid lipids [45]. The multiple type consists out of lipids, oil and water. Oil is excessively added to the solid lipid matrix, which results in the formation of oil nano-compartments in this matrix [45]. In the application of drug transport, the drugs are carried in dissolved state in the oil compartments [43]. NLCs are an interesting carrier option for GBM-related drug transport systems, because of the hydrophobic nature of TMZ. NLCs have a large loading capacity compared to other carrier systems and are capable of controlled and sustained drug release. Besides that, NLC-encapsulated drugs are not recognized by efflux pumps when traversing the endothelial cell membrane, which prevents the efflux of drugs at the BBB [43].

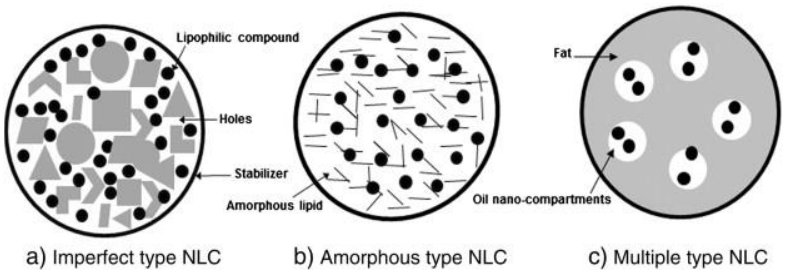

Figure 4, Structure of an SLN and of the three types of NLC's. (a) Structure of the imperfect type NLC, with an imperfect crystal lattice structure. (b) Structure of the amorphous type NLC, in which the solid lipids in the core have been converted into an amorphous form, rather than in a crystal structure. (c) Structure of the multiple type NLC, containing an increased liquid lipid concentration. (Figure taken from: [46])

3. Transport

Nanoparticle based drug delivery across the BBB can be done by either active or passive transport mechanisms (figure 5). Lipid soluble carrier particles that have a very small core-size, like gold-nanoparticles, are able to conduct passive transport, which is simple diffusion through the barrier (figure 5.a.) [25]. Active transport mechanisms are receptor- and adsorption-mediated transcytosis and carrier-mediated transport [25][26]. Receptor-mediated transcytosis makes use of the natural ligand-receptor systems present on the cell membranes of the endothelial cells of the BBB (figure 5.e.). Nanocarriers are coupled to specific ligands, like antibodies or peptides, to be actively transported over the cell membrane by a receptor [25]. Adsorption-mediated transcytosis utilizes the negative charge of the cell membranes of the endothelial cells (figure 5.d.). Positively charged nanoparticles can bind to these membranes, enter the cell and cross the BBB [25]. Carrier-mediated transport is the facilitated diffusion of hydrophobic compounds across the phospholipid bilayer of the endothelial cell by a transporter molecule (figure 5.b.) [47]. In order to get all essential nutrients, specific transporters are present at the BBB to transport, among other substances, essential amino acids and glucose [48]. Coupling of therapeutic molecules to such a nutrient could lead to the transport of the drug across the BBB [48]. Typically, a transporter molecule exposes a binding site on either the in- or outside of the membrane. Binding of a substrate to the transporter results in a conformational change, which facilitates the transport of the substrate to the other side of the membrane [47].

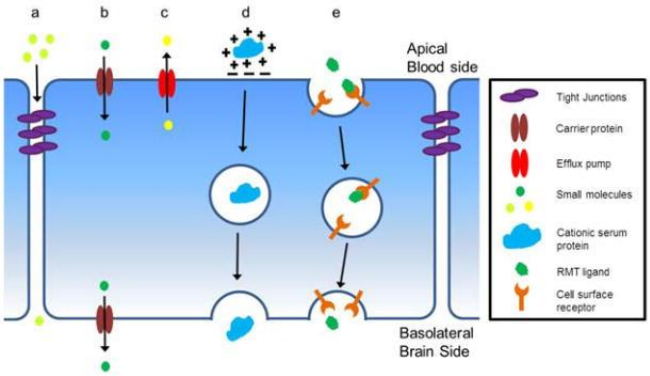

Figure 5, Overview of the different transport mechanisms across the BBB. (a) Restricted diffusion of small hydrophilic molecules by tight junctions. (b) Carrier-mediated transport of small molecules. (c) Efflux of small molecules by efflux pumps. (d) Adsorptive-mediated transcytosis of a cationic molecule. (e) Receptor-mediated transcytosis of a receptor-specific ligand. (Figure taken from: [49])

3.1. Adsorption-mediated transcytosis

Cationic peptides and proteins have an increased ability to cross cell-membranes, compared to anionic compounds [50]. To cross over from the blood stream to the brain, the BBB must be crossed, which means the crossing of the cell-membranes of the capillary-endothelial cells. The luminal surface of the endothelial cells, as well as the abluminal surface, have affinity to bind cationic compounds and this enhances the transport of molecules over the BBB [50]. Liposomes with a cationic character have shown to be more effective in crossing the BBB compared to anionic liposomes [26]. Cationic liposomes interact electrostatically to the negatively charged cell membranes of the endothelial cells in the BBB, which results in the invagination of the cell membrane and the adsorptive-mediated endocytosis of the liposomes [26]. Transcytosis happens when the endosome fuses together with the abluminal surface of the endothelial cell and releases the liposome into the CNS [26]. Cationic proteins and cell-penetrating peptides (CPPs) are used to utilize this mechanism in anionic nanocarriers, or to increase the cationic nature of an already positively charged carrier molecule [50]. Cationic proteins are most often polyamines, and these are used to cationize a given protein like albumin by amidation of the protein's accessible carboxyl groups [51]. The extent to which a protein can be cationized, therefore depends on the number of accessible carboxyl groups in the protein [50].
Nanocarriers can subsequently be coupled to albumin, which gets transported over the BBB using the adsorptive-mediated transcytosis mechanism [51].

CPPs can be either covalently or non-covalently added to a nanocarrier or directly to the drug that has to be transported. Covalent conjugation is the chemical cross-linking of the cargo molecule with the CPP. Non-covalent conjugation utilizes electrostatic and hydrophobic interactions to link the cargo with the CPP [52]. Covalent conjugation directly to the therapeutic molecule has the risk of inducing a change in biochemical activity of this molecule. This risk is avoided by non-covalent conjugation, or by conjugation to a drug-loaded nanocarrier [52]. Direct penetration by CPPs is a passive process, which operates by the interactions between the CPPs and the phosphate groups in the lipid bilayer of the cell membrane [53]. Another proposed mechanism involves transport by inducing the formation of aqueous pores, through which the peptides can cross [53]. CPPs directly linked to therapeutic molecules result in a very small delivery system compared to other delivery systems, which combine low toxicity with high transport efficiency [53]. CPPs have shown the ability to carry macromolecules across cell-membranes and this could be applied to the BBB [53]. A major disadvantage of this transport mechanism is the non-specific uptake by tissues everywhere in the body

[26]. The adsorptive property of the cationized surface of the liposomes or proteins is not only restricted to cell membranes in the BBB, but is found in all living cells [50]. This drastically increases the amount of administered drug-loaded nanocarriers in order to achieve therapeutic conditions in the brain, which is observed to increase endothelial damage of blood vessels [26][50].

3.2. Receptor-mediated transcytosis

Receptor-mediated transcytosis is the other mechanism used for the transport of nanocarrier systems across the BBB. Different carrier systems use this mechanism and a wide variety of ligand-receptor couples are utilized. Receptor-mediated transcytosis is used in both liposome, polymeric, as well as in nanostructured lipid nanocarrier systems and could enhance the drug delivery accuracy compared to adsorptive-mediated endocytosis [54]. When a ligand on the surface of a nanocarrier binds to a receptor on the cell surface of an endothelial cell, the ligand-receptor complex is internalized via endocytosis [26]. The cell membrane starts budding inwards on the location of the ligand-receptor complex, which leads to a vesicle in the cytoplasm of the endothelial cell. This vesicle now contains the targeted receptor, along with the ligand, coupled to the nanocarrier. The vesicle is transported through the cytoplasm and fuses with the abluminal cell membrane, where the vesicle's cargo is released [26]. Receptor-mediated endocytosis can be clathrin dependent or clathrin-independent. In clathrin-dependent endocytosis, vesicle formation is mediated by the protein clathrin [54]. Caveolae-mediated endocytosis is an example of clathrin-independent endocytosis, which uses the integral membrane protein caveolin for vesicle formation [55]. Receptors that are present in the cell-membranes of the endothelial cells in the CNS and that are most linked to BBB crossing of nanocarrier systems are transferrin receptors, insulin receptors and low-density lipoprotein receptors [54][56].

3.2.1. Transferrin receptors

Transferrin receptors (TfRs) are transmembrane glycoprotein that are present in all cells of the human body and serve the function of importing iron into cells [57]. TfRs are especially highly expressed by red blood cells, endothelial cells in the brain and in some types of cancerous cells and much less by other bodily cells [58]. TfR levels in the brain are estimated to be 2 to 7 times larger than in other cells, resulting in a transferrin affinity of 10 to 100 times larger compared to non-brain cells [58]. Transferrin is an iron transport protein, circulating in the blood which can activate the transferrin receptors. When transferrin binds to a TfR in the membrane of a brain-endothelial cell, the iron is transferred through the BBB towards the neurons [57]. TfRs function as the major iron transport mechanism in the brain. Because of their abundant presence in the brain compared to other tissues, TfRs could possibly be utilized in the transport of nanocarriers across the BBB. The downside of using transferrin to enhance cell crossing is the potential competition with endogenous transferrin for binding the TfRs at the BBB, preventing free transferrin uptake and consequently impairing iron transport [58].

Antibodies against TfRs are proteins that naturally have a high affinity to TfRs, with the mouse antibody OX26 as being the most studied antibody for this mechanism [54]. OX26 targets the Tf receptors in endothelial cell membranes and can successfully be transported across the cell membrane. Studies have shown the ability of liposomes to cross the BBB, using the transferrin receptors when conjugated with OX26 antibodies [61]. Other studies, however, claim that it is impossible to reach therapeutic drug concentrations in the brain when using OX26 in receptor-mediated transcytosis of nanocarriers [62].

A third class of TfR targeting molecules are peptides, like the peptide T7 [58]. T7 has the same affinity to TfRs as transferrin. The big difference between transferrin and T7 is that there is no competition between the peptide and endogenous transferrin. This indicates that T7-peptides have affinity for a different binding site at the TfR than transferrin-peptides, which mainly enhances the ligands distribution in tumor tissue in the brain [63][64].

Transferrin receptors on the tumor membrane can also be activated by ligands on the surface of the nanocarrier, resulting in the formation of a clathrin coated vesicle inside the tumor cell. This vesicle now contains TfRs and the drug-loaded nanocarrier. The cargo attached to the TfRs is released in response to a drop in pH, after which the TfRs return to the cell membrane to be reused again later, leaving the nanocarrier inside the tumor cell [59][60]. Besides transferrin, transferrin-binding peptides can be used to target nanocarriers to TfRs to prevent competition with natural transferrin.

3.2.2. Low density lipoprotein receptors

Low density lipoproteins (LDLs) contain the majority of our body's cholesterol. LDLs are transport proteins that have the function of transporting fat molecules to cells [54]. Lipoproteins consist of lipids, bound to apoproteins, which are recognized by low density lipoprotein receptors (LDLRs), which facilitate the endocytosis of the LDL into the endothelial cell [65]. Lipoprotein transport occurs abundantly in the BBB, because of the critical need of essential lipids for brain cells. LDLRs are mainly located on the luminal side of endothelial cells, which enhances the uptake of ligands from the blood circulation [54]. Apoproteins often involved in the activation of the LDL receptors are ApoE and ApoB100, which could be used to coat nanocarriers and induce clathrin-dependent transport across the BBB [54][65]. Another ligand studied is melanotransferrin, which shows greater brain transport quantities compared to the use of transferrin ligands. Melanotransferrin is a ligand, very similar to transferrin, that targets LDL receptors instead of Tf receptors, increasing the blood-brain transport effectiveness [54]. LDL receptors are expressed in all cells, but are more abundantly present in liver cells, adrenal cells and tumor cells. Tumor tissue expresses higher LDLR levels because of the increased need for cholesterol, to be able to keep up with the fast growth rate of the cancerous cells [65]. A proposed strategy to increase the specificity of this transport pathway is by the oral administration of the bile acid sodium taurocholate and hydrocortisone sodium succinate, which suppresses the receptor-mediated uptake of LDL of both the liver and the adrenal gland, without changing the tumor activity [66].

3.2.3. Insulin receptors

Insulin is a polypeptide hormone produced by the Islets of Langerhans in the pancreas and is an important metabolism regulator of fats, carbohydrates and protein. After the intake of food, the concentration of glucose in the blood increases. In response to this, beta cells in the Islets of Langerhans increase their insulin release into the bloodstream causing the uptake of glucose into cells [67]. Insulin receptors (IR) are present in the BBB for the uptake of insulin into the brain. In the brain however, insulin mainly functions as a neuroregulatory peptide rather than as a glucose transport-mediator, since the majority of glucose transport across the BBB is insulin-independent [68]. Using the insulin receptor to transport drug-loaded nanoparticles across the BBB is complicated, because the exact mechanism of insulin transport by this receptor is unknown [68][69]. A research by Ulbrich et al., published in 2010, showed the enhanced transcytosis of nanoparticles loaded with the hydrophobic drug loperamide across the BBB when conjugated to insulin or to the anti-insulin receptor monoclonal

antibody 29B4 [70]. The downside of the use of exogenous insulin as a ligand to target IRs, is that it could cause hypoglycemia in the patient, because of the induced glucose uptake by cells [68]. Furthermore, a research performed by Padridge et al., 2018, to study the insulin receptor-mediated cargo-delivery through the BBB, using human anti insulin receptor monoclonal antibodies (HIRmAbs), shows the first clinical use of receptor-mediated transcytosis for drug delivery into the brain by the insulin receptor [71].

3.3. Carrier-mediated transcytosis

Carrier-mediated endocytosis is typically divided into three steps. First, a substrate molecule binds to the luminal side of a transporter/carrier molecule in the membrane of an endothelial cell. Next, in a response to this binding, the conformation of the carrier molecule changes. The third step is the release of substrate molecules on the other side of the plasma membrane. Carrier molecules are able to facilitate passive transport when transport happens along the concentration gradient [72]. Transporting against the concentration gradient requires an active process, in which the conformational change is driven by ATP, by light or by an ion-gradient [72]. Using ATP or light as an energy source enables the active transport of solutes from low concentrations in the blood to high concentrations in the cell. Ion-gradients can be used in coupled-transport of a solute with an ion. In this process, an ion is transported from a high to a low concentration, which enables the transporter to transport a solute against its electrochemical gradient at the same time. The conformational change makes that the solute-binding site at the luminal side of the carrier molecule becomes unavailable and opens up at the intracellular side of the membrane. Glucose is an example of a solute that can be transported by carrier molecules, using a Na^+ ion-gradient (figure 6).

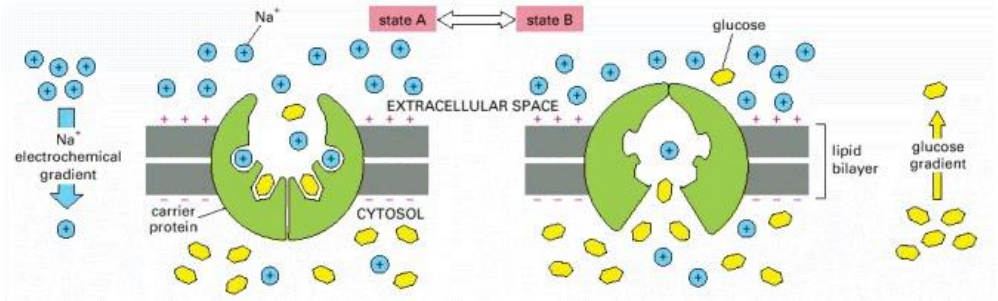

Figure 6, Glucose transport from the extracellular space to the cytosolic side of the lipid bilayer using a Na^+-gradient driven, transmembrane carrier molecule. The conjugation of both Na^+ ions and glucose molecules induces a conformational change of the carrier molecule from state A to state B, resulting in the co-transport of Na^+ and glucose. Glucose is transported against its electrochemical gradient, facilitated by the downhill transport of Na^+. (Figure taken from: [72])

Another mechanism of glucose transport into cells is by passive facilitated diffusion using transmembrane glucose transporters (GLUTs) [54]. Glucose uptake is facilitated by stimulating the accumulation of GLUTs at the luminal cell membrane of the endothelial cell. There are multiple types of GLUT, of which some depend on insulin concentrations in the blood for their functioning. GLUT-1 is an insulin-independent transport protein and is mainly expressed in red blood cells and in endothelial cells in the blood brain barrier. Besides that, upregulation of GLUT-1 is often observed in brain tumor cells [73]. The transport protein has the highest affinity for D-glucose substrates and glucose analogues, as well as oxidized vitamin-C molecules [73]. The GLUT transporters can be targeted by nanocarriers by conjugating D-glucose to the surface. Free D-glucose crosses the cell membrane by passive facilitated diffusion, but when conjugated to a nanocarrier, the transport involves the process of transcytosis [74]. Conjugation of a nanocarrier to D-glucose has already shown positive results for

liposome uptake in the brain via GLUT-mediated transcytosis. GLUT-1 transporters have shown to transport glucose-coated nanocarriers three times faster across brain endothelial cells than across normal endothelial cells, because of their abundant presence in the brain [73]. GLUT-1 furthermore can enhance the transport of glycosylated nanoparticles, with high specificity and high efficiency in brain tumor accumulation [73].

4. Drug-loading

The purpose of a nanocarrier system is to transport a load to a desired location in the body. The load is not naturally present in the carrier structure and thus has to be put there artificially. The loading procedure depends on the type of cargo loaded and the type of nanocarrier used. Within one nanocarrier type, often different loading-strategies are possible. The loading capacity of nanocarriers is also a characteristic that shows great variety among the different systems. The loading capacity is, together with transport efficiency, an important factor in determining the quantity of carriers needed in order to reach therapeutic drug concentrations in the brain. Therapeutic concentrations are often indicated by indicating the half maximal inhibitory concentration (IC_{50}) of a drug, which is the drug concentration at which 50% of the cells in the targeted tissue are inhibited. The IC_{50} value of a drug depends on the treatment time of a cell with this drug. For TMZ, the IC_{50} values for GBM cells are found to range from 1631 µM for a treatment time of 24 hours, down to 57.48 µM for a treatment time of 96 hours [75]. In the process of loading drugs into nanocarriers, two values are of importance, i.e., the encapsulation efficiency and loading efficiency.

4.1. Encapsulation efficiency

Encapsulation efficiency (EE%), also called entrapment efficiency, indicates the percentage of drug quantity that is successfully entrapped into the nanocarrier during the loading process [76]. The encapsulation efficiency is calculated by calculating the ratio between the total weight of entrapped drug after the loading procedure and the total weight of drug that was added before the loading procedure (Eq. 1) [45]. The efficiency of nanoparticle-loading is important because it determines the characteristics of the release process [15]. The encapsulation efficiency differs for every type of nanocarrier and depends strongly on both the characteristics of the drug that is loaded into the carrier and on the technique used to produce and load the nanoparticles [77][9].

Equation 1, Encapsulation efficiency (%EE) (Taken from: [78])

$$\%EE = \frac{\text{Total amount of drug taken} - \text{unentrapped drug}}{\text{Total amount of drug taken}} \text{ X } 100$$

4.2. Loading efficiency

The loading efficiency (%D) of a drug-carrier system refers to the ratio of mass of the drug entrapped in the carrier, compared to the mass of the total carrier system (Eq. 2) [76]. The loading efficiency determines the amount of nanocarriers that have to be administered to the patient per treatment, in order to achieve therapeutic drug concentrations at the target site [76].

Equation 2, Loading efficiency (%D) (Taken from: [78])

$$\%D = \frac{\text{Total amount of drug taken} - \text{Unentrapped drug}}{\text{Total amount of drug taken} + \text{Amount of excipient added}} X \ 100$$

4.3. Liposome loading

The loading of drugs into liposomes can be done by either active/remote loading, or passive loading [76]. Active loading means that the drugs are loaded into the liposome after the liposome is produced. In passive loading, the drugs are loaded into the liposome during the formation of the liposome itself [76]. A passive loading technique often applied for the loading of hydrophobic drugs, like TMZ, is the ethanol injection method [79][80]. In this method, a solution containing ethanol and lipids is injected into an aqueous medium. Because of the fast injection, phospholipids get dispersed all over the medium and their reciprocal affection results in the formation of small bilayer fragments, which later arrange into liposome molecules [80].

An active or remote loading technique is drug loading into already formed liposomes by the appliance of transmembrane gradients [76]. The drugs have to pass the membrane of the liposome to get in and then have to be trapped inside. For this to happen, the drug has to change its degree of ionization in the process of passing the liposomal membrane based on a pH stimulus. Amphiphilic weak acids or bases are the only compounds capable of doing this [76]. The pH- and ion-gradient that exists between the in and outside of the liposome is the driving force for the loading procedure. The drugs are applied to the external environment of the liposomes and because of the gradients present, diffusion of the drugs to the interior of the liposomes takes place. Encapsulation and loading efficiencies depend on the magnitude of the gradients applied and on the exact drug that is being encapsulated. Drugs carrying a positive charge greater than 1, showed a high encapsulation efficiency [76].

The encapsulation efficiency of TMZ into liposomes depends on the liposome preparation technique used and the dimensions of the liposomes. Using the ethanol injection method (Kim et al., 2015), the encapsulation efficiency of TMZ-loaded liposomes, with a particle size of 41.4 ± 9.2 nm, was found to be 45.23 ± 4.34 % [81]. Using bigger liposome particles, typically increases the encapsulation efficiency, but decreases the ability to penetrate the BBB. A study by Patel et al., from 2015, presents encapsulation efficiencies of 78.25±0.98%, when using the ethanol injection method with liposomes particle size of 105.7±3.9 nm [82]. The TMZ loading efficiency of liposomes was measured using the passive loading method of pro-liposomes (Gao et al., 2015) and was found to be 2.81 ± 0.20% for liposomes with dimensions of 156.70 ± 11.40 nm [83].

4.4. Polymer loading

The drug loading procedures in polymer nanoparticles are very diverse. A proposed drug loading mechanism is using a spontaneous emulsification solvent diffusion method (Niwa et al., 1993). In the case of a hydrophobic drug and a PLGA nanocarrier system, the drug and PLGA are dissolved in a mixture of acetone and chloroform. The mixture is poured into an aqueous alcohol solution. High-speed stirring of this solution results into the emulsification of the PLGA mixture into the aqueous alcohol solution. The PLGA mixture arranges into drug-loaded PLGA nanospheres with a size of 200 to 300 nm [80]. This same emulsion-solvent evaporation method was used by Sayiner et al., 2010, to

produce TMZ-loaded PLGA nanoparticles. The PLGA particles used in this study ranged in size from 100 to 200 nm and showed an encapsulation efficiency of 55 to 70 % [84].

Another research, dedicated on assessing the capability of TMZ-loaded PLGA nanoparticles in the function of drug carrier system in the treatment of GBM, proposed and assessed a different PLGA loading mechanism (Anata et al., 2016). PLGA was loaded with TMZ using a TMZ saturated aqueous phase, which resulted in nanoparticles with dimensions smaller than 200 nm. The maximal loading efficiency achieved by this method was 4.4%. Because the loading mechanism shows a poor encapsulation efficiency, PLGA nanoparticles were concluded to not be suitable for treatment of GBM by single drug (TMZ) delivery [85].

4.5. NLC loading

The commonly used methods of drug-loaded NLC preparation are by cold/hot high-pressure homogenization (HPH) or hot emulsification-ultrasound [43]. In all these approaches in general, the higher the solubility of the drug in the solid/liquid lipid mixture, the higher the encapsulation and loading efficiency [43][45]. Entrapment efficiency is for NLCs also dependent on the degree of crystallization of the lipid core [45]. HPH is the most common and reliable method of NLC preparation known so far and can be divided into the hot and the cold approach.

In hot HPH, the mixture of the liquid and solid lipids is heated until all solid lipids are melted. The drug is added to the heated mixture and disperses. Separately, a mixture of surfactant and deionized water is heated to the same temperature as the lipid mixture and is subsequently added to the lipid mixture. The two liquid mixtures are made into a pre-emulsion, after which it passes through the high-pressure homogenizer. The homogenizer forces the liquid through a system, resulting in great forces on the sample. The forces homogenize the mixture and reduce the particle size of lipids present in the liquid. Passing the pre-emulsion multiple times through the homogenizer results in a smaller particle size. The homogeneous emulsion is cooled by stirring, which leads to recrystallization of the solid lipids and solid drug-loaded NLC particles [43]. Limitations of this method are that it is only suitable for drugs that are not heat-sensitive and that it could cause the decomposition of hydrophilic drugs [43].

Cold HPH deals with the limitations of hot HPH. The main difference of cold HPH compared to hot HPH is the cooling process. In hot HPH, the emulsion is slowly cooled by stirring. In cold HPH, the emulsion is very rapidly cooled after homogenization by using liquid nitrogen or dry ice. This approach minimizes the thermal exposure of the drug and is therefore better suited for use with heat-sensitive compounds. Cold HPH shows higher entrapment efficiencies and a more uniform distribution of drug molecules in the mixture [43][45]. The downside of cold HPH is that there is a broader distribution of NLC particle sizes, compared with hot HPH [45].

Another method is the hot emulsification-ultrasound method, which is very similar to HPH. The liquid and solid lipids are again mixed and heated up to just above the melting temperature of the solid lipids. The surfactant is heated to this same temperature and added to the lipid mixture, together forming the pre-emulsion. The pre-emulsion is stirred with a sufficient speed to create a homogeneous emulsion. Alternating high- and low-pressure waves are applied to the emulsion in a process called ultrasonication, which causes a decrease in particle size. The emulsion is cooled at room temperature, causing the lipids to solidify and form NLCs [43][45]. The thermal exposure of the drug is much smaller than in hot HPH and this method results in particles with dimensions of 30-100 nm, depending on the solid lipid concentration and the specific surfactant used [45].

A study to the efficacy of TMZ loaded NLCs to enhance brain targeting (Khan et al., 2016), prepared TMZ-NLCs using the cold HPH method. The NLCs produced all had dimensions within the nanometer range and showed an entrapment efficiency of 81.64 ± 3.71% [86]. Studies to co-loading of

NLCs with TMZ and DNA (Chen et al., 2015) or TMZ and vincristine (Zhang et al., 2017) also showed high encapsulation and loading efficiencies for TMZ. The dual-loading of TMZ and DNA into NLC nanoparticles showed for TMZ an encapsulation efficiency of 80.5 ± 2.8% [87]. The co-loading of TMZ and vincristine resulted for TMZ in an EE% of 83.4 ± 2.9% and a %D of 10.1 ± 0.7% [88].

5. GBM-specific environment

In addition to the successful loading of nanocarriers with a drug, the nanocarrier needs to be able to be transported through the blood brain barrier and into tumor cells. There, the drug has to be released from the carrier in order to destroy the cell. The characteristics of drug release are strongly dependent on the properties of the drug and the amount of drug loaded into the nanoparticle. The entrapment efficiency and loading efficiency, again have a role in the functioning of the release mechanism. An important property of a nanocarrier system in the application of drug release in the brain, is the ability of sustained and controlled release [89]. Drug release can be induced by a variety of triggers in the GBM tissue, or happen by simple diffusion through carrier membrane. Diffusion is observed in the release of hydrophobic drugs from liposome nanocarriers. The drugs encapsulated in the carrier slowly diffuse through the outer membrane, because of the drug concentration difference between the interior and the exterior environment of the particle. The typical drug-release profile for diffusion is biphasic, consisting of an initial drug release burst, followed by a sustained release pattern [90]. Release by diffusion is not location specific and the release of drugs will not be restricted to only the tumor area. To restrict nanocarrier drug-release to only the target tissue, tissue specific characteristics have to be utilized. GBM-tissue differs from the surrounding tissue with respect to pH, redox potential and enzymatic activity, which could all be used as a release-trigger [91][92][93]. Besides the natural differences in the microenvironment of tissues, external stimuli could be applied to artificially change the environmental conditions in a restricted area [89].

5.1. Acidity

Making use of the differences between the cellular microenvironment of tumor tissue and healthy tissue can help to restrict drug release to only the malignant tissue. A stimulus that could be used for the spatial specific delivery of drugs to tumors is pH. The extracellular environment of cancerous tissue is known to have an acidic pH, in contrast to the neutral pH of its surrounding tissue [91]. Nanocarrier systems can make use of this, by making the drug release pattern pH-dependent. The exposure to an acidic pH could weaken the structural integrity of nanocarriers by impairing cross-links or changing the spatial conformation of lipids and polymers (figure 7). Weakening of the structural integrity of the nanocarrier, eases the diffusion of the encapsulated drug, enhancing drug release [91]. Both liposomes, polymer nanocarriers and NLCs are explored that show an increased drug release rate under acidic pH compared to a neutral or basic pH [94][95][96].

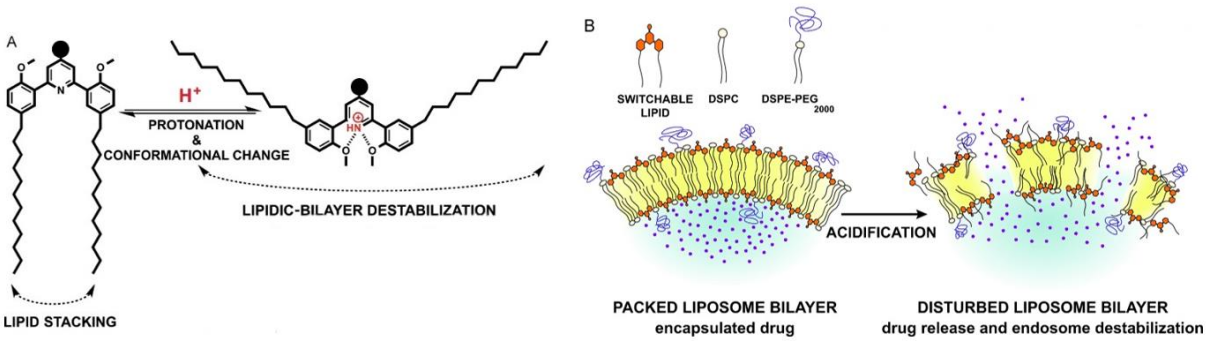

Figure 7, Conformational change of liposomal lipids, induced by protonation in an acidic environment, resulting in the destabilization of a liposome bilayer [97]. (A) The long chains of the lipid molecule change their conformation due to protonation by protons in an acidic environment. (B) Destabilization of the liposome bilayer, consisting of pH sensitive lipids, along with distearoylphosphatidylcholine (DSPC) and pegylated 1, 2-Distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-PEG) phospholipids, resulting in release of the encapsulated drug.

5.2. Redox potential

Another characteristic of GBM tissue that could potentially be used as a stimulus for drug release is the redox state. The redox state indicates the tendency of a tissue to oxidize or reduce substrates. The redox state of tumors depends on the ratio NADPH/$NADP^+$ and reduced/oxidized glutathione (GSH/GSSG). Reducing environments contain a much higher GSH concentration than NADPH concentration and in tumor tissues, the GSH concentrations are reported to be up to four times higher than in healthy tissues [92]. Redox-responsive nanocarriers are carriers that contain for example disulfide or diselenide bonds. Glutathione is capable of breaking down disulfide bonds, resulting in the break-down of the carrier system and subsequent release of its cargo. Multiple reduction-sensitive structures exist that utilize this mechanism, which can be added to a nanocarrier in various locations to realize a highly efficient release mechanism with a low toxicity [92].

5.3. Enzymatic activity

Enzyme expression is a third notable difference in the microenvironment of tumor tissues with respect to other tissues. Cancerous tissues have shown to express increased concentrations of enzymes like proteases, phosphatases and glycosidases [93]. Enzyme-based drug release makes use of enzyme-catalyzed chemical reactions in an enzyme-sensitive component of the nanoparticle. Enzyme cleavable links are implemented in carrier systems to make their structural integrity enzyme responsive. Bonds that can be cleaved by enzymes are e.g., disulfide, ester and amide bonds. Enzymes often used in this application are the matrix metalloprotease enzymes (MMPs). The desired release pattern of a nanocarrier is often a sustained release. In order to achieve this, it is important to limit the destruction done to the structure of the carrier. Rapid cleavage and degeneration by enzyme activity leads to a rapid burst pattern in drug release, which may not be beneficial for treatment [93]. Unstable drug-loaded liposomes can be stabilized by the surface-conjugation of stabilizing polymers with amide bonds. Enzymatic degradation of these bonds by enzymes in the tumor tissue diminishes the stability of the liposome and enhances drug release. Surface-localized enzyme-sensitive components are easily accessible to enzymes, resulting in a short response time of these components after entering the target tissue [98].

5.4. External stimuli

Besides using tissue related stimuli, external stimuli could also be used to induce drug release. Examples of external stimuli are light, ultrasound and magnetic fields [89]. Nanoparticles composed of materials that efficiently convert infrared light into heat, have shown to be successfully triggered to release their cargo in response to irradiation with IR light (de Solorzano et al., 2020). Ultrasound can induce mechanical or thermal effects in nanocarriers. High intensity ultrasounds lead to thermal degradation of carriers, inducing drug release. Lower intensity ultrasound induces drug release by creating mechanical stress in the material. An alternating magnetic field has shown the ability to cause mild hyperthermia in TMZ-loaded, lipid-based, magnetic nanoparticles (Tapeinos et al., 2019). The hyperthermia causes the slow degeneration of the carrier, causing sustained drug release [99].

6. Carrier mediated dual-drug delivery

6.1. Chemosensitizers

Chemosensitizers are compounds that make tumor cells more sensitive to chemotherapeutic drugs, which helps in the battle against treatment resistance [100]. Combining the use of chemosensitizers with chemotherapy increases the effectivity of treatment drastically, which makes that lower drug doses have to be administered in order to achieve therapeutic concentrations [101]. Multiple different chemosensitizers are known and these improve chemotherapy treatment in various ways [100]. In the treatment of GBM with TMZ, curcumin, a highly hydrophobic chemical produced by plants of the Curcuma longa species, is proposed to be used as chemosensitizer [101]. Curcumin (CUR) is believed to enhance TMZ effectivity by downregulating ATP-binding cassette transport proteins, also called efflux pumps [102]. Other curcumin tumor-inhibiting mechanisms are the activation of apoptotic pathways, induction of G2/M cell cycle arrest, induction of autophagy and disruption of molecular signaling [101][103]. Preclinical in vitro and in vivo studies have shown a synergistic effect between CUR and TMZ in which the combination of these agents caused a significant decrease in the IC_{50} value for GBM cells, compared to the IC_{50} for each of these agents individually (figure 8) [101][104]. Additionally, a study by Dilnawaz et al., from 2010, showed that the simultaneous co-delivery of both therapeutic agents to the brain using an iron-oxide nanocarrier further increases treatment efficiency, by decreasing the IC_{50} from 0.8 µg/mL to 0.1 µg/mL [105].

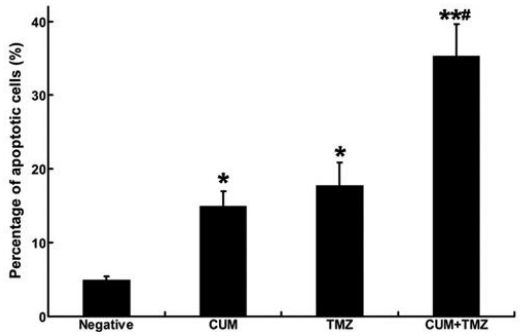

Figure 8, Induced apoptosis of GBM cells by CUR, TMZ or TMZ+CUR administration [104].
The percentage of apoptotic GBM-cells, when untreated, treated with subtoxic levels of CUR (1.25 µg/mL), TMZ-treated (15.625 µg/mL) and treated with a combination of these two agents, TMZ+CUR (TMZ, 15.625 µg/mL; CUR, 1.25 µg/mL).
** P<0.05 w.r.t. control group, **P<0.01 w.r.t. control group, #P<0.05 w.r.t. CUR and TMZ group*
(Figure taken from: [104])

The downside of CUR is its very poor bioavailability, due to the compound's rapid metabolism, chemical instability and rapid systemic elimination [106]. This is the main reason for the KWF Dutch Cancer Society to discourage further research to curcumin by ending any financial support to curcumin-research groups [107]. Using nanocarriers to deliver CUR to the target tissue however, has shown to drastically increase its bioavailability and enhance its therapeutic activity, which explains the continued interest to use CUR in anti-cancer treatment, when formulated in nanocarriers [108][109].

6.2. TMZ/CUR-loaded nanocarriers

Combining TMZ with a chemosensitizer enhances the effectivity of GBM treatment. Nanocarrier delivery of both the drug and the chemosensitizer is currently the most efficient way of transporting compounds from the blood to a cancerous region in the brain. The ability of a nanocarrier to carry both TMZ as well as curcumin would mean a more effective treatment with a lower administered drug concentration, meaning less side-effects [101]. A research conducted to the dual loading of nanostructured lipid carriers (NLCs) (Xu et al., 2020) showed promising results. The cold high pressure homogenization method was used to mix the solid and liquid lipids and the curcumin and temozolomide were added to this mixture in a weight-ratio TMZ/CUR/lipid of 2:1:40. After combining this solution with the surfactant mixture, the emulsion is cooled by quickly immersing it into ice-water. Magnetic stirring subsequently results in the formation of NLCs loaded with both TMZ and CUR, with a particle size of 78.49 nm ± 0.38. The encapsulation efficiency of both TMZ and CUR were around 70%. Drug-release from the NLCs happens according to a simple diffusion mechanism. The drug release study shows a much higher initial release rate of CUR compared to the initial release rate of TMZ. A sequential release pattern is proposed (figure 9), explained by the highly hydrophobic character of CUR. CUR is more hydrophobic than TMZ, which could have led to CUR arrangement nearby the outer lipid walls, rather than in the center of the carrier. The location of CUR inside the NLC could account for faster initial diffusion rates of CUR.

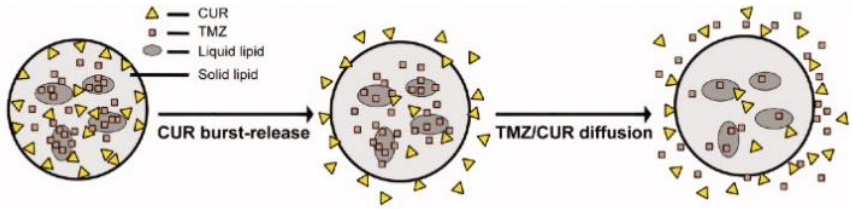

Figure 9, Sequential CUR/TMZ release from TMZ/CUR-loaded NLCs.
(Figure taken from: [110])

In vitro and in vivo testing has shown the capability of TMZ/CUR-NLCs to cross the BBB, accumulate in the tumor tissues in the brain and to sequentially release CUR and TMZ to enhance the apoptosis ratio of GBM cells (figure 10). Besides that, no increased toxic effects of the NLCs at therapeutic concentrations to non-target tissues were observed [110]. This research points out the ability of TMZ/CUR-NLCs to enhance the inhibition of GBM tissues compared to single-drug alternatives, without increasing toxic effects, which makes the usage of NLCs in dual-treatment mechanisms very interesting for further clinical investigation.

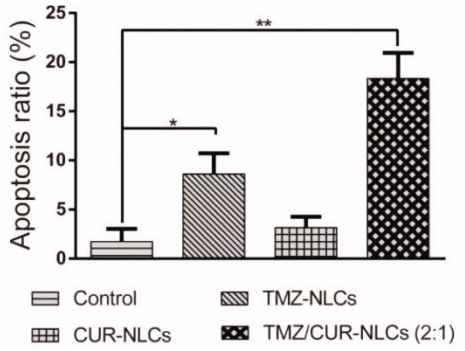

Figure 10, Apoptosis ratio of GBM cells in TMZ/CUR-NLC treatment, compared to TMZ-NLC and CUR-NLC treatment. Apoptosis ratio in percentages of GBM-cells that are untreated, treated with CUR-NLCs (3.33 µg/mL of CUR), treated with TMZ-NLCs (6.67 µg/mL of TMZ) and treated with TMZ/CUR-NLCs (TMZ, 6.67 µg/mL; CUR, 3.33 µg/mL) .
**P<0.5, **P<0.01*
(Figure taken from: [110])

7. Discussion

GBM-patient survival rates and quality of life would benefit from the use of nanocarrier drug-delivery systems. Nanocarrier drug-delivery systems are able to increase GBM treatment-effectivity and efficiency, while at the same time decreasing treatment-toxicity to non-target tissues. The ideal nanocarrier for the use in GBM treatment is non-toxic to healthy tissues, able to carry TMZ, able to efficiently cross the BBB, has a great affinity for GBM-tissue and is capable of GBM tissue-induced, controlled and sustained release of TMZ. Transporting TMZ encapsulated in a nanocarrier has multiple advantages over transport of TMZ in free form. The nanocarrier prevents the premature hydrolysis of TMZ into MTIC in the blood, which increases the drug's stability and also enhances the BBB crossing-efficiency of the drug. The GBM tissue-specificity of encapsulated TMZ molecules is higher than that of free TMZ molecules. Additionally, nanocarriers allow for the co-delivery of TMZ with a chemosensitizer, like CUR, to GBM tissue. These factors all enable the administered dose of TMZ to be lowered, while still reaching therapeutic TMZ concentrations in the GBM tissue, meaning a significant decrease in detrimental side effects induced by the treatment.

The central question in this paper is the question of which nanocarrier system would be best suited for the co-transport of temozolomide and curcumin to the brain, in order to improve the effectivity and efficiency of the treatment of glioblastoma multiforme. In my opinion, based on the currently available literature discussed in this paper, the best suited nanocarrier system for this application would be an NLC carrier, since they have a large loading capacity and are capable of controlled and sustained drug release. Both liposomes and polymer nanoparticles with dimensions small enough to cross the BBB, often have a small drug loading capacity, which makes it harder to reach therapeutic drug conditions in the brain. Encapsulation efficiencies of liposomes and polymeric nanoparticles are typically lower than that of the NLCs. Another pre of using NLCs as nanocarriers is their proven ability of dual-drug delivery. Loading the NLCs with a combination of TMZ and CUR will further enhance the efficiency and effectivity of GBM treatment. To achieve the requirement of tissue-specific targeting, receptor-mediated transcytosis must be chosen over adsorptive-mediated transcytosis. Transferrin receptors are receptors that are very abundantly present in the brain and much less in other tissues, which enhances the tissue-specificity of carriers targeting this receptor. The ligand best suited for targeting this receptor is the T7 peptide, which has a very high affinity for TfRs,

while avoiding competition with endogenous transferrin-peptides. Conjugation of T7 peptides to the NLC surface can be done in combination with PEG conjugation in order to improve NLC circulation time. For the release of the therapeutic agents, the extraordinary high glutathione concentrations in tumor tissues can be utilized. Including disulfide bonds in the NLCs will make them susceptible for GSH cleavage, which makes that they lose their structure inside the tumor and subsequentially release their cargo.

More than 150 years after its discovery, long-term survival after diagnosis with GBM is still extremely rare. Recent developments in the field of biomedical engineering have given perspective for more effective treatment strategies, by controlled and localized drug-delivery using nanocarriers. Very few currently available drug-delivery systems have already progressed into clinical trials, which indicates the importance of the continuation of extensive research in this topic. Future research should focus on the development and testing of reduction sensitive, pegylated NLC nanocarriers, stabilized by disulfide bonds, dual loaded with TMZ and CUR by cold high-pressure homogenization, with T7-peptides conjugated to the surface. Experimentation has to point out whether production of this proposed nanocarrier system is feasible and whether the intended gain in treatment effectivity can be practically achieved. Development of the ideal nanocarrier system to deliver chemotherapeutic agents would increase the GBM patient-survival rates and additionally increase the patient's quality of life during and after treatment, finally enabling us to fight back at the most aggressive and detrimental type of brain cancer known to this day.

8. References

1. Wirsching, H., Galanis, E., & Weller, M. (2016). Glioblastoma. *Handbook of Clinical Neurology*, 381-397. doi:10.1016/b978-0-12-802997-8.00023-2

2. Thakkar, J. P., Dolecek, T. A., Horbinski, C., Ostrom, Q. T., Lightner, D. D., Barnholtz-Sloan, J. S., & Villano, J. L. (2014). Epidemiologic and molecular prognostic review of glioblastoma. *Cancer Epidemiology Biomarkers & Prevention*, 23(10), 1985-1996. doi:10.1158/1055-9965.epi-14-0275

3. Harter, D., Wilson, T., & Karajannis, M. (2014). Glioblastoma multiforme: State of the art and future therapeutics. *Surgical Neurology International*, 5(1), 64. doi:10.4103/2152-7806.132138

4. Ballabh, P., Braun, A., & Nedergaard, M. (2004). The blood–brain barrier: An overview. *Neurobiology of Disease*, 16(1), 1-13. doi:10.1016/j.nbd.2003.12.016

5. Munoz, J. L., Rodriguez-Cruz, V., Greco, S. J., Nagula, V., Scotto, K. W., & Rameshwar, P. (2014). Temozolomide induces the production of epidermal growth factor to regulate mdr1 expression in glioblastoma cells. *Molecular Cancer Therapeutics, 13*(10), 2399-2411. doi:10.1158/1535-7163.mct-14-0011

6. Liu, S., Agalliu, D., Yu, C., & Fisher, M. (2012). The role of pericytes in blood-brain barrier function and stroke. *Current Pharmaceutical Design, 18*(25), 3653-3662. doi:10.2174/138161212802002706

7. Obermeier, B., Daneman, R., & Ransohoff, R. M. (2013). Development, maintenance and disruption of the blood-brain barrier. *Nature Medicine, 19*(12), 1584-1596. doi:10.1038/nm.3407

8. Omidi, Y., & Barar, J. (2012). Impacts of blood-brain barrier in drug delivery and targeting of brain tumors. *BioImpacts : BI*, *2*(1), 5–22. https://doi.org/10.5681/bi.2012.002

9. Pullagurla, S. R., Baird, A. E., Adamski, M. G., & Soper, S. A. (2015). Current and future bioanalytical approaches for stroke assessment. *Bioanalysis, 7*(8), 1017-1035. doi:10.4155/bio.15.40

10. Fernandes, C., Costa, A., Osório, L., & Costa, R. (n.d.). Glioblastoma. In 1305710984 960503312 S. D. Vleeschouwer (Editor), *Glioblastoma* (p. Chapter 11).

11. Barani, I. J., & Larson, D. A. (2014). Radiation therapy of Glioblastoma. *Cancer Treatment and Research*, 49-73. doi:10.1007/978-3-319-12048-5_4

12. Miller, J. J., & Reardon, D. A. (2018). The use of temozolomide for Chemoradiation and adjuvant therapy of high-grade gliomas. *Handbook of Brain Tumor Chemotherapy, Molecular Therapeutics, and Immunotherapy*, 411-417. doi:10.1016/b978-0-12-812100-9.00031-0

13. National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 5394, Temozolomide. Retrieved April 26, 2021 from https://pubchem.ncbi.nlm.nih.gov/compound/Temozolomide.

14. Kwok, K. K., Vincent, E. C., & Gibson, J. N. (2017). Antineoplastic drugs. *Pharmacology and Therapeutics for Dentistry*, 530-562. doi:10.1016/b978-0-323-39307-2.00036-9

15. Agarwala, S. S., & Kirkwood, J. M. (2000). Temozolomide, a Novel alkylating agent with activity in the central nervous system, may improve the treatment of advanced metastatic melanoma. *The Oncologist, 5*(2), 144-151. doi:10.1634/theoncologist.5-2-144

16. Khosa, A., Krishna, K. V., Dubey, S. K., & Saha, R. N. (2019). Lipid nanocarriers for Enhanced delivery of temozolomide to the brain. *Drug Delivery Systems,* 285-298. doi:10.1007/978-1-4939-9798-5_15

17. Baker, S. D., Wirth, M., Statkevich, P., Reidenberg, P., Alton, K., Sartorius, S. E., Dugan, M., Cutler, D., Batra, V., Grochow, L. B., Donehower, R. C., & Rowinsky, E. K. (1999). Absorption, metabolism, and excretion of 14C-temozolomide following oral administration to patients with advanced cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research*, *5*(2), 309–317.

18. Stupp, R., Mason, W. P., Van den Bent, M. J., Weller, M., Fisher, B., Taphoorn, M. J., . . . Mirimanoff, R. O. (2005). Radiotherapy plus Concomitant and adjuvant temozolomide for glioblastoma. *New England Journal of Medicine*, 352(10), 987-996. doi:10.1056/nejmoa043330

19. Singh, N., Miner, A., Hennis, L., & Mittal, S. (2020). Mechanisms of temozolomide resistance in glioblastoma - a comprehensive review. *Cancer Drug Resistance*. doi:10.20517/cdr.2020.79

20. Lee, S. Y. (2016). Temozolomide resistance in glioblastoma multiforme. *Genes & Diseases*, 3(3), 198-210. doi:10.1016/j.gendis.2016.04.007

21. Munoz, J. L., Rodriguez-Cruz, V., Greco, S. J., Nagula, V., Scotto, K. W., & Rameshwar, P. (2014). Temozolomide induces the production of epidermal growth factor to regulate mdr1 expression in glioblastoma cells. *Molecular Cancer Therapeutics, 13*(10), 2399-2411. doi:10.1158/1535-7163.mct-14-0011

22. Kazda, T., Dziacky, A., Burkon, P., Pospisil, P., Slavik, M., Rehak, Z., . . . Lakomy, R. (2018). Radiotherapy of glioblastoma 15 years after the landmark Stupp's trial: More controversies than standards? *Radiology and Oncology*, 52(2), 121-128. doi:10.2478/raon-2018-0023

23. Azad, T. D., Pan, J., Connolly, I. D., Remington, A., Wilson, C. M., & Grant, G. A. (2015). Therapeutic strategies to improve drug delivery across the blood-brain barrier. *Neurosurgical Focus*, 38(3). doi:10.3171/2014.12.focus14758

24. Zhang, H., Van Os, W. L., Tian, X., Zu, G., Ribovski, L., Bron, R., . . . Zuhorn, I. S. (2021). Development of curcumin-loaded zein nanoparticles for transport across the blood–brain barrier and inhibition of glioblastoma cell growth. *Biomaterials Science*. doi:10.1039/d0bm01536a

25. Teleanu, D., Chircov, C., Grumezescu, A., Volceanov, A., & Teleanu, R. (2018). Blood-Brain delivery methods using nanotechnology. *Pharmaceutics*, 10(4), 269. doi:10.3390/pharmaceutics10040269

26. Vieira, D., & Gamarra, L. (2016). Getting into the brain: Liposome-based strategies for effective drug delivery across the blood-brain barrier. *International Journal of Nanomedicine*, Volume 11, 5381-5414. doi:10.2147/ijn.s117210

27. Baeza, A., Ruiz-Molina, D., & Vallet-Regí, M. (2016). Recent advances in porous nanoparticles for drug delivery in antitumoral applications: Inorganic nanoparticles and nanoscale metal-organic frameworks. *Expert Opinion on Drug Delivery*, 14(6), 783-796. doi:10.1080/17425247.2016.1229298

28. Zhan, W. (2019). Delivery of liposome encapsulated temozolomide to brain tumour: Understanding the drug transport for optimisation. *International Journal of Pharmaceutics*, 557, 280-292. doi:10.1016/j.ijpharm.2018.12.065

29. Rahbar Takrami, S., Ranji, N., & Sadeghizadeh, M. (2019). Antibacterial effects of curcumin encapsulated in nanoparticles on clinical isolates of pseudomonas aeruginosa through downregulation of efflux pumps. *Molecular Biology Reports, 46*(2), 2395-2404. doi:10.1007/s11033-019-04700-2

30. Fang, C., A. Al-Suwayeh, S., & Fang, J. (2012). Nanostructured lipid Carriers (nlcs) for drug delivery and targeting. *Recent Patents on Nanotechnology, 7*(1), 41-55. doi:10.2174/187221051130701004 1

31. Sharma, S., Italiya, K., Mittal, A., & Chitkara, D. (2017). New strategies for cancer management: How can temozolomide carrier modifications improve its delivery? *Therapeutic Delivery, 8*(7), 475-477. doi:10.4155/tde-2017-0016

32. Petrak, K. (2005). Essential properties of drug-targeting delivery systems. *Drug Discovery Today, 10*(23-24), 1667-1673. doi:10.1016/s1359-6446(05)03698-6

33. Johnsen, K. B., & Moos, T. (2016). Revisiting nanoparticle technology for blood–brain barrier transport: Unfolding at the endothelial gate improves the fate of transferrin receptor-targeted liposomes. *Journal of Controlled Release, 222*, 32-46. doi:10.1016/j.jconrel.2015.11.032

34. Lee, M. (2020). Liposomes for enhanced bioavailability of water-insoluble drugs: In vivo evidence and recent approaches. *Pharmaceutics, 12*(3), 264. doi:10.3390/pharmaceutics12030264

35. Hatakeyama, H., Akita, H., Maruyama, K., Suhara, T., & Harashima, H. (2004). Factors governing the in vivo tissue uptake of transferrin-coupled polyethylene glycol liposomes in vivo. *International Journal of Pharmaceutics*, 281(1-2), 25-33. doi:10.1016/j.ijpharm.2004.05.025

36. Patel, T., Zhou, J., Piepmeier, J. M., & Saltzman, W. M. (2012). Polymeric nanoparticles for drug delivery to the central nervous system. *Advanced Drug Delivery Reviews, 64*(7), 701-705. doi:10.1016/j.addr.2011.12.006

37. Garg, U., Chauhan, S., Nagaich, U., & Jain, N. (2019). Current advances in Chitosan Nanoparticles based drug delivery and targeting. *Advanced Pharmaceutical Bulletin, 9*(2), 195-204. doi:10.15171/apb.2019.023

38. Yan, G., Li, A., Zhang, A., Sun, Y., & Liu, J. (2018). Polymer-Based nanocarriers for co-delivery and combination of Diverse therapies against cancers. *Nanomaterials, 8*(2), 85. doi:10.3390/nano8020085

39. Lee, C., & Ooi, I. (2016). Preparation of temozolomide-loaded nanoparticles for Glioblastoma multiforme Targeting—Ideal versus reality. *Pharmaceuticals, 9*(3), 54. doi:10.3390/ph9030054

40. Cai, Q., Wang, L., Deng, G., Liu, J., Chen, Q., & Chen, Z. (2016). Systemic delivery to central nervous system by engineered PLGA nanoparticles. *American journal of translational research*, *8*(2), 749–764.

41. Lara-Velazquez, M., Alkharboosh, R., Norton, E. S., Ramirez-Loera, C., Freeman, W. D., Guerrero-Cazares, H., Forte, A. J., Quiñones-Hinojosa, A., & Sarabia-Estrada, R. (2020). Chitosan-Based Non-viral Gene and Drug Delivery Systems for Brain Cancer. *Frontiers in neurology*, *11*, 740. https://doi.org/10.3389/fneur.2020.00740

42. Yingchoncharoen, P., Kalinowski, D. S., & Richardson, D. R. (2016). Lipid-Based drug delivery systems in Cancer therapy: What is available and what is yet to come. *Pharmacological Reviews, 68*(3), 701-787. doi:10.1124/pr.115.012070

43. Salvi, V. R., & Pawar, P. (2019). Nanostructured lipid carriers (nlc) system: A novel drug targeting carrier. *Journal of Drug Delivery Science and Technology, 51*, 255-267. doi:10.1016/j.jddst.2019.02.017

44. De Jesus, M. B., & Zuhorn, I. S. (2015). Solid lipid nanoparticles as nucleic acid delivery system: Properties and molecular mechanisms. *Journal of Controlled Release, 201*, 1-13. doi:10.1016/j.jconrel.2015.01.010

45. Chauhan, I., Yasir, M., Verma, M., & Singh, A. P. (2020). Nanostructured lipid carriers: A Groundbreaking approach for Transdermal drug delivery. *Advanced Pharmaceutical Bulletin, 10*(2), 150-165. doi:10.34172/apb.2020.021

46. Tamjidi, F., Shahedi, M., Varshosaz, J., & Nasirpour, A. (2013). Nanostructured lipid carriers (NLC): A potential delivery system FOR BIOACTIVE food molecules. *Innovative Food Science & Emerging Technologies, 19*, 29-43. doi:10.1016/j.ifset.2013.03.002

47. Ohtsuki, S., & Terasaki, T. (2007). Contribution of carrier-mediated transport systems to the blood–brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. *Pharmaceutical Research, 24*(9), 1745-1758. doi:10.1007/s11095-007-9374-5

48. Tsuji, A. (2005). Small molecular drug transfer across the blood-brain barrier via carrier-mediated transport systems. *NeuroRX, 2*(1), 54-62. doi:10.1602/neurorx.2.1.54

49. Lajoie, J. M., & Shusta, E. V. (2015). Targeting Receptor-Mediated transport for delivery of biologics across the blood-brain barrier. *Annual Review of Pharmacology and Toxicology, 55*(1), 613-631. doi:10.1146/annurev-pharmtox-010814-124852

50. Hervé, F., Ghinea, N., & Scherrmann, J. (2008). CNS delivery via adsorptive transcytosis. *The AAPS Journal, 10*(3), 455-472. doi:10.1208/s12248-008-9055-2

51. Thöle, M., Nobmann, S., Huwyler, J., Bartmann, A., & Fricker, G. (2002). Uptake of cationized Albumin Coupled Liposomes by cultured Porcine brain microvessel endothelial cells and Intact brain capillaries. *Journal of Drug Targeting*, 10(4), 337-344. doi:10.1080/10611860290031840

52. Gagat, M., Zielińska, W., & Grzanka, A. (2017). Cell-penetrating peptides and their utility in genome function modifications (review). *International Journal of Molecular Medicine*. doi:10.3892/ijmm.2017.3172

53. Zou, L., Ma, J., Wang, T., Yang, T., & Liu, C. (2013). Cell-Penetrating Peptide-Mediated Therapeutic molecule delivery into the central nervous system. *Current Neuropharmacology, 11*(2), 197-208. doi:10.2174/1570159x11311020006

54. Xiao, G., & Gan, L. (2013). Receptor-Mediated endocytosis and Brain delivery of Therapeutic Biologics. *International Journal of Cell Biology, 2013*, 1-14. doi:10.1155/2013/703545

55. Kiss, A. L., & Botos, E. (2009). Endocytosisviacaveolae: Alternative pathway with distinct cellular compartments to avoid Lysosomal degradation? *Journal of Cellular and Molecular Medicine, 13*(7), 1228-1237. doi:10.1111/j.1582-4934.2009.00754.x

56. Pulgar, V. M. (2019). Transcytosis to cross the blood brain Barrier, new advancements and challenges. *Frontiers in Neuroscience, 12*. doi:10.3389/fnins.2018.01019

57. Leitner, D. F., & Connor, J. R. (2012). Functional roles of transferrin in the brain. *Biochimica Et Biophysica Acta (BBA) - General Subjects, 1820*(3), 393-402. doi:10.1016/j.bbagen.2011.10.016

58. Mo, X., Liu, E., & Huang, Y. (2019). The intra-brain distribution of brain targeting delivery systems. *Brain Targeted Drug Delivery System,* 409-438. doi:10.1016/b978-0-12-814001-7.00016-0

59. Mayle, K. M., Le, A. M., & Kamei, D. T. (2012). The intracellular trafficking pathway of transferrin. *Biochimica Et Biophysica Acta (BBA) - General Subjects, 1820*(3), 264-281. doi:10.1016/j.bbagen.2011.09.009

60. Steere, A. N., Byrne, S. L., Chasteen, N. D., & Mason, A. B. (2012). Kinetics of iron release from transferrin bound to the transferrin receptor at endosomal ph. *Biochimica Et Biophysica Acta (BBA) - General Subjects, 1820*(3), 326-333. doi:10.1016/j.bbagen.2011.06.003

61. Gosk, S., Vermehren, C., Storm, G., & Moos, T. (2004). Targeting Anti—Transferrin Receptor Antibody (ox26) and OX26-conjugated liposomes to brain Capillary endothelial cells using in Situ Perfusion. *Journal of Cerebral Blood Flow & Metabolism, 24*(11), 1193-1204. doi:10.1097/01.wcb.0000135592.28823.47

62. Johnsen, K. B., & Moos, T. (2016). Revisiting nanoparticle technology for blood–brain barrier transport: Unfolding at the endothelial gate improves the fate of transferrin receptor-targeted liposomes. *Journal of Controlled Release, 222*, 32-46. doi:10.1016/j.jconrel.2015.11.032

63. Bi, Y., Liu, L., Lu, Y., Sun, T., Shen, C., Chen, X., . . . Jiang, C. (2016). T7 Peptide-functionalized Peg-plga micelles loaded with Carmustine for Targeting therapy of glioma. *ACS Applied Materials & Interfaces, 8*(41), 27465-27473. doi:10.1021/acsami.6b05572

64. Lee, J. H., Engler, J. A., Collawn, J. F., & Moore, B. A. (2001). Receptor mediated uptake of peptides that bind the human transferrin receptor. *European Journal of Biochemistry, 268*(7), 2004-2012. doi:10.1046/j.1432-1327.2001.02073.x

65. Almer, G., Mangge, H., Zimmer, A., & Prassl, R. (2015). Lipoprotein-Related and Apolipoprotein-mediated delivery systems for Drug targeting and imaging. *Current Medicinal Chemistry, 22*(31), 3631-3651. doi:10.2174/0929867322666150716114625

66. Hynds, S. A., Welsh, J., Stewart, J. M., Jack, A., Soukop, M., McArdle, C. S., . . . Shepherd, J. (1984). Low-density lipoprotein metabolism in mice with soft tissue tumours. *Biochimica Et*

Biophysica Acta (BBA) - Lipids and Lipid Metabolism, 795(3), 589-595. doi:10.1016/0005-2760(84)90189-9

67. Donner T, Sarkar S. Insulin – Pharmacology, Therapeutic Regimens, and Principles of Intensive Insulin Therapy. (Updated 2019 Feb 23). In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK278938/

68. Gray, S. M., Meijer, R. I., & Barrett, E. J. (2014). Insulin regulates brain function, but How does it get there? *Diabetes, 63*(12), 3992-3997. doi:10.2337/db14-0340

69. Rhea, E. M., Rask-Madsen, C., & Banks, W. A. (2018). Insulin transport across the blood-brain barrier can occur independently of the insulin receptor. *The Journal of Physiology, 596*(19), 4753-4765. doi:10.1113/jp276149

70. Ulbrich, K., Knobloch, T., & Kreuter, J. (2010). Targeting the insulin receptor: Nanoparticles for drug delivery across the Blood–brain barrier (BBB). *Journal of Drug Targeting, 19*(2), 125-132. doi:10.3109/10611861003734001

71. Pardridge, W. M., Boado, R. J., Giugliani, R., & Schmidt, M. (2018). Plasma pharmacokinetics OF Valanafusp Alpha, a human insulin Receptor Antibody-Iduronidase Fusion protein, in patients with Mucopolysaccharidosis Type I. *BioDrugs, 32*(2), 169-176. doi:10.1007/s40259-018-0264-7

72. Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. Carrier Proteins and Active Membrane Transport. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26896

73. Patching, S. G. (2016). Glucose transporters at the Blood-Brain Barrier: Function, regulation and gateways for drug delivery. *Molecular Neurobiology, 54*(2), 1046-1077. doi:10.1007/s12035-015-9672-6

74. Wang, H., Zhang, Z., Guan, J., Lu, W., & Zhan, C. (2021). Unraveling glut-mediated transcytosis pathway of glycosylated nanodisks. *Asian Journal of Pharmaceutical Sciences, 16*(1), 120-128. doi:10.1016/j.ajps.2020.07.001

75. Khazaei, M., Pazhouhi, M., & Khazaei, S. (2019). Temozolomide and tranilast synergistic antiproliferative effect on human glioblastoma multiforme cell line (U87MG). *Medical journal of the Islamic Republic of Iran*, *33*, 39. https://doi.org/10.34171/mjiri.33.39

76. Zucker, D., Marcus, D., Barenholz, Y., & Goldblum, A. (2009). Liposome drugs' loading Efficiency: A working model based on loading conditions and drug's physicochemical properties. *Journal of Controlled Release, 139*(1), 73-80. doi:10.1016/j.jconrel.2009.05.036

77. Gonzalez Gomez, A., Syed, S., Marshall, K., & Hosseinidoust, Z. (2019). Liposomal Nanovesicles for Efficient encapsulation of Staphylococcal Antibiotics. *ACS Omega, 4*(6), 10866-10876. doi:10.1021/acsomega.9b00825

78. S, T., P, M., & V, P. (2014). Slns based on co-processed lipids for topical delivery of terbinafine hydrochloride. *Journal of Pharmaceutics and Drug Development, 1*(6). doi:10.15744/2348-9782.1.604

79. Jaafar-Maalej, C., Diab, R., Andrieu, V., Elaissari, A., & Fessi, H. (2009). Ethanol injection method for hydrophilic and lipophilic drug-loaded liposome preparation. *Journal of Liposome Research, 20*(3), 228-243. doi:10.3109/08982100903347923

80. Niwa, T., Takeuchi, H., Hino, T., Kunou, N., & Kawashima, Y. (1993). Preparations of biodegradable nanospheres of water-soluble and insoluble drugs with d,l-lactide/glycolide copolymer by a novel spontaneous emulsification solvent diffusion method, and the drug release behavior. *Journal of Controlled Release, 25*(1-2), 89-98. doi:10.1016/0168-3659(93)90097-o

81. Kim, S., Rait, A., Kim, E., DeMarco, J., Pirollo, K. F., & Chang, E. H. (2015). Encapsulation of temozolomide in a tumor-targeting nanocomplex enhances anti-cancer efficacy and reduces toxicity in a mouse model of glioblastoma. *Cancer Letters, 369*(1), 250-258. doi:10.1016/j.canlet.2015.08.022

82. Patel, B. K., & Parikh, R. H. (2016). Formulation development and evaluation of temozolomide loaded hydrogenated soya phosphatidylcholine liposomes for the treatment of brain cancer. *Asian Journal of Pharmaceutical and Clinical Research*, *9*(3), 340-344. Retrieved from https://innovareacademics.in/journals/index.php/ajpcr/article/view/11459

83. Gao, J., Wang, Z., Liu, H., Wang, L., & Huang, G. (2015). Liposome encapsulated of temozolomide for the treatment of Glioma tumor: Preparation, characterization and evaluation. *Drug Discoveries & Therapeutics, 9*(3), 205-212. doi:10.5582/ddt.2015.01016

84. Sayiner, O., Arisoy, S., Comoglu, T., Ozbay, F. G., & Esendagli, G. (2020). Development and in vitro evaluation of temozolomide-loaded plga nanoparticles in a thermoreversible hydrogel system for local administration in glioblastoma multiforme. *Journal of Drug Delivery Science and Technology, 57*, 101627. doi:10.1016/j.jddst.2020.101627

85. Ananta, J. S., Paulmurugan, R., & Massoud, T. F. (2016). Temozolomide-loaded plga nanoparticles to treat glioblastoma cells: A biophysical and cell culture evaluation. *Neurological Research, 38*(1), 51-59. doi:10.1080/01616412.2015.1133025

86. Khan, A., Imam, S. S., Aqil, M., Ahad, A., Sultana, Y., Ali, A., & Khan, K. (2016). Brain targeting of Temozolomide via the Intranasal route Using Lipid-Based Nanoparticles: Brain PHARMACOKINETIC and Scintigraphic analyses. *Molecular Pharmaceutics, 13*(11), 3773-3782. doi:10.1021/acs.molpharmaceut.6b00586

87. Chen, Z., Lai, X., Song, S., Zhu, X., & Zhu, J. (2015). Nanostructured lipid carriers Based temozolomide and Gene co-encapsulated nanomedicine for gliomatosis Cerebri combination therapy. *Drug Delivery, 23*(4), 1369-1373. doi:10.3109/10717544.2015.1038857

88. Zhang, J., Xiao, X., Zhu, J., Gao, Z., Lai, X., Zhu, X., & Mao, G. (2018). Lactoferrin- and rgd-comodified, temozolomide and vincristine-coloaded nanostructured lipid carriers for gliomatosis cerebri combination therapy. *International Journal of Nanomedicine, Volume 13*, 3039-3051. doi:10.2147/ijn.s161163

89. Lee, J. H., & Yeo, Y. (2015). Controlled drug release from pharmaceutical nanocarriers. *Chemical Engineering Science, 125*, 75-84. doi:10.1016/j.ces.2014.08.046

90. Hasan, M., Elkhoury, K., Kahn, C. J., Arab-Tehrany, E., & Linder, M. (2019). Preparation, characterization, and Release kinetics of Chitosan-Coated Nanoliposomes encapsulating

curcumin in simulated environments. *Molecules, 24*(10), 2023. doi:10.3390/molecules24102023

91. Zhang, X., Lin, Y., & Gillies, R. J. (2010). Tumor ph and its measurement. *Journal of Nuclear Medicine, 51*(8), 1167-1170. doi:10.2967/jnumed.109.068981

92. Guo, X., Cheng, Y., Zhao, X., Luo, Y., Chen, J., & Yuan, W. (2018). Advances in redox-responsive drug delivery systems of tumor microenvironment. *Journal of Nanobiotechnology, 16*(1). doi:10.1186/s12951-018-0398-2

93. Li, M., Zhao, G., Su, W., & Shuai, Q. (2020). Enzyme-responsive nanoparticles for anti-tumor drug delivery. *Frontiers in Chemistry, 8*. doi:10.3389/fchem.2020.00647

94. Pinheiro, M., Ribeiro, R., Vieira, A., Andrade, F., & Reis, S. (2016). Design of a nanostructured lipid carrier intended to improve the treatment of tuberculosis. *Drug Design, Development and Therapy, Volume 10*, 2467-2475. doi:10.2147/dddt.s104395

95. Lee, Y., & Thompson, D. (2017). Stimuli-responsive liposomes for drug delivery. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology, 9*(5). doi:10.1002/wnan.1450

96. Khanal, S., Adhikari, U., Rijal, N., Bhattarai, S., Sankar, J., & Bhattarai, N. (2016). Ph-Responsive plga nanoparticle for controlled payload delivery of diclofenac sodium. *Journal of Functional Biomaterials, 7*(3), 21. doi:10.3390/jfb7030021

97. Viricel, W., Mbarek, A., & Leblond, J. (2015). Switchable Lipids: Conformational change for FAST pH-Triggered Cytoplasmic delivery. *Angewandte Chemie, 127*(43), 12934-12938. doi:10.1002/ange.201504661

98. De la Rica, R., Aili, D., & Stevens, M. M. (2012). Enzyme-responsive nanoparticles for drug release and diagnostics. *Advanced Drug Delivery Reviews, 64*(11), 967-978. doi:10.1016/j.addr.2012.01.002

99. Tapeinos, C., Marino, A., Battaglini, M., Migliorin, S., Brescia, R., Scarpellini, A., . . . Ciofani, G. (2019). Stimuli-responsive lipid-based magnetic nanovectors increase apoptosis in glioblastoma cells through synergic intracellular hyperthermia and chemotherapy. *Nanoscale, 11*(1), 72-88. doi:10.1039/c8nr05520c

100. Caster, J. M., Sethi, M., Kowalczyk, S., Wang, E., Tian, X., Nabeel Hyder, S., . . . Wang, A. Z. (2015). Nanoparticle delivery of chemosensitizers improve chemotherapy efficacy without incurring additional toxicity. *Nanoscale, 7*(6), 2805-2811. doi:10.1039/c4nr07102f

101. Klinger, N. V., & Mittal, S. (2016). Therapeutic potential of curcumin for the treatment of brain tumors. *Oxidative Medicine and Cellular Longevity, 2016*, 1-14. doi:10.1155/2016/9324085

102. Rahbar Takrami, S., Ranji, N., & Sadeghizadeh, M. (2019). Antibacterial effects of curcumin encapsulated in nanoparticles on clinical isolates of pseudomonas aeruginosa through downregulation of efflux pumps. *Molecular Biology Reports, 46*(2), 2395-2404. doi:10.1007/s11033-019-04700-2

103. Ryskalin, L., Biagioni, F., Busceti, C. L., Lazzeri, G., Frati, A., & Fornai, F. (2020). The multi-faceted effect of curcumin in Glioblastoma from rescuing cell clearance to Autophagy-Independent Effects. *Molecules, 25*(20), 4839. doi:10.3390/molecules25204839

104. Yin, H., Zhou, Y., Wen, C., Zhou, C., Zhang, W., Hu, X., . . . Shao, J. (2014). Curcumin sensitizes glioblastoma to temozolomide by simultaneously generating ROS and disrupting AKT/mTOR signaling. *Oncology Reports, 32*(4), 1610-1616. doi:10.3892/or.2014.3342

105. Dilnawaz, F., Singh, A., Mohanty, C., & Sahoo, S. K. (2010). Dual drug loaded superparamagnetic iron oxide nanoparticles for targeted cancer therapy. *Biomaterials, 31*(13), 3694-3706. doi:10.1016/j.biomaterials.2010.01.057

106. Anand, P., Kunnumakkara, A. B., Newman, R. A., & Aggarwal, B. B. (2007). Bioavailability of curcumin: Problems and promises. *Molecular Pharmaceutics, 4*(6), 807-818. doi:10.1021/mp700113r

107. Kwf checkt. Retrieved May 02, 2021, from https://www.kwf.nl/onderzoek/dit-onderzoek-maken-we-mogelijk/kwf-checkt

108. Mohanty, C., Das, M., & Sahoo, S. K. (2012). Emerging role of nanocarriers to increase the solubility and bioavailability of curcumin. *Expert Opinion on Drug Delivery, 9*(11), 1347-1364. doi:10.1517/17425247.2012.724676

109. Kumar, A., Ahuja, A., Ali, J., & Baboota, S. (2014). Curcumin-loaded lipid nanocarrier for improving bioavailability, stability and cytotoxicity against malignant glioma cells. *Drug Delivery, 23*(1), 214-229. doi:10.3109/10717544.2014.909906

110. Xu, M., Li, G., Zhang, H., Chen, X., Li, Y., Yao, Q., & Xie, M. (2020). Sequential delivery of dual drugs with nanostructured lipid carriers for improving synergistic tumor treatment effect. *Drug Delivery, 27*(1), 983-995. doi:10.1080/10717544.2020.1785581