CHAPTER I

INTRODUCTION

Macrophages can be found in all organs of the body. The precursors of macrophages are monocytes, promonocytes, and monoblasts. During maturation, these cells acquire many functions, some of which are related to phagocytic capacity. These include host defense against microorganisms, the ability to initiate immune responses through stimulation of and/or antigen presentation to immunocompetent T cells, secretion of various mediators, and control of tumor growth (Sigal and Ron, 1994). During a local infection or inflammation, chemotactic factors, other mediators, and many secretory enzymes are produced by macrophages. These substances can cause cell destruction or more inflammation (Giese, 1979). Although macrophages constitute primary defense against infection, a variety of bacterial diseases originated from facultative or obligate intracellular parasites often use macrophages as the reservoirs. Recent studies have described the important role of monocytes (immature macrophages) in viral infectious diseases such as HIV-1 infection. The viruses can replicate themselves in the macrophages (Sigal and Ron, 1994). Many studies suggest that *Mycobacterium avium-M. intracellulare* complex (MAC), a common complication in Acquired Immune Deficiency Syndrome, can replicate within macrophages (Armstrong et al.,1985; Horsburgh, 1991). Therefore, it seems clear that macrophages are the prominent target of treatment for inflammation or infectious diseases. Many studies have shown that direct delivery of antibiotics to macrophages is a more effective therapy for MAC (Ashtekar, Duzgunes, and Gangadharam, 1991; Duzgunes et al., 1996; Kesavalu et al., 1990; Majumdar et al., 1992; Oh, Nix, and Straubinger, 1995).

Liposomes are simple vesicles in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules (usually phospholipids). These components are biodegradable so liposomes are widely studied as drug carriers. They can be constructed so that they entrap quantities of materials both within their aqueous compartment and within the membrane (New, 1997). Numerous studies demonstrate

that liposomes are effectively phagocytosed by macrophages. Monkkonen et al. (1995) found that the potency of liposomal clodronate was greater than that of the free drug on a murine macrophage cell line, RAW264. Duzgunes et al. (1996) reported that liposome-encapsulated sparfloxacin was more effective than free sparfloxacin in treating MAC infections in the murine macrophage-like cell line J774. An understanding of the mechanism of liposome uptake by cells and the method of drug delivery by liposomes is still emerging (Allen, Williamson, and Schlegel, 1988; Lee, Nir, and Papahadjopoulos, 1993; Papahadjopoulos et al., 1991). Many studies attempted to search for the effect of liposome composition on macrophages. Early studies indicated that negatively charged liposomes containing phosphatidylserine (PS), phosphatidylglycerol (PG), or phosphatidic acid (PA) were taken up faster and at greater extent than neutral liposomes or positively charged liposomes by phagocytic cells (Allen et al., 1991; Lee, Hong, and Papahadjopoulos, 1992; Lee et al., 1993). Several in vitro studies have shown different rates of liposome uptake by different cell types. Lee et al. (1993) investigated the interaction of liposomes of different surface properties with two mammalian cell lines, CV1 (an African green monkey cell line) and J774 (a murine macrophage-like cell line). Inclusion of 9 mol% PS, PG, or PA increased the extent of CV1 uptake of phosphatidylcholine (PC)/cholesterol (CH) liposomes but did not enhance the uptake by J774 cells. In contrast, liposome uptake by CV1 cells was not promoted when monosialoganglioside G_{M1}, phosphatidylinositol, or phosphatidylethanolamine conjugated to polyethylene glycol was incorporated into PC/CH liposomes (Lee, Hong, and Papahadjopoulos, 1992). CH is an important component which is often added to leaky bilayers to reduce leakage rate (New, 1997). However, addition of CH to liposomes decreased their uptake by mouse bone marrow macrophages and rabbit peritoneal macrophages (Allen et al., 1991; Foong, and Green, 1988). On the other hand, Katragadda, Bridgman, and Betageri (2000) could not reach any conclusion regarding the effect of CH on cellular uptake of U-937 human macrophages. Thus, the effect of cholesterol on cellular uptake of liposomes may depend on the cells used.

Rattana Rattanatraiphop (2000) studied the effects of formulation factors on physical properties and in vitro biological activity of propylthiouracil (PTU) liposomes. The assessment of biological activities of PTU in terms of antiproliferative effect on BALB/c 3T3 mouse fibroblasts showed that PTU liposomes were not better

than free PTU. However, a sustained-release characteristic could be seen with PTU liposomes though the underlying mechanism was not investigated. Design of an appropriate liposomal carrier system for drug delivery requires better understanding of the mechanisms by which liposomes enhance uptake and/or therapeutic response of the drug.

This present study was aimed at exploring the effects of formulation factors on role of liposomes as a drug carrier system for macrophage cells. The U-937 monocytic cell line was used since it is of human origin. It still expresses many of the monocyte-like characteristics, which are seen in only a few human cell lines. The cells are known to retain phagocytic activity, a process expected to facilitate liposome uptake. Effects of various negatively charged lipids (PS, PG, and dicetylphosphate) as well as addition of CH were investigated. Specific receptors for some of these negatively charged lipids were found on the surface of macrophages. Thus, it was expected that enhanced delivery would be seen with these lipids. PTU was selected as a model drug. The drug possesses antiproliferative activity, which can be used as an evidence of its uptake by living cells (Elias, Goodman, and Rohan, 1993a; Elias, Goodman, and Rohan, 1993b). Preliminary investigation for possible mechanism by which liposomes interacted with U-937 cells was also carried out. The insights regarding the formulation factors and the mechanism by which liposomes interact with cells would be significant in design and development of liposomal delivery systems.