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Liposome as carrier therapy for Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is the most common type of dementia. Approximately 50 million people suffer from Alzheimer's and it is expected to increase more than 100 million by 2050. According to the Diagnostic and Statistical Manual of Mental Disorders V (DSM-V), the diagnosis of AD has an onset of memory impairment and cognitive decline. Effective treatment of AD is a major challenge in the field of pharmacy and science due to the restriction from blood brain barrier (BBB). This causes poor access to pills or oral administration. Liposome as a part of nanoparticle showed to be a potential AD treatment because of its biocompatibility, flexibility, and capability to carry various therapeutic molecules across the BBB and into brain cells. Several types of liposomes provide varied therapy effect. Some factors which determine liposomes effectiveness are its structure, mechanism of action; formed; and delivery, and its preparation method. This article reviewed liposomes characteristics which has potency as drug delivery system for AD.

ABSTRAK

Alzheimer disease (AD) adalah jenis demensia paling umum. Jumlah kasus AD sebanyak 50 juta yang diperkirakan akan meningkat hingga 100 juta pada tahun 2050. Menurut *Diagnostic and Statistical Manual of Mental Disorders* V (DSM-V) diagnosis AD memiliki gejala awal gangguan memori dan penurunan kognitif. Terapi AD yang efektif menjadi tantangan di bidang farmasi dan ilmu pengetahuan karena keterbatasan obat menembus sawar otak *(blood brain barrier)*. Hal ini menyebabkan kesempatan mendapatkan pengobatan dengan pil atau secara oral menjadi buruk. Liposom merupakan suatu nanopartikel yang potensial untuk pengobatan AD karena bersifat biokompatibel, fleksibel, dan dapat membawa molekul terapeutik melintasi sawar otak hingga sel otak. Beberapa jenis liposom memberi variasi efek pada pengobatan AD. Faktor yang memengaruhi efektivitas liposom sebagai pembawa terapi AD, antara lain struktur, mekanisme aksi; pembentukan; dan penghantaran obat serta cara penyiapannya. Makalah membahas karakteristik liposom yang berpotensi sebagai system pembawa obat untuk AD.

INTRODUCTION

Alzheimer's disease (AD) is the most common type of dementia. Approximately 80% of patients with dementia was reported worldwide.[1] Effective therapy for AD is a major challenge in the fields of pharmacy and science. There are six FDA-approved drugs such as donepezil, and available in clinics. However, they are just for symptomatic relief only. Some factors became constraints in the discovery and development of AD drugs. The mechanism and pathogenesis of AD

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have not been well understood due to it is a highly complex and progressive neurodegenerative disease. Moreover, the blood-brain barrier (BBB) limits the efficacy of the drugs for AD.[2] Drug delivery systems of drugs for AD face problems of bioavailability, biocompatibility, toxicity, efficacy and release control.[3]

Nanoliposomes as drug delivery systems for AD have not yet reached clinical trials. However, nanoliposomes are biocompatible, highly flexible, and have the potential to carry a wide variety of therapeutic molecules across the BBB enter brain cells. Current treatment modifications include the use of brain-penetrating peptides, along with targeting ligands. Several modifications of drug delivery system into liposomes were introduced for AD therapy to facilitate passage across the BBB, and evaluation of novel therapeutic agents to block and/or aggregation in AD.[4] This article reviewed liposomes characteristics which has potency as drug delivery system for AD.

DISCUSSION

Structure of liposome

Liposomes are the most widely used carriers in the health sector. Liposomes can deliver compounds that are both hydrophobic and hydrophilic. Encapsulation with liposomes can prevent degradation. The size varies between 20 nm and has a multi lamellar or unilamellar structure (FIGURE 1).[5] The hydrophilic heads of lipid molecules can be divided into three types according to their charge i.e. cationic, neutral and anionic. The cationic head helps attract liposomes to the negatively charged cell membrane containing primary and secondary amines resulting in a "proton sponge effect" to decrease the therapeutic effect of the lysosomes. Cationic liposomes containing drugs or molecular probes can absorb negatively charged macromolecules, to achieve the purpose of double charge. Neutral charge can be phosphatidylethanolamine (PE), phosphatidylcholine (PC) and cholesterol, such as EPC, DPPC (1.2-dipalmitoyl-sn-glycero-3-phosphocholine), DSPE (1.2-distearoyl-sn-glycero-3-phosphoethanolamine), DOPE (1.2-dioleoyl-sn-glycerol-3-phosphoethanolamine) and so on. While phosphatidylserine (PS) is an example of anionic lipid.[6]

The hydrophobic tail can affect the volume of the hydrophobic cavity and the ratio of the hydrophobic portion of the liposome, thus playing a role in determining the structure of the liposome. The hydrophobic tail consists of fat and cholesterol. In gene delivery, single-tailed lipid molecules are less efficient than double-tailed lipid molecules with aliphatic hydrocarbon tails.[6]

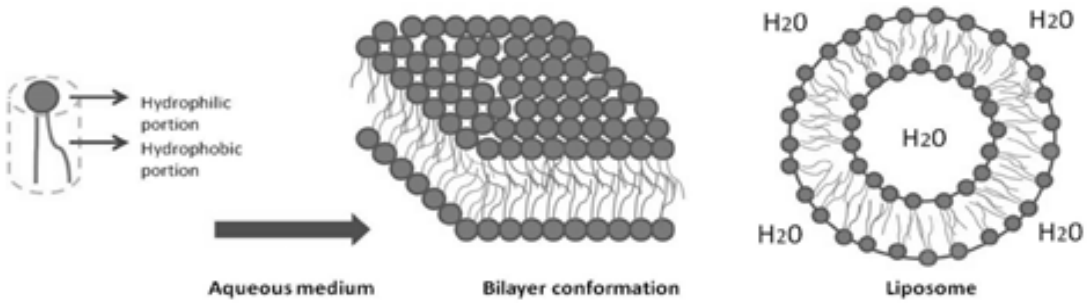

FIGURE 1. Structure and formation of liposome.[8]

Mechanism of liposome formation

Liposomes are formed by phospholipids which have a hydrophilic head and a hydrophobic tail (FIGURE 1). The hydrophilic part is composed of phosphoric acid bond to a water-soluble molecule while the hydrophobic part consists of two fatty acid chains with 10-24 carbon atoms and 0-6 double bonds in each chain. A flat sheet is formed when dispersed in an aqueous medium, the polar heads facing out from the aqueous region while the fatty acid groups face each other to form micelles called as liposomes. Phospholipids have a natural tendency to form liposomes, which can be used as drug targeting molecules.[7]

Liposomes also have good emulsifying properties to stabilize emulsions. Liposomes also play a role in drug coating to provide hydrophilicity to hydrophobic drugs. Variations in the aliphatic and alcohol chains lead to the occurrence of phospholipid varieties. In addition, different sources of phospholipids increase the type of phospholipid.[7]

The bilayer is formed due to the presence of conditions such as unfavorable interactions between the hydrophilic and hydrophobic phases which can be minimized by folding into closed concentric vesicles. In addition, the large free energy difference between hydrophilic and hydrophobic environments can be reduced by the formation of large vesicles because the spherical structure has minimum surface tension and maximum stability.[7]

Drug delivery system of liposome

Modern drug delivery systems have involved nanoparticles such as soluble polymers, micro particles, cells, cells ghosts, lipoproteins, liposomes, micelles, dendrimers, hydrogels, and carbon nanotubes. These particles act as carriers that have specificity to slow down degradability and are sensitive to pH or temperature with the target of conjugation with specific antibodies. Drug targeting can be performed into two categories, namely passive and active. Passive targeting leads to preferential drug accumulation at the target site, whereas active targeting involves functionalizing the surface of the drug carrier with ligands that are selectively identified by receptors on the cell surface.[3]

Formulation of liposome as drug delivery system is important to overcome the limitations of recent conventional drugs for AD. Formulation is prepared by selecting the appropriate composition for the function and in the targeting. Phospholipids, selection of head group and chain length, ratio of components of liposomes and modification of liposomes are important choices in determining the stability, safety, and efficiency of liposomes.[12] There are several examples of liposom formulation for many types of targets such as fungi, bacteria and cancer (TABLE 1).

TABLE 1. Drug delivery using liposome formulation

Liposome formulation	Drug	Target	Mode of action
Fungi[9]			
▪ Dimyristoyl phosphatidylcholine cholesterol or ergosterol	*N*-Methyl-*N*-*D*-fructosyl amphotericin B methyl ester (MFAME)	Antifungal	Decreasing amphotericin toxicity
▪ Hydrogenated soy phosphatidylcholine cholesterol and distearoyl glycerol	Amphotericin B	*Aspergillus fumigatus*	Targeted drug delivery at the site of infection
▪ Soya lecithin, cholesterol tocopheryl acetate	Ketoconazole	*Candida* spp., Microsporum	Improves therapeutic response and reduces side effects
Bacteria[9,10]			
▪ Partially hydrogenated egg phosphatidylcholine and cholesterol	Gentamicin	*Klebsiella pneumonia*	Increase the survival rate of animal models and therapeutic efficacy
▪ Partially hydrogenated egg phosphatidylcholine and cholesterol	Polymyxin B	*Pseudomonas aeruginosa*	Decreas number of lung bacteria and lung impairment by bacteria, increas bioavailability
▪ Solid supported liposomes (SSLs) encapsulating triclosan or penicillin	Zinc citrate	Immobilized biofilms *S. oralis*	Prevent bacterial growth and plaque accumulation in dental preparations
Cancer[9,11]			
▪ Soybean phosphatidylcholine or hydrogenated soybean phosphatidylcholine and cholesterol (PEGLigated)	Topotecan HCl	Anticancer	Increase stability and efficacy with accumulation in tumor cell
▪ Soybean phosphatidylcholine (S100PC) and 1,2-distearoyl-*sn* glycero-3 phosphoethanolamine [methoxy (polyethylene glycol)-cholesterol $(CH)_4$	Paclitaxel	Anticancer	Increased water solubility
▪ Cholesterol phosphatidylserine, phosphatidylglycerol or cardiolipin. Saturated or unsaturated phospholipid acyl chains	Doxorubicin	Anticancer	Reduction of cardiotoxicity and increased antitumor activity
▪ Distearoyl phosphatidylcholine (DSPC) : cholesterol	Daunorubicin (DNR)	Anticancer	Minimise RES uptake and prolong drug circulation time cause can be phagocytosed by monocytes and increased anthracycline dose, no cardiotoxicity

Drug delivery systems are easily detected by macrophage and monocytes so they can be removed from the bloodstream. Phagocytes can be found in liver, spleen, and bone marrow. Absorption phenomenon into the cell is called endocytosis, which is further divided into phagocytosis and pinocytosis. The process of phagocytosis is carried out through several specialized cells using specific and nonspecific receptors. FIGURE 2 show the general process of macrophage capture mechanism. From its competition, liposomes have become one of the best delivery systems in colloid systems and are clinically applicable because of its membrane structure.[13]

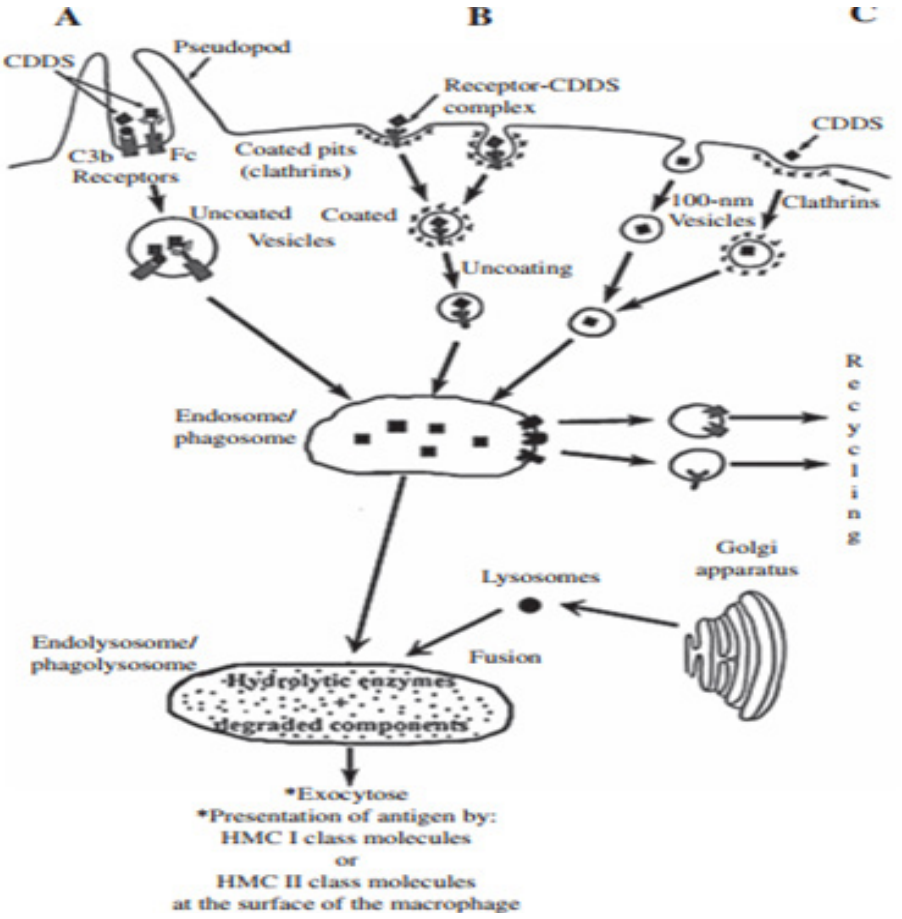

FIGURE 2. Mechanism of liposome delivery in cells.[13]

Reticuloendothelial system (RES), opsonization, and immunogenicity is a challenge in drug delivery system. This factor can be overcome by increasing the permeability and EPR (retention effect). When liposomes insert into the body, they will change into opsonin with high density lipoprotein (HDL) and low density lipoprotein (LDL) which circulate around the bloodstream. Opsonization will help RES in identifying and removing liposomes. The HDL and LDL interact with liposomes and decrease their stability.[14]

Mechanism of membrane fusion and optimization

Cell membranes can fuse with liposomes. There are four stages in fusion i.e. adsorption, fusion, and lipid exchange. At adsorption stage, the contact happens between cell membranes and the membrane target which construct liposomes. Endocytosis occurs when liposomes have been engulfed by the cell followed by the internalization of liposomes into the cell. Fusion is the combination of the lipid bilayer membrane between the liposome and the cell. The mechanism of membrane fusion is presented in FIGURE 3. The membranes appear to unite and form channels or gaps and special pathways. This pathway will be used for lipid exchange.[7,15]

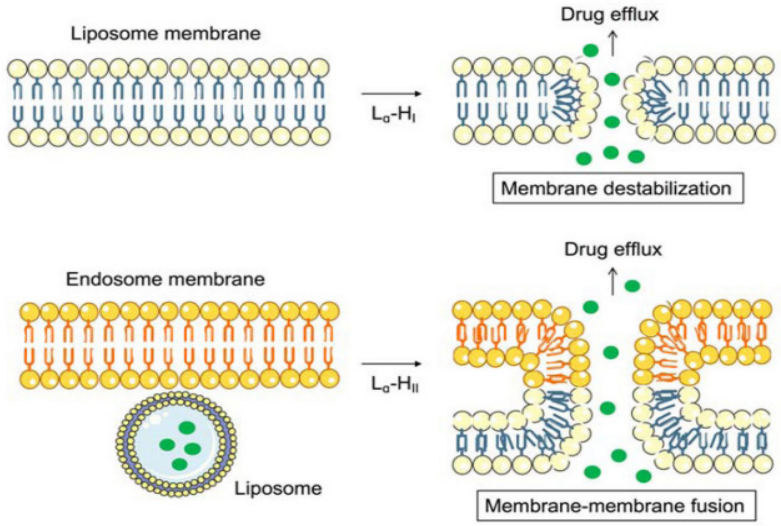

FIGURE 3. Mechanism of membrane fusion.[16]

The function of liposomes can be optimized in several ways. There are four ways to optimize the function of liposome: increasing entrapment efficiency, regulating drug release rate, *in vivo* manipulation of liposome, and enhancing intracellular delivery system with payload liposome.[16] The entrapment efficiency was increased by encapsulating a weak acid or base with a high ratio of drug and lipid. This can increase efficiency by 90%. The rate of drug release can be regulated by involving the role of cholesterol which will give a rigid effect and change the fluid phase of the phospholipid bilayer into a solid bilayer. *In vivo*, liposomes can be manipulated by changing size, fluidity, and surface charge. The payload liposome system can be performed using a sensitive pH or lipid cation during endocytosis. Cell penetration can lead to translocation of cellular membranes, increasing the delivery of liposomes via the endocytosis pathway.

Liposome preparation

There are three basic strategies for liposome preparation namely mechanical method, organic solvent method and detergent removal method.[8] 1). **Mechanical method**. Lipid hydration (while stirring) and sizing was carried out to obtain the vesicle population. Thin-film: from the lipid solution in chloroform, the flask was vacuumed for 2 h, to ensure that all organic solvents were removed. Then, the aqueous solution is added. After mechanical agitation of the flask, multilamellar vesicles (MLV) were formed. To make the homogeneous population, there are several methods: sonication (based on high energy cavitation, yielding an SUV with a diameter of 30 nm), extrusion (producing vesicles around 100 nm (LUV)), microfluidization (producing liposomes with a diameter of 50-500 nm) and homogenization with high pressure (producing liposomes as desired). 2). **Organic solvent method**. Lipids are dissolved in organic solvents by dispersion and aqueous phase. It consists of ethanol injection method (it is possible to prepare SUV without sonication), ether injection method, reverse phase evaporation (producing large unilamellar vesicles and oligo lamellar vesicles). 3). **Detergent removal method.** This methodology is based on the formation of mixed micelles from phospholipids and surfactants. After the formation of the lipid film, a detergent buffer solution was added. The whole system is stirred until complete dissolve. Furthermore, the detergent must be removed by gel chromatographic filtration, dialysis, or adsorption.

Types of liposome

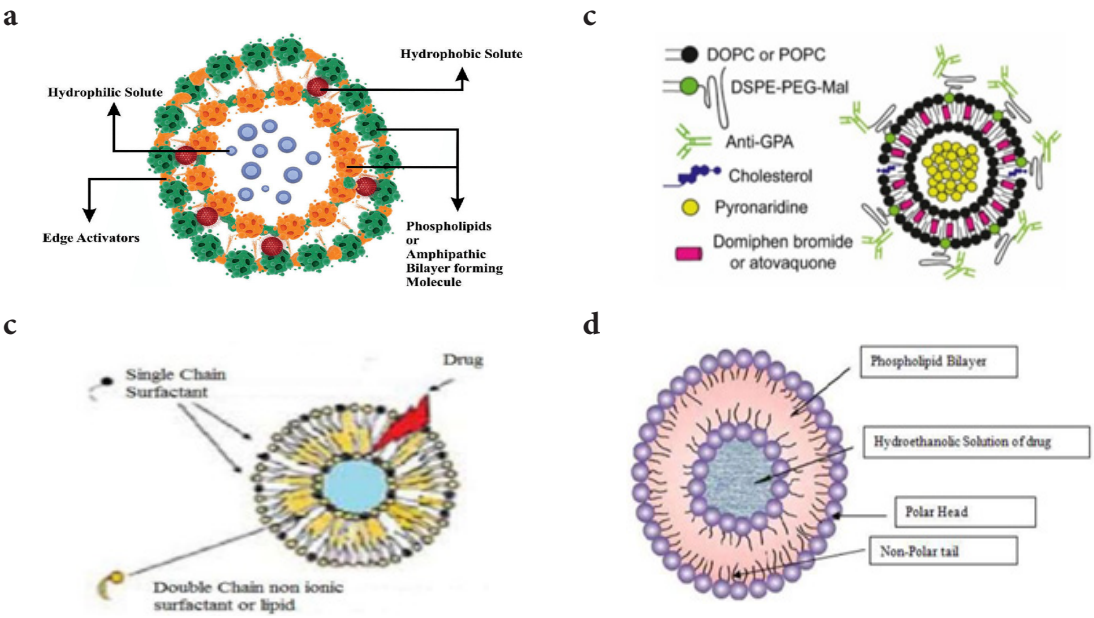

FIGURE 4. Types of liposome (a) transferosome[17] (b) immunoliposome for targeted antimalaria[19] (c) pharmacosome[22] (d) ethosomes[24]

Transferosome

Transferosomes are a special type of liposome, consisting of phosphatidylcholine and edge activator. When placed on a skin surface that is partially dehydrated by loss of water evaporation, lipid vesicles will escape from the dehydrated area by moving along this gradient. Transferosomes composed of surfactants are optimized to achieve maximum flexibility, thereby making full use of the transepidermal osmotic gradient (water concentration gradient). Transferosomes overcome the difficulty of skin penetration by squeezing themselves along the intracellular sealing lipids of the stratum corneum.[18]

Immunoliposome

Immunoliposomes are emerging as a promising strategy for targeted delivery using antigen-antibody, with consequent reduction of side effects. The antibody molecules contain functional chemical groups, such as amines and carboxylates, which are susceptible to modification for targeting purposes, and sulfhydryl groups act as targeting groups and have been widely reported. However, this group is rarely used in antibody molecules and must be generated by reduction of the disulfide group or through a suitable isolating agent.[20]

Escheriosome

Escheriosomes are one type of liposomes grafted from polar lipids extracted from *E. coli.* This vesicular form elicits a high cytotoxic T lymphocyte (CTL) response. Escheriosome showed its ability to deliver molecules which are trapped directly in antigen presenting cells (APC) and to process the trapped antigen via endocytic pathway thereby leading to antigen presentation by MHC-I. The expression through MHC-I will activate CD8+ lymphocyte. Escheriosome has role as new immune-adjuvant and emerge as an effective tool for generating protective immunity so it is interest the scientists in its use in vaccination.[21]

Pharmacosome

Pharmacosomes are colloidal dispersions of drugs covalently bound to lipids and maybe as ultrafine vesicular, micellar or hexagonal aggregates, depending on the chemical structure of

the drug-lipid complex. This system is formed by linking a drug *(*pharmacon*)* with a carrier (soma), so it is called a pharmacosome. Once absorbed, the rate of degradation into active drug molecules depends to a large extent on the size and functional group of the drug molecule, lipid chain length, and spacers.[23]

Ethosome

The ethosomes consist of hydroalcoholic or hydro/glycolic phospholipids in which the alcohol concentration is relatively high. Ethosome consists of phospholipid with various chemical structure like phosphatidylcholine, phosphatidylacid, phosphatidylserine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidyl inositol, alcohol (ethanol orisopropylalcohol), water and propyleneglycol.[25]

Liposome in biomedical fields

Liposomes provide benefits in analysis, such as increased affinity for chromatography, sensors, and immunoassays.[26] Liposomes are nanoparticles with a diameter of less than 20 nm. The benefit of this size is that liposomes can leave the bloodstream to reach target cells, such as cancer cells in cancer therapy. Liposomes have also been used for the encapsulation of mRNA-based COVID19 vaccines.[27] The composition of liposomes, which is similar to cell membranes, provides benefits in the form of ease of penetration and the release of certain molecules that can be carried out without the promoter protein. These proteins potentially become allergens or irritants. Another benefit of liposomes is that they can deliver drugs more stable, like hydrophilic, hydrophobic drugs, or both at the same time.[8] Liposome encapsulation in the drug provides benefits in the form of increased efficiency and reduced toxicity. For example, doxorubicin which is encapsulated by liposome has less cardiac toxicity.[28] The description of liposome's role as drug carrier and its advantages or disadvantage are summarised in TABLE 2 and 3.

TABLE 2. Drug delivery using liposome formulation

Liposome application	Description	References
Drug carrier	Liposomes increasing the solubility, sensitivity, and stability of hydrophobic drugs release. Liposomes reducing the drug side effect.	[6]
Vaccine carrier	Liposomes encapsulated the vaccine then make it easier to penetrate and reach lymphatic system. Liposomes change the integrity and conformation of cell membranes. Liposomes become adjuvants vaccine that are biodegradable, non-toxic, and do not induce antibodies. One example is the virosome.	[6,29]
Cancer drug therapy	Liposomes encapsulated the cancer drug. Liposomes reduce cancer drug side effect, cardiotoxicity. For example: Doxil, daunorubicin, and mepact.	[30-32]

TABLE 3. Drug delivery using liposome formulation[5,13]

Advantages	Disadvantages
Increasing drug stability and biocompatibility	Short time storage
Biodegradable	More expensive cost
Easier to penetrate into cells	More sensitive to osmotic pressure
Protecting drug (e.g. antimicrobials) from chemical substance, immune properties, and enzyme	Lower solubility
Higher activities to against intracellular pathogens	Easier to undergo oxidation and hydrolysis reactions
Easily to modify (changing membrane charge, adding protein and antibodies, increasing affinity)	Changing easily by sterilization with high temperatures and radiation methods that affects liposome structure and function
Less toxic	Sensitive on radiation method

CONCLUSION

The restriction of the BBB in AD therapy can be potentially solved by using liposomes as drug delivery systems. Liposome is adequate to cut through BBB and penetrate to membrane cell due to its small size and similar composition of membrane structure. Besides increasing drug stability, liposome enhance drug efficiency and reduce its toxicity to not target cells. In the future, further research is needed to enhance its specificity as drug delivery for AD.

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