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Drug Permeation across the Blood-Brain Barrier: Applications of Nanotechnology

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

The blood-brain barrier (BBB) is a neurobiological frontier that isolates brain tissues from the blood vascular system. Its main role is to protect the brain and the central nervous system from external fluctuations in hormones, nutrients and drugs, while allowing the passage of water and small lipophilic molecules. Diffusion across the BBB can occur through several biological mechanisms, but the most common one is simple diffusion, which mainly depends on the size, lipid solubility and concentration gradient of the molecule. Because of the highly dense network of capillary endothelium cells found in the BBB, most of the drugs are not able to cross this physiological barrier. Delivering therapeutic agents to the brain is thus a big challenge, which may prevent treatment of important neurological diseases. In order to overcome this difficulty, researchers have used nanotechnology to help the passage of drugs across the BBB. Nanotechnology has significantly contributed to the field of biotechnology by improving the strategies for drug delivery, and by providing novel carriers for safe and effective brain targeting. The aim of this review is to

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discuss in more details the anatomical structure and the functions of the BBB, as well as its significance in neurological diseases. A closer look will be given at the transport mechanisms across the BBB. This review finally explores the most recent advances in the field of nanotechnology for drug delivery in the brain, and gives meaningful examples of delivery systems developed including the micelles, liposomes, dendrimers, microcapsules and polymeric nanoparticles.

Keywords: Blood-brain barrier; drug delivery; nanotechnology; neurological disease.

1. INTRODUCTION

The blood-brain barrier (BBB) is a highly selective permeability structure designed to regulate brain homeostasis and the transport of essential molecules to the brain [1,2]. It acts as a tight connection between the circulating blood and the brain extracellular fluid. Its role is also to protect the neurons from bacterial infections and from fluctuations in hormones, metabolites and nutrients [3,4]. By controlling the environment of the nervous tissue of the brain, it is able to maintain a proper neuronal transmission. Recent advances in molecular neuroscience and biochemistry have shaped our understanding of the physiology of the BBB and its relationship with pathological conditions of the brain. However, treatment of diseases of the brain and the central nervous system still remains a big challenge, mostly because of the fact that therapeutic molecules cannot easily cross the BBB, and fail to reach specific part of the brain [1,5]. Therefore, there is an urgent need to use novel approaches for better drug targeting and delivering to specific regions of the brain.

The transport of pharmaceutical agents across the BBB rely on a variety of mechanisms, one of which is the transmembrane passive diffusion, which depends on the concentration gradient, as well as the size and charge of the molecule [6]. However, this process is limited because it mostly favors lipid-soluble molecules [7]. Other methods such as carrier and receptor-mediated transport are also employed to deliver drugs to the brain [8], but most of them may do damage and cause toxicity to the BBB. The optimal method of delivery into the brain should be able to accurately target specific areas without damaging the BBB. Among the numerous approaches available, nanotechnology has provided the possibility to deliver therapeutic agents into neuronal tissues and cells with high safety and specificity [1,3,9].

This review talks about the characteristics of the BBB from a physiological and biological point of view, and describes the physical barriers that therapeutic agents need to cross in order to reach the brain. Moreover, it discusses the transport mechanisms across the BBB and explains how the use of nanotechnology could enhance the delivery of pharmaceutical agents to specific areas of the brain. Finally, a closer look is given at the impact that nanotechnology has on the treatment of brain diseases and disorders.

2. PROPERTIES OF THE BLOOD-BRAIN BARRIER

2.1 Function and Physiology of the Blood-Brain Barrier

The BBB is constituted of a vast network of microcapillaries that isolate brain tissues from the substances that circulate into the vascular system [10]. The molecular architecture of this barrier gives it specific characteristics that enable only fat-soluble molecules and compounds with a low molecular mass to pass directly through its walls [6,11]. Therefore, the BBB protects the neuronal tissue from abrupt changes in metabolites and exogenous compounds circulating in the blood. It also prevents the transport of ionic currents and hydrophilic compounds to the brain, and protects the individual from several pathological conditions [3]. Moreover, it plays an important role during inflammation of the brain and the central nervous system [12]. By becoming more permeable during inflammation, it allows phagocytes and other immune cells to cross the barrier and to fight against bacteria and foreign molecules.

The structural bases of the BBB are the endothelial cells, the pericytes and the astrocyte end-feet. The astrocytes are important structures in the brain that help modulate the permeability of the BBB [13]. They can also up regulate many features of the BBB, leading to a tighter physical barrier and increased expression of transporters and specialized enzymes [13]. Tight junctions, which mostly consist of transmembrane proteins, connect the endothelial cells [14]. Their role is to

restrict the passage of bacteria through the BBB, while allowing the transport of small hydrophobic molecules such as O_2 and CO_2 [14]. The main function of the pericytes is to wrap around endothelial cells and to maintain the protection and functionality of the brain and nervous system [15]. A lack of pericytes in the central nervous system can cause BBB to break down [15]. Moreover, the elastic properties of the pericytes enable them to significantly reduce neuronal inflammation by allowing the release of toxic substances from the brain. The astrocyte end-feet, which also encircled the endothelial cells, play a major role in maintaining the BBB and in facilitating blood flow in the brain [16]. They are important in regulating the neuronal metabolism and in discharging used or unwanted substrates [16]. Other structures such as the extracellular base membrane, the microglia and the surrounding neurons also form part of the BBB.

2.2 Physical Barriers

The BBB prevents molecules from entering and leaving the brain by forming a physical barrier. This barrier is larger due to the establishment of a network of brain capillaries that isolate the brain, and the presence of tight junctions that separate the capillary endothelial cells [17]. Tight junctions, also known as occluding junctions, generate a barrier to the extracellular fluid by closely contacting adjacent endothelial cells [18]. They practically control which substances can pass through the BBB. They are composed of a series of transmembrane proteins that are highly interconnected. Claudins and occludins are such proteins that form the integrity of the tight junctions [19,20]. Claudins are small molecules that have four transmembrane domains. They form the primary seal of the tight junctions through the use of disulfide bonds [21]. Occludins also span the plasma membrane four times but have a different function than the claudins. Although they do not directly participate in the formation of tight junctions, their main role is to maintain the shape and permeability of the barrier [1,22]. Junctional adhesion molecules (JAM), which are mainly expressed by leukocytes and endothelial cells, also play a key part in the formation of tight junctions [23,24]. They appear to facilitate the interaction between leukocytes and endothelial cells, suggesting a potential role in mediating the immune activity of the BBB during pathological conditions of the brain [25].

Another important complex of proteins that form part of the physical barrier are the adherens junctions [26,27]. They perform several tasks including the stabilization of cell-cell adhesion, and the regulation of intracellular signaling and transcriptional regulation [28]. The adherens junctions are located just below the tight junctions, and can occur in epithelial and endothelial tissues [29]. They are composed of cadherins, which are part of the transmembrane glycoproteins that form homodimers with other cadherin molecules and leads to the generation of a tightening complex [30,31]. A down-regulation of the cadherins often results in a loss of cell-cell adhesion, which can lead to cell proliferation and tumor invasiveness [32]. The adherens junctions are also composed of other cytoskeleton proteins, such as the catenins, which links the actin cytoskeleton with the cadherin [33].

The catenin family is composed of the p120-catenin, the α-catenin and the β-catenin [34,35,36]. By binding to the cadherin, the p120-catenin helps stabilize the formation of cell-cell contacts at the plasma membrane. The p120-catenin also serves as a regulator of cell motility through the actin cytoskeleton [28]. The α-catenin is a protein that binds to actin filaments and plays a major role in maintaining the structural integrity of the adherens junctions. Moreover, the α-catenin binds to the β-catenin [37], which is involved in the regulation of efflux transporters of the BBB and in strengthening the cell-cell connections mediated by the cadherin. Taken all together, the BBB consists of several physical connections that create a nearly impermeable boundary between the brain and the bloodstream, and that help maintain the necessary extracellular environment of the central nervous system.

3. TRANSPORT MECHANISMS ACROSS THE BLOOD-BRAIN BARRIER

Transport across the BBB occurs through different mechanisms, the most common ones being simple diffusion, carrier-mediated transport, facilitated diffusion and receptor-mediated endocytosis. The characteristics and biological properties of each mechanism are illustrated in Table 1.

Table 1. Mechanisms of transport through the blood-brain barrier

Transport mechanism	Characteristics	Molecules transported
Simple diffusion	Passive and transcellular transport	Small, non-polar and lipophylic molecules *Example: steroid hormones and psychoactive drugs*
Carrier-mediated transport	Passive transport using transport proteins	Essential amino acids, peptides, hormones, glucose and small drugs
Facilitated-diffusion	Passive transport through transmembrane proteins	Water, ions, amino acids, nutrients and monocarboxyates
Receptor-mediated endocytosis	Active transport by transcytosis across the barrier	Macromolecules, plasma proteins, hormones and peptides. *Example: insulin, leptin, transferrin and Vitamin B6*

3.1 Simple Diffusion

Several molecules, including psychoactive drugs, move through the BBB by simple diffusion [38,39]. This mechanism relies on the size and lipid solubility of the substance, but also on the concentration gradient across the barrier. This is the simplest and most frequent form of transport since there is no energy needed. While electrostatically charged molecules cannot pass through the barrier by simple diffusion, lipophilic substances and small molecules like O_2 and CO_2 canrapidly penetrate the BBB [38]. Simple diffusion can be divided into paracellular and transcellular diffusion. Paracellular diffusion refers to the transport of substances between cells, through the intercellular space. In contrast, transcellular diffusion is the passage of molecules across cells, through the apical and basolateral membranes. Because of the presence of tight junctions, passage between the cells could not occur in the BBB.

3.2 Carrier-Mediated Transport

In the case of carrier-mediated transport, the solute binds to a transport protein located on one side of the membrane. A transport protein is an integral carrier protein that spans the membrane of the endothelial cells. When the solute binds to the carrier protein, a conformational change occurs in the protein, which result in the transport of the solute through its concentration gradient. This type of diffusion is passive and does not require any form of energy. It mostly contributes to the transport of substances such as the essential amino acids, small peptides, hormones and glucose [40,41]. In the case of glucose, this molecule passes through the BBB by binding to specific glucose transport proteins that are found on the membrane of endothelial cells [42].

3.3 Facilitated Diffusion

Facilitated diffusion is a type of carrier-mediated endocytosis, which refers to the passage of molecules or ions across a biological membrane through specific transmembrane integral proteins [43]. During this process, molecules and ions move down their concentration gradient in a passive way. Channels that open and close to regulate the flow of substances across the membrane control this type of transport. For instance, water molecules can pass through aquaporins, which are proteins that form pores in the membrane of the endothelial cells [44,45]. They selectively allow the passage of water molecules through the BBB, while preventing the transport of other ions and solutes [45].

3.4 Receptor-Mediated Endocytosis

Large molecules and aggregates that cannot pass through transmembrane proteins can penetrate the BBB by receptor-mediated endocytosis [46]. During this process, the molecule binds to receptors located on the surface of endothelial cells and gets incorporated by endocytosis. The molecule then gets transported across the interior of the cell by transcytosis. Alternatively, the substance can get incorporated into the endothelial cell by merging with the membrane and forming a vesicle that migrates inside the cell and releases its content upon reaching the other side of the barrier. Receptor-mediated transcytosis can mediate the transport of plasma proteins such as insulin, and circulating peptides [47]. This process uses

energy, mostly from adenosine triphosphate, in order to drive the movement of molecules across the barrier and against their concentration gradient [48].

4. USE OF NANOTECHNOLOGY-BASED DELIVERY PLATFORMS

Because passage of drugs to the central nervous system through the BBB is very limited, new approaches need to be employed in order to improve drug delivery to the brain. Nanocarriers are an emerging class of delivery platform that can easily transport drugs to the brain and several other parts of the body [1,9,49]. They exhibit properties that make them very suitable for drug delivery across the BBB. Such properties include their small size, their biocompatibility, their prolonged blood circulation time and their nontoxicity [1,50]. Several studies have focused on the development of such nanotechnology-based systems for the delivery of pharmaceutical agents, peptides, recombinant proteins, as well as vaccines and nucleotides [50,51,52]. The most commonly used delivery systems are micelles, liposomes, dendrimers, microcapsules and functionalized nanoparticles.

4.1 Polymeric Micelles

Micelles have recently emerged as a promising tool for the delivery pharmaceutical drugs through the BBB [53,54]. They are spherical aggregates of molecules dispersed in a liquid colloid [1,55]. They are prepared from a certain type of amphiphilic co-polymers that consist of both hydrophilic and hydrophobic units of monomers, and are a perfect choice to deliver drugs with poor liquid solubility. The molecular geometry and properties of the molecules that form the micelles, such as concentration, pH, and ionic strength, dictate the overall size and shape of the final structure. An advantage of using micelles for drug delivery is that they have the ability to significantly increase the bioavailability and solubility of pharmaceutical drugs [1]. However, because of their sensitivity to environmental changes such as dilution and ionic strength, micelles are often conjugated to some organic compounds that can provide higher stability. Several studies used micelles in order to deliver hydrophobic drugs across the BBB, one of which was able to demonstrate that molecular compounds with poor solubility could be loaded into very small negatively charged micelles that could easily penetrate the BBB [56]. In this study, steroidal compounds were delivered to the brain through the use of ligand-modified nanomicelles. Lactoferrin and sodium alginate–cholesterol derivatives were used to develop the nanomicelles, and they were shown to significantly increase the drug loading capacity and prolong the release profile [56]. Polymeric micelles can also improve the pharmacological activity of drugs by enhancing pharmacokinetics, and have been extensively used to deliver anticancer agents to the tumor site of the patient [57]. They are also used for other medical applications, such as cancer chemotherapy [58], due to their ability to accumulate and persist longer in tumoral tissue [55,58]. Taken all together, polymeric micelles can be used as an efficient drug delivery platform that could aid in the treatment of neurological diseases.

4.2 Liposomes

Liposomes are spherical vesicles of a lamellar phase liquid that are artificially prepared and that can be used for administration of pharmaceutical compounds [59]. They consist of naturally occurring phospholipids and cholesterol, and are traditionally prepared by sonication (disruption of biological membranes) [60]. Because liposomes have a relatively high systemic plasma clearance, they get rapidly removed form the blood circulation by macrophages [50,59]. With the advances in pharmaceutical research, it is now possible to increase their half-life by incorporating polyethylene glycol (PEG) to theirmembrane [61,62]. This coating also enables the liposomes to avoid being detected by the body's immune system. Another advantage in using liposomes is that they can be coated with several biological molecules, such as ligands, which enable them to target specific sites in the brain. A recent study demonstrated that coupling the liposomes with PEG and targeting molecules could significantly enhance drug delivery across the BBB [63]. The scientists conducting this research aimed at developing a liposome-based vehicle coupled with PEG and another small molecule, OX26, which is a very efficient targeting antibody in brain drug delivery [63]. The therapeutic efficacy of this delivery platform was then tested by intravenously injection in an animal model of high-grade glioma, one of the most frequent types of intracranial tumor [63]. They showed that the modified liposomes were able to cross the BBB and could effectively target the brain glioma cells, both in-vitro and in-vivo [63]. Another study has coupled the liposomes with PEG and glutathione, in order to deliver methylprednisolone, a

corticosteroid drug, in an animal model of neuro-inflammation [64]. Their results showed that surface-modified liposomes are effective in delivering the drug to the central nervous system, and in treating neuro-inflammatory disorders such as multiple sclerosis [64]. Such targeting delivery system could indeed open up new treatment strategies that are non-invasive and efficient in treating brain diseases.

4.3 Dendrimers

Dendrimers are branched molecules that have received a large attention because of their ability to cross the BBB and several other target points [65,66]. Their small size and shape allow them to penetrate brain endothelial cells and deliver drugs to targeted sites of the central nervous system [67]. Moreover, their encapsulation ability, their low toxicity, as well as their water solubility make them appropriate candidates for evaluation as drug delivery vehicles. Applications of dendrimers typically involve the delivery of pharmaceutical drugs through the BBB to treat neurological disorders such as Alzheimer's disease, stroke, and multiple sclerosis [68,69]. Manipulations can be made during the preparation of dendrimers in order to increase its functionality and specificity, and to make it more efficient in targeting the desired area of the brain. The drug can be either encapsulated in the dendrimers or attached to the periphery by covalent or ionic interactions [69,70]. There exist many types of dendrimers used in biomedical research, such as the poly-(propylene-imine) (PPI), polyether-copolyester (PEPE) and PEG dendrimers [65,67]. However, the most widely studied are the poly-(amidoamine) (PAMAM) dendrimers [71]. Studies have shown that PAMAM dendrimers can diffuse in the central nervous system and penetrate living neurons [71,72]. Moreover, in-vitro studies demonstrated that this drug delivery system presents little cytotoxicity in brain capillary endothelial cells, and can be effectively used to deliver anticancer drugs to the brain [73,74]. Taken all together, dendrimers are an excellent choice of a delivery system because of their potential to deliver therapeutic drugs to the brain, and because they have shown great promise in the treatment of neurological diseases

4.4 Microcapsules and Polymeric Nano-particles

Microencapsulation is a technique during which a coating surrounds droplets of a solution in order to generate spherical microcapsules, using different types of proteins and polymers. It is widely accepted that microencapsulation is a promising tool in biomedical research that enables the delivery of pharmaceutical drugs to targeted areas of the body [75,76]. The coating of the microcapsules is generally made of sodium alginate, and it's used to protect the entrapped material from the external environment [75]. Studies have shown that by using microcapsules of very small size (100-200 microm), scientists could bypass the BBB and deliver therapeutic agents to specific areas of the brain [77]. They demonstrated that small microcapsules loaded with a recombinant gene product display high stability and mechanical strength, and were very efficient in treating neurological deficits in rodents [77].

Another carrier of choice for drug delivery across the BBB is the polymeric nanoparticle [78,79]. Nanoparticles are solid colloidal particles of very small size (1-1000nm) that have been frequently used as a drug delivery platform because of their high loading capacity, bioavailability, high stability, and targeted delivery [80]. They can be made of natural or synthetic polymers that constitute a thin envelope surrounding the entrapped material [81,82]. Moreover, the surface of the nanoparticle can be coated with molecules that help increase its half-life. Examples include PEG-coated nanoparticles, which exhibits enhanced stability and better accumulation into the brain [78]. The nanoparticle can also be coated with small ligands, such as antibodies, proteins and peptides, in order to target specific sites within the brain or even specific organelles within the cell [83,84]. A recent study using nanoparticles conjugated with a trans-activating transcriptor peptide has shown that coated nanoparticles were able to cross the BBB more efficiently and accumulate in neuronal cells in much higher proportions [85]. In summary, nanoparticles can be coated to move freely across the BBB, and their ability to enhance drug delivery and therapeutic efficacy make them an ideal choice for the treatment of neurological disorders.

5. CONCLUSION

Because of the enormous challenge faced by pharmaceutical companies and academics to develop therapies that could effectively treat brain diseases, a lot of attention is given on the understanding of the physiology and transport mechanisms across the BBB. Nanotechnology

emerges as one of the most important tool in biotechnology due to its ability to enable drug targeting and delivery to specific areas of the brain [50]. In this review, we examined the functions and physiological aspects of the BBB as well as the role of its physical barriers, such as the tight and adherens junctions, in creating an impermeable boundary between the brain and the bloodstream. We also looked at the different transport mechanisms that could be used to penetrate the BBB. While this barrier restricts access to most foreign molecules such as drugs, it facilitates the penetration of nutrients essential for normal metabolism to reach brain tissues [71]. The main goal of this review was to study the feasibility of a new nanotechnology-based strategy to cross the BBB and deliver therapeutic agents to the brain. All of the studies presented in this review have illustrated the benefits of using nanocarriers for drug delivery, which include micelles, liposomes, dendrimers, microcapsules and polymeric nanoparticles. These delivery systems show superior stability, high biocompatibility, and enhanced drug distribution and release across the BBB. However, our knowledge of the neurobiological processes that regulate the function of the BBB is still very limited, and more work needs to be done to fully understand its implication in neurological disorders such as brain tumor and inflammation. Moreover, future research needs to focus on investigating the safety and clinical efficiency of using nanotechnology in humans for drug delivery across the BBB as a long-term therapy for patients with severe neurological disorders.

CONSENT AND ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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