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CHAPTER I

INTRODUCTION

General

Liposomes were discovered in the early 1960s by the British scientist, Alec Bangham. In the course of his research on the effect of phospholipids on the clotting of blood, Bangham would created reagents by adding water to a phospholipid film. He soon recognized that the phospholipid films formed closed spherical structures that encapsulated part of the liquid medium in their interior. These are so-called liposomes [Lasic, 1992].

Liposomal membrane is semi-permeable membrane, in which the rate of diffusion of molecules and ions across the membrane varies considerably. For amphiphilic molecule which has a high solubility in both organic and aqueous media, a phospholipid membrane acts as a very thin barrier, in contrast with polar solutes such as glucose that can pass very slowly across the membrane. Small molecules with neutral charge (e.g. water and urea) can diffuse across membrane quite rapidly, while penetration of charge ions differs greatly, depending on their characteristics. Protons and hydroxyl ions are be able to diffuse across the membrane fairly quickly, probably as a result of transfer of hydrogen bonds between water molecules which are located as deep in the bilayer interior as the lower carbonyl group. Sodium and potassium ions,

on the other hand, transverse the membrane very slowly, not only with respect to protons but also to anions such as chloride and nitrite. Entrapped macromolecules, e.g. enzymes, nucleic acids, polymers and viruses or viral components are unable to cross the lipid bilayer due to their large size [Kirby, 1980].

Sterols are essential components of most natural membranes and incorporation of sterols into liposome bilayers can cause various changes to membrane's properties. In mammals, the predominant sterol is cholesterol which by itself does not form bilayer structures; however, it can be incorporated into phospholipid membranes in very high concentrations up to 1:1 or even 2:1 molar ratios of cholesterol to phospholipid [New, 1992].

As illustrated in Figure 1, the structure of cholesterol has most likely involved for an optimal interaction of the sterol with various phospholipids in the membranes [Demel, 1976]. Cholesterol, bearing the isooctyl side chain at C-17, can reduce the solute permeability of phosphatidylcholine liposome. The tetracyclic ring structure provides rigidity to cholesterol molecule whereas the 3β-hydroxy group shows an important amphiphilic function [Yeagle, 1985].

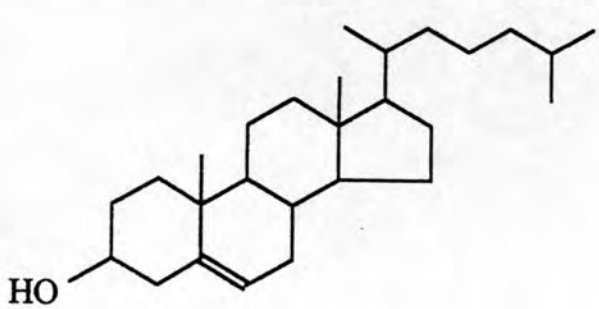

Figure 1. Structure of cholesterol.

The importance of cholesterol is normally visualized in an association with the other membrane lipids and in theoretical considerations, both hydrogen bonding and London-Van der Waals interactions are thought to be of importance. The role of cholesterol in the liposome model system can be interpreted as reducing the chain mobility, consequently by altering the permeability of the structure [De Gier, 1968]. Cholesterol interactes with phospholipids to decrease the area occupied by phospholipid molecules; moreover, it also controls the fluidity of bilayers [Chauhan, 1986].

Patel, et al. (1984) had reported that hydration of an ethoxylate derivative of cholesterol, triethoxycholesterol, resulted in the formation of stable, rigid, bilayer-like structures capable of encapsulating polar compounds. They had studied on the stability and tissue distribution of these liposomes in mice indicated that these bilayer system might be suitable as a drug delivery system.

In 1985, Patel et al. (1985) had shown that cholesterol derivatives with side chains ending in hydroxyl group reduced the permeability of unilamellar vesicles. However, addition of cholesterol derivatives with terminal amino group made the vesicle more permeable. Vesicles prepared with a short-chain amino cholesterol derivatives were found to be less permeable in phosphate-buffered saline, but not in bovine serum, while long chain amino cholesterol containing vesicles were very permeable in both media.

Generally, substances such as drug can be entrapped within liposome by two different pathways. Passive loading is conventional technique that the interested substances will be introduced to liposomes before or at some stage during the manufacture of the liposomes and their position may be located at liposome membrane or within internal aqueous media of vesicle depending on their solubilities. Another approach is active loading technique in which certain types of compound with ionizable groups, and those which display both lipid and water solubility (amphipathic compound, e.g. lipophilic amine) can be introduced into preformed liposomes with an imposed pH gradient. These compounds are often difficult to retain inside liposomes, prepared by passive loading, since their lipophilicity leads to their being able to pass into and out of membranes readily, and thus equilibrating between the liposome interior and exterior; therefore, significant leakage of entrapped compounds on prolong storage are visibly shown. In the active loading approach, however, amphipathic compound can permeate easily across the membrane in response to the pH gradient as the uncharged form and be converted to the fully charged species inside the liposome so that it is unable to escape from the liposome, since its lipophilicity is very much reduced. The compound, therefore, accumulates inside the liposome as long as a pH difference is maintained between the inside and outside of the membrane, resulting in high trapping efficiency. Since long-term stability is achieved; thus, the problem of passive loading techniques is resolved.

Madden, et al. (1990) had shown that several biogenic amines and antineoplastic agents could be accumulated by vesicles in response to an imposed proton gradient. This "active loading" technique allowed independent variation of any liposomal parameter and in addition much higher drug to lipid ratios could be achieved in comparison to conventional techniques. Furthermore, as the transmembrane distribution of the drug was determined by the proton gradient, it may be possible to control the rate of drug leakage in the circulation by changes in the buffering capacity of the intravesicular medium.

Nichols and Deamer, (1976) had shown that liposomes were prepared with pH gradients across their membranes (acidic interiors with respect to the external buffer). These liposomes efficiently concentrated several catecholamines (dopamine, norepinephrine, and epinephrine) added to the external buffer. They had suggested that pH gradients might contribute to uptake of catecholamines by sub-cellular storage sites.

Mayer, et al. (1986) had shown that adriamycin could be loaded into LUVs exhibiting pH gradients (inside acidic) in the absence of ionophores, resulting in trapping efficiencies of over 90%. Interior adriamycin concentration was found more than 50 mM and dramatic reduction in efflux rate was also obtained.

Mayer, et al. (1988) had employed dibucaine as a model lipophilic amino-containing compound and characterized the uptake behavior in response to pH gradients. Rapid uptake levels reaching interior concentrations in excess

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of 120 mM could be achieved, in combination with trapping efficiencies more than 90%. The transbilayer concentration gradients obtained and the influence of interaction buffering capacity were shown to be consistent with transport of the neutral form of the amine.

Objective and Scope of the Research

The objective of this research was to studies influence of cholesterol analogues on transmembrane pH gradients in trapping of amine drug in liposome by using propranolol as a model of amino-containing drug. In addition, these studies defined conditions requiring to achieve liposomal preparations for vesicle systems exhibiting high drug-to-lipid ratios with appropriate stability. The investigation presented here might provide some evidences that could be applied successfully when liposome was used as a carrier for potent or cytotoxic drug; since, restricted controlled of therapeutic dose with prolong integrity of drug carrier before reaching target organ is essentially necessary and important.