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Review article

Recent advancements in liposomes targeting strategies to cross blood-brain barrier (BBB) for the treatment of Alzheimer's disease

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ABSTRACT

In this modern era, with the help of various advanced technologies, medical science has overcome most of the health-related issues successfully. Though, some diseases still remain unresolved due to various physiological barriers. One such condition is Alzheimer; a neurodegenerative disorder characterized by progressive memory impairment, behavioral abnormalities, mood swing and disturbed routine activities of the person suffering from. It is well known to all that the brain is entirely covered by a protective layer commonly known as blood brain barrier (BBB) which is responsible to maintain the homeostasis of brain by restricting the entry of toxic substances, drug molecules, various proteins and peptides, small hydrophilic molecules, large lipophilic substances and so many other peripheral components to protect the brain from any harmful stimuli. This functionally essential structure creates a major hurdle for delivery of any drug into the brain. Still, there are some provisions on BBB which facilitate the entry of useful substances in the brain via specific mechanisms like passive diffusion, receptor-mediated transcytosis, carrier-mediated transcytosis *etc.* Another important factor for drug transport is the selection of a suitable drug delivery systems like, liposome, which is a novel drug carrier system offering a potential approach to resolving this problem. Its unique phospholipid bilayer structure (similar to physiological membrane) had made it more compatible with the lipoidal layer of BBB and helps the drug to enter the brain. The present review work focused on various surface modifications with functional ligand (like lactoferrin, transferrin *etc.*) and carrier molecules (such as glutathione, glucose *etc.*) on the liposomal structure to enhance its brain targeting ability towards the successful treatment of Alzheimer disease.

1. Introduction

With changes in modern lifestyle, the busy lifestyle also brings some disorders associated with increasing age of the human being. One such epidemic condition considered worldwide as the reason of death is Alzheimer disease [1]. As per the World Alzheimer report 2016, now around 47 million peoples, all over the world suffering from dementia which is estimated to increase by 131 million till 2050 [2,3]. Alzheimer or dementia is a neurodegenerative disorder [3–5] which directly and severely affect the functioning of central nervous system triggering memory impairment, progressive cognitive neuronal dysfunctioning, thinking and behavioral disturbance and much more similar problems uproar in the daily life of the patient [6]. The exact cause of Alzheimer is not yet identified but some factors considered to be more responsible for the dementia are aging, genetic mutation, and family background. The people with age > 65 years are more likely to be susceptible for the Alzheimer's, as the frequency of neuronal degeneration increases with increasing age factor. Genetically, the gene APOE[1]-e4 is responsible for the incidence of this brain disorder [7–10]. The symptoms of Alzheimer's initially involves the loss of memory followed by decreasing ability of the person to recognize the relatives, friends, children's, spouse's further leading to interruption in daily routine activity like walking, eating, dressing *etc.* and finally the patient become bed ridden in last stages, ultimately fatal [11]. The histopathology of Alzheimer

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include the formation of neurofibrillary tangles and accumulation of β amyloid plaque [12] accompanying with neuronal inflammation, increased oxidative stress and reduced level of neurotransmitters (ACh[2], BuCh[3]) [11]. The formation of β amyloid plaque in the neuronal extracellular space is considered the major cause of neurodegeneration and memory impairment [13]. Normally, a protein APP[4], present on the surface of the neuron, is cleaved from a proteolytic enzyme β secretase, results in the formation of small fragments of β amyloid peptide [14]. These fragments have a tendency to normally get dissolved and eliminated from the brain but in elderly peoples or person with some abnormalities, this peptide gets accumulated and forms a large aggregate that causes nerve degeneration and may lead to Alzheimer disease [15].

The major obstacle encountered in the treatment of Alzheimer's and other brain disorders is BBB[5], that prevent the transfer of most of the drug, peptides and large molecules across the endothelial cell lining [16–18] to protect the brain from undesirable side effects of the same [19]. The BBB structurally composed of BCECs[6], astrocytes, pericytes, neuronal cells, basement membrane *etc.* [20,21]. The BCECs closely attached to each other through tight junction that confines paracellular transfer of small drug molecules and the presence of degrading enzyme limits the transport of various molecules from the periphery to the brain [22]. All such arrangements have been made to maintain the homeostasis inside the brain and keep it unaffected from the harmful stimuli of drug and toxic compounds. But there are some provisions like paracellular diffusion for hydrophilic substances, the transcellular pathway for small lipoidal molecules and although some other mechanisms like carrier-mediated transcytosis, receptor-mediated transport, cell-mediated endocytosis, adsorptive transcytosis *etc.* [23] that helps in transfer of essential components through specific mechanisms to the brain [24,25]. The BBB creates major hurdle in the effective treatment of various CNS[7] disorders. All over the world, the researchers are trying to make attempts to formulate a drug molecule and delivery system, which can able to deliver the drug into the brain by crossing the BBB and maintain the higher concentration inside the brain. In this track, various novel drug delivery systems like nanoparticle, liposome, dendrimers *etc.* offers good stratagem due to their unique capability to target BBB [26].

Drug delivery to the brain is a very complex phenomenon that can be done by three major routes like, injecting/inserting drug via intracerebral or intracerebroventricular injection (invasive approach), systemic administration via oral or i.v.[8] and intranasal administration of the active compounds. The first approach is invasive, painful and causes patient inconvenient hence, used in very severe condition or only when the patient is hospitalized. While the rest two approaches are very popular, among which the systemic drug delivery encounters the interruption through BBB that limits the drug concentration, bioavailability, and effectiveness of drug and also increases the systemic side effect. To overcome such problems various novel approaches such as nanocarriers are used to enhance the drug property and pharmacokinetic behavior. The last approach as mentioned above i.e. i.n.[9] administration offers a distinct and attractive approach that bypasses the BBB and directly deliver the drug to the brain via olfactory region thereby enhancing the drug bioavailability and activity. Hence, become popular among researchers for the treatment of CNS disorders like Alzheimer's, Parkinson's disease *etc.*

Another factor to be taken into account for the delivery of drugs to the brain systemically is the development of several types of NCs[10] to assist their translocation across the blood-brain-barrier, the basic barrier between blood circulation and CNS. The most commonly developed types of nanocarriers, for such applications, are liposomes, solid lipid nanoparticles, albumin nanoparticles and polymeric nanoparticles [27–35]. In general, NC-assisted drug delivery possesses many advantages; increased drug bioavailability and stability and at the same time decreased peripheral toxicity. Between the different types of NCs, the ones that have easily modified by its surfaces, such as liposomes, hold an additional advantage since they may be modified in order to efficiently target a particular site of interest (*e.g.* BBB). Thus, targeted liposomal NCs are considered to have great future perspectives for the diagnosis and treatment of brain located diseases. Several types of liposome formulations have been developed up-to-date, as systems to facilitate the translocation of drugs across the BBB, by employing a number of different strategies.

The possible mechanism for the transportation of drug across the BBB is due to the phospholipid bilayer of liposome facilitating the permeation of drug across various biological membranes. However, it does not allow to cross BBB. Hence, various surface modifications have been made to that enables the transfer of liposomal carrier via BBB [36]. There are a number of receptors present on the surface of BBB, particularly for different proteins, peptides, antibodies *etc*. Such molecules are used as surface-active ligands and assist the translocation via receptor-mediated transcytosis. At the same time, the cationic liposomes cross the BBB via absorption mediated transcytosis. One more strategy is carrier mediated transcytosis that utilizes some nutrients like glucose; glutathione *etc.* binds to the surface of liposome and facilitate its translocation [37,38]. Once the liposome enters into the brain it releases the entrapped drug to the target site initially through passive diffusion where the drug release is triggered by general passive efflux [37]. This does not control the release rate hence, some more progressive approaches have been developed that responds to the changes in the physiological environment and release the drug in a controlled manner. In such system, the drug release from the liposomal vesicle is triggered by a change in pH, enzymatic stimulus or change in the level of some redox agents like glutathione [39–42]. In the case of Alzheimer's disease, once the drug release it performs its particular function like disaggregation of β amyloid plaque by binding with the β amyloid peptides, increases the ACh in the brain, reduces inflammatory reaction in the brain, promotes neuronal health depending upon the nature of drug and thereby treat Alzheimer's disease.

Among all the modification in the liposomes, one strategy applies cationic liposomal drug vehicles that would be able to take advantage of the BBB's negative charge, and consequently trigger the cell internalization processes through electrostatic interactions [43–45]. However, major drawbacks of this strategy are the nonspecific uptake of cationic NCs by peripheral tissues, together with their binding to serum proteins, which result in the requirement for administration of high doses of NCs to reach therapeutic efficacy; such doses cannot be administered in most cases, due to toxicity. Another strategy to target BBB includes the surface functionalization of liposomes with PEG[11] or polysaccharides, as a method to improve their pharmacokinetic profile, allowing longer time-periods in circulation and increased distribution of such “stealth” liposomes into the brain (by preventing their fast clearance through the RES[12]). However, although succeeding to dramatically improve liposome circulation time, the strategy of “stealth” liposomes does not provide any certainty that liposomes will be transcytosed across the BBB. To provide such additional properties to (“stealth”) liposomes, several modern methods for functionalization of the liposomal surface with biologically active ligands, such as peptides,

[2] Acetylcholinesterase
[3] Butyl cholinesterase
[4] Amyloid precursor protein
[5] Blood brain barrier
[6] Brain capillary endothelial cells
[7] Central nervous system
[8] Intravenous
[9] Intranasal
[10] Nanocarriers
[11] Polyethylene glycol
[12] Reticuloendothelial system