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Review Article

SPHINGOSOMES: APPLICATIONS IN TARGETED DRUG DELIVERY

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ABSTRACT

Sphingosomes are vesicular drug delivery systems in which an aqueous volume is entirely enclosed with sphingolipid bilayer membranes. Sphingolipids are developed as bioeffector molecules, which regulate cell growth, proliferation and anti-cancer therapeutics. Sphingosomes have become an enhanced area of interest because of their applicability in improving the *in vivo* delivery of various chemotherapeutic agents, biological macromolecules and diagnostics. The present review emphasizes on the applicability of sphingosomes in drug delivery technology.

Keywords: sphingosome, vesicular drug delivery, sphingolipids, targeted drug delivery.

INTRODUCTION

The ideal goal of targeted drug delivery is to optimize therapeutic index of the drug by localizing its activity at the site or organ of action[1]. Drugs can be targeted to specific organs[2], systems, cells or even specific intracellular organelles[3] or molecules[4]. Drug targeting can be achieved by physical, biological[5] or molecular systems that result in high concentrations of pharmaceutically active agents at the targeted site, thus lowering its concentrations in the rest of the body. Targeted drug delivery results in reduction in the dose and increased efficacy of the treatment with a significant reduction of drug toxicity. Various approaches are available for drug targeting which can control the release, distribution of the therapeutic molecule[6]. Vesicular drug delivery systems have practically showed applicability in targeted drug delivery in the uptake and transport of active agents to the tissue or organ through biological membranes[7]. Biologic origin of these vesicles was first reported in 1965 by Bingham and was given the name Bingham bodies. Drugs that are encapsulated in the vesicular structures are predicted to achieve specific uptake, tissue specific distribution, decrease of interaction with the blood components and reduction in toxicity[8].

The vesicular systems are highly ordered assemblies of one or several concentric lipid bilayer formed, when certain amphiphillic building blocks are confronted with water. Vesicular drug delivery systems can incorporate both hydrophilic and lipophilic drugs. They improve the bioavailability of poorly soluble drugs, delay elimination of rapidly metabolizable drugs, prolong the existence of drugs in the systemic circulation. A wide number of vesicular drug delivery systems such as liposomes, sphingosomes, pharmacosomes, niosomes, virosomes, transferosomes etc., were developed[9,10].

Liposomes, the phospholipid bilayered vesicles have gained much importance as potential drug carrier systems in targeted drug delivery[11]. The major limitations of using conventional liposomes as drug

delivery vehicles are rapid clearance from blood, restricted control of encapsulated molecule release, low or non reproducible drug loading, physical and chemical instability, and large-scale sterile preparation. Many of these problems have been addressed during the past two decades of research.

Liposomal formulations containing sphingolipids are more propitious than the phospholipid liposomes in improving the efficacy, circulation time, encapsulation efficiency, resistant to oxidation, hydrolysis, increased stability towards acids and flexible to couple with site specific ligand to achieve active targeting[8,12]. These sphingolipids have been nowadays used for preparing stable liposomes, which are called as sphingosomes[13].

Sphingosomes

Sphingosome are concentric, bilayered vesicle in which an aqueous volume is enclosed by a membranous lipid bilayer mainly composed of natural or synthetic sphingolipid[8]. Sphingosomes comprise of sphingolipid and cholesterol, an interior aqueous environment having pH less than that of exterior. The drug is encapsulated inside the lipid bilayer and is delivered to the host at a predetermined rate thereby improving the efficacy, increasing the circulation time and reducing the toxicity. Sphingosomes can be utilized for therapeutic, cosmetic and diagnostic purpose for the delivery of active to the target site or organ. They can be administered by variety of routes like oral, parenteral, inhalation, transdermal etc. Sphingolipid present in the sphingosomes offer several advantages to these vesicular systems for targeting both by passive and active targeting mechanism [14-16].

Composition of sphingosomes

Sphingosomes are the liposomal preparations which mainly differ in the lipid composition. They are having one or more membranes which comprise sphingolipids and cholesterol. The sphingolipid and cholesterol are typically present at a percentage molar ratio from 75:25 to 30:50 and most preferable ratio is 55:45. Other lipids may also be present provided they should not adversely affect the stability of the drug. Generally inclusion of other lipids will result in a decrease in sphingolipid/cholesterol ratio[17].

Sphingolipids

Sphingolipids represent major class of phospholipids which are not simply regarded as structural components of biological membranes but are also involved in transmission of signal and in the recognition of cells. They are complex family of compounds share a common structural feature, a sphingoid base backbone[18] as shown in Fig.1. Sphingolipids is derived from amino alcohol with 18 carbon atom and unsaturated carbon chains called sphingosine. Biologically it is synthesized from serine and a long-chain fatty acyl-CoA, then converted into ceramides, phosphosphingolipids, glycosphingolipids, and other species, including protein adducts[19,20]. The classification of sphingolipids is given in Table 1**.** **S**phingolipids serves various functional roles in biological systems[18,21]. They protect cell membrane surface by the formation of stable outer leaflet of plasma membrane. Complex glycosphingolpids play vital role in cell physiology by acting as antigens, modulators in signal transduction, binding agents in microbial toxins and growth factors and mediators in cell adhesion. Ceramide functions as a second messenger and helps in signaling of proliferation, differentiation, apoptosis and regulation and function of immune system. Sphingosine-1-Phosphate functions as both first and second messenger and regulates various cellular processes such as angiogenesis, vascular maturation, cardiac development, calcium homeostasis, cell growth. They help in predicting the cell pathway during transport all the way through cell membrane.

Theoretical aspects of sphingosomes

Formation of ordered membranes[22-30]

In a conventional lipid-bilayer assembly, the hydrophobic acyl chains of a lipid molecule

associate and interact with those of the neighboring molecules, and the polar head-groups orient themselves to the exterior of the assembly. Sphingosomes are forming ordered membranes as the sphingolipids in general show a preference for partitioning into ordered domains. The head group structures and acyl chain compositions of naturally occurring sphingolipids vary greatly. The ceramide moieties with the long-chain base and long saturated N-acyl chains promote the partitioning of sphingolipids into ordered membrane domains. The polar head group, which varies from the single hydroxyl of ceramide and the phosphocholine group of sphingomyelin, to large assemblies of carbohydrates for the complex glycosphingolipid, will undoubtedly also affect the partitioning of these lipids.

Stability against hydrolysis

Liposome dispersions are thermodynamically unstable. The total free energy of a dispersed system can always be lowered by reduction in the interfacial area. This tendency to aggregation is due to attractive van der Waals forces between the negatively charged group on the liposome surface. In sphingosomes the negative charge is shielded by a bulky hydrophilic group and is attributable for prevention of vesicle aggregates during preparation and storage and, perhaps, also immediately after injection. Liposomal phospholipid can undergo chemical degradation such as oxidation and hydrolysis due to ester linkages. Sphingosomes structurally they are stable to hydrolysis[31]. Sphingolipids are biologically inert macromolecules, back bone contains amide and ether linkages which have resistant to hydrolysis.

Interaction between cholesterol and sphingolipids

Cholesterol prefers to interact with sphingolipid over phosphatidylcholine which contains an acyl-chain. It has long been appreciated that there is a positive correlation between the concentrations of cholesterol and sphingolipids in various membrane fractions[22]. Cholesterol desorption or exchange studies, both in monolayer membranes[32], and in bilayer systems[33], has showed that cholesterol desorbs more slowly from sphingomyelin-rich membranes, or has trapped more avidly in sphingomyelin-containing acceptor vesicles than is the case with acyl-chain containing phosphatidylcholines. These interactions also have a positive application for sphingosome in improving the biological effectiveness.

Encapsulation [34,35]

Sphingosomes has high entrapment efficiency of drug in response to transmembrane pH gradient. This not only accomplishes efficient drug encapsulation but also decreases the rate of drug efflux from the vesicles.

Circulation time [36,37]

The major barrier to using liposomes for systemic drug delivery has been the rapid clearance of liposomes from the bloodstream by the reticuloendothelial system. Increased rigidity of the sphingosomal walls prolongs the circulating life of sphingosomes and significantly extends the duration of drug release. In addition the sphingosome surface negative charge is shielded by a bulky hydrophilic group and reduces the rate of their clearance by the reticuloendothelial system and increases the biological half life.

Drug loading in tumor [36]

Sphingosomes readily extravasate through the pores of leaky tumor vessels created during angiogenesis and readily accumulate within the tumor. Once lodged within the interstitial space, these resilient sphingosomes slowly release the encapsulated drug. Slow release of the drug from extravasated sphingosomes increases drug levels within the tumor, extends drug exposure through multiple cell cycles, and significantly enhances tumor cell killing.

Sphingosomes preparation and drug loading

Sphingosomes are lamellar vesicle systems whose preparation is similar to that of liposomes. The preparation of sphingosomes mainly involve loading of the drug into vesicles. For drug loading suitable conventional passive loading and active entrapment methods are being used[38]. The general methods for the preparation are listed below.

1. Lipid Hydration Method[38,39]
2. Solvent Spherule Method[40,41].
3. Sonication Method[42]
4. French Pressure Cell Method[43].
5. Solvent Injection Methods
   5a. Ether Infusion Method[44,45].
   5b. Ethanol Injection Method[46]
6. Detergent Removal Methods[47-49]
7. Reverse Phase Evaporation Method[50]
8. Calcium-Induced Fusion Method[51].
9. Microfluldization Method[52]
10. Freeze-Thaw Method[53-55]

Sphingosomes Characterization

Sphingosomes are vesicular systems, which has to be characterized for morphological, biophysical, drug loading, drug release and stability. Biophysical properties of the final drug product are recommended to be analyzed by gravimetric analysis of lipids in the formulation, lamellarity, particle size and size distribution, phase transition temperature, vesicle charge, osmotic and pH properties, and light scattering index . Particle sizing, size distribution can be analyzed by Dynamic light scattering (DLS), electron microscopy using either cryo-fixation techniques or negative staining, atomic force microscopy (AFM)[56] and ultracentrifugation[57]. Lamellarity of liposomes can be detected using nuclear magnetic resonance (NMR) spectroscopy, small angle x-ray scattering and cryo-electron microscopy. Zeta potential measurements can be used to determine the electrophoretic mobility (microelectrophoresis) of liposomal vesicles and thus identify their surface charge density[56,57]. Therapeutic efficacy and the in vivo performance of these drug delivery systems are estimated by some of the most critical parameters such as drug loading and in vitro release from liposomal vesicles[58,59].

Research work on sphingosomes

Sphingosomes have gained much attention in the past few years because of their potential applications. Many of their applications are based on the functional properties of the sphingolipids. The therapeutics applications of sphingosomes are summarized in Table 2.

There is an increasing interest in the concept of sphingosomes due to their usefulness in stabilization and increasing the circulation time of liposomes for the design of targeted drug delivery systems. There are several reports on the preparation and applications of sphingosomes in drug delivery. Modrak *et al.*[60] has filed a patent on sphingomyelin containing preparation for the treatment of rheumatoid arthritis, in which they investigated that sphingomyelin is used in the manufacture of medicament that is used for rheumatoid arthritis, an autoimmune disease.

Modrak *et al.*[61] filed a patent of sphingomyelin containing preparation for the enhancement of tumour therapy, they claimed that therapeutically effective amount of sphingomyelin is used in the preparation of a medicament for enhancing cytotoxic tumour theapy, wherein sphingomyelin is administered in conjunction with an anthracyclin.

Igor V. *et al.*[62] investigated on the comparative study of drug loading and retention of liposome encapsulated vincristine, vinorelbine and vinblastine. They found that vinca alkaloids exhibited different retention properties, the more hydrophobic drugs were released more rapidly. They found that the retention characteristics of vinca drugs having low drug to lipid ratio can be improved when ionophore technique is used for loading.

Modrak, *et al.*[63] filed a patent of Sphingomyelin enhancement of tumour therapy, they investigated that co-administration of 5-Fluoro uracil and sphingomyelin reduced the rate of colonic tumour growth to a greater degree and for

longer time. Webb *et al.*[17] filed a patent of sphingosome preparations, they proved the formulation have enhanced stability and improved drug delivery of ciprofloxacin, swainsonine, vincristine and vinblastine.

Hope M. *et al.*[64] filed a patent where they reported a method for loading a therapeutic agent into preformed liposomes having a methylamine concentration gradient across the lipid bilayer of the liposomes, which is more stable, more cost effective and easy to prepare.

This review summarizes the emerging popularity and valuable potential offered by the sphingosomes in the field of vesicular drug delivery. Sphingosomes represent an attractive class of liposomal drug delivery systems with improved stability and circulation time for targeted drug delivery. These sphingosomes offer potential applications as vesicular carrier for delivery of various therapeutic agents to the target tissue or organ for diagnostic, cosmetic, tumour therapy, cancer therapy, gene therapy, immunology and enzyme delivery.

Table 1: Classification of sphingolipid[20]

Sphingoid bases
- a. Sphing-4-enines (sphingosines)
- b. Sphinganines
- c. 4-Hydroxysphinganines (phytosphingosines)
- d. Hexadecasphinganine (Sphingoid base homologs and variants)
- e. Sphingoid base 1-phosphates
- f. Lysosphingomyelins and lysoglycosphingolipids
- g. *N*-Methylated sphingoid bases
- h. Sphingoid base analogs

Ceramides
- a. N-Acylsphingosines (ceramides)
- b. N-Acylsphinganines (dihydroceramides)
- c. N-Acyl-4-hydroxysphinganines (phytoceramides)
- d. Acylceramides
- e. Ceramide 1-phosphates

Phosphosphingolipids
- a. Ceramide phosphocholines (sphingomyelins)
- b. Ceramide phosphoethanolamines
- c. Ceramide phosphoinositols

Phosphonosphingolipids

Neutral glycosphingolipids
- a. Simple Glc series (GlcCer, LacCer, etc.)
- b. GalNAcβ1-3Galα1-4Galβ1-4Glc- (globo series)
- c. GalNAcβ1-4Galβ1-4Glc- (ganglio series)
- d. Galβ1-3GlcNAcβ1-3Galβ1-4Glc- (lacto series)
- e. Galβ1-4GlcNAcβ1-3Galβ1-4Glc- (neolacto series)
- f. GalNAcβ1-3Galα1-3Galβ1-4Glc- (isoglobo series)
- g. GlcNAcβ1-2Manα1-3Manβ1-4Glc- (mollu series)
- h. GalNAcβ1-4GlcNAcβ1-3Manβ1-4Glc- (arthro series)
- i. Gal- (gala series)
- j. Other

Acidic glycosphingolipids
- a. Gangliosides
- b. Sulfoglycosphingolipids (sulfatides)
- c. Glucuronosphingolipids
- d. Phosphoglycosphingolipids
- e. Other

Basic glycosphingolipids

Amphoteric glycosphingolipids

Arsenosphingolipids

Table 2: Therapeutic applications of sphingosomes

Formulation	Application
Cancer therapy	
Vincristine (vincristine sulphate liposome injection)	Non-Hodgkins lymphoma[65]
Vincristine in combination with Rituximab	Large B-cell lymphoma[66]
Vinorelbine Navelbine® single or in combination with cisplatin.	Non-small cell lung cancer, metastatic breast cancer[67,68]
Alocrest (vinorelbine tartrate liposome injection)	Non-small cell lung cancer, breast cancer[69]
Topotecan Hyacamtin®	Relapsed small-cell lung cancer, relapsed ovarian cancer[70]
Tumour therapy	
5-Fluoro uracil in combination with sphingomyelin	Colonic tumour[63]
Swasinosine in combination with interferon.	Colon cancer and melanoma[17]
Drug vehicles	
Prostaglandins, amphoterecin B, methotrexate, cisplatin, vincristine, vinblastine, doxorubicin, Camphothecin, ciprofloxacin, progesterone,	Proliferative disease, immune disease, infectious disease, vascular disease, rheumatoid disease and inflammatory disease[16]
Cosmetic	
i.Beclomethasone	Skin / Dermal therapy[11]
ii.SPHINGOSOMES™ MOIST	skin cleansing and make-up removal efficiency[71]
Ocular drug delivery	
Idoxuridine	acute and chronic herpetic keratitis[11]
Enzyme Delivery	
Streptokinase, Urokinase	Treatment of malnutrition[13]
Antifugal therapy	
Sphingosine and sphinganine, free sphingolipids of the stratum corneum	Treating infectious disease[72]
Gene therapy	
sphingosine 1-phosphate analogs	radiation-induced lung injury (RILI)[73]
Immunology	
Ceramides, sphingosine 1-phosphate	Regulation of immune response[74]

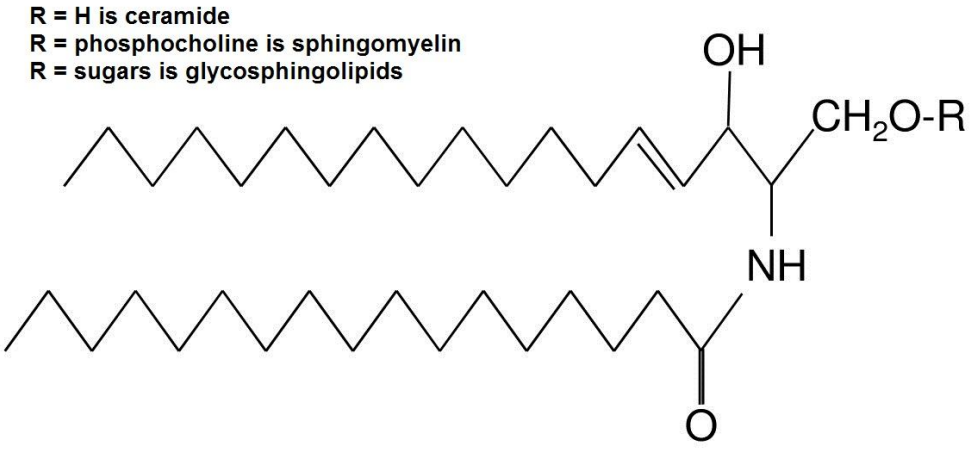

Fig. 1: General structure of sphingolipid

CONCLUSION

An extensive research is going on sphingolipids and sphingosomal drug delivery systems. There is a great potential in utilizing these sphingosomes in ecology, biotechnology, medicine and pharmaceutical technology. However these techniques are not effectively applied for the development of drug delivery systems. They may be considered as efficient vesicular drug delivery systems due to improved drug loading, stability, release and targeting to specific tissue or organ. Hence the sphingosomes have great potential in the design of novel vesicular targeted drug delivery systems.

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