POLYMERIZABLE FATTY ACIDS, PHOSPHOLIPIDS AND POLYMERIZED LIPOSOMES THEREFROM

This is a continuation of application Ser. No. 09/002,145, filed Dec. 31, 1997, now U.S. Pat. No. 6,187,335.

1. INTRODUCTION

The present invention relates to novel polymerizable fatty acids and phospholipids useful for preparing polymerizable liposomes for oral and/or mucosal delivery of vaccines, allergens, diagnostics and therapeutics. In particular, the present invention relates to polymerizable fatty acids having a polymerizable group, a surfactant group, and a functional group, such as octadecadienoyl-polyethylene glycol-succinic acid (ODPEGSu) compounds, and polymerizable liposomes prepared therefrom. The present invention further relates to polymerizable fatty acids coupled to targeting ligands with an affinity for human and mammalian intestinal M cells and similar cells in the nasopharyngeal cavity, such as lectins or proteins or peptides which can bind to M cells, and to polymerizable liposomes incorporating them. The invention also relates to negatively charged polymerizable lipids, specifically derivatives of polymerizable liposomes which have phosphatidyl inositol (PI), phosphatidyl glycerol (PG) or phosphatidyl serine (PS) groups on a polymerizable backbone, and to liposomes prepared therefrom. The invention still further relates to the use of the polymerized liposomes of the present invention as, or in, pharmaceutical compositions for oral delivery of a variety of diagnostic or therapeutic agents, including drugs, allergens and vaccines. The liposomes of the present invention provide increased stability in the gastrointestinal (G-I) tract, and increased flexibility in targeting liposomes to particular cells to enhance the uptake of encapsulated therapeutic agents.

2. BACKGROUND OF THE INVENTION

2.1. Drug Delivery

Drug delivery takes a variety of forms, depending on the agent to be delivered and the administration route. The most convenient way to administer drugs into the body is by oral administration. However, many drugs, in particular proteins and peptides, are poorly absorbed and unstable during passage through the gastrointestinal (G-I) tract. The administration of these drugs is generally performed through parenteral injection.

Although oral vaccination is more convenient, vaccines are generally given through injection. This is particularly true with killed or peptidic vaccines, because of their low absorbability and instability in the G-I tract. A problem with systemic immunization is that it may not effectively induce mucosal immune responses, particularly production of IgA, that are important as the first defense barrier to invaded microorganisms. For this reason, it would be beneficial to provide oral vaccination, if the problems of low absorbability and instability could be overcome.

Controlled release systems for drug delivery are often designed to administer drugs to specific areas of the body. In the gastrointestinal tract it is important that the drug not be eliminated before it has had a chance to exert a localized effect or to pass into the bloodstream.

Enteric coated formulations have been widely used for many years to protect drugs administered orally, as well as to delay release. Several microsphere formulations have been proposed as a means for oral drug delivery. For example, PCT/US90/06433 by Enzytech discloses the use of a hydrophobic protein, such as zein, to form microparticles; U.S. Pat. No. 4,976,968 to Steiner et al. discloses the use of "proteinoids" to form microparticles; and European Patent Application 0,333,523 by the UAB Research Foundation and Southern Research Institute discloses the use of synthetic polymers such as polylactic acid-glycolic acid to form microspheres.

Particles less than ten microns in diameter, such as the microparticles of EPA 0,333,523, can be taken up by cells in specialized areas, such as Peyer's patches and other intestinal mucosal lymphoid aggregates, located in the intestine, especially in the ileum, into the lymphatic circulation. Entrapping a drug or antigen in a microparticulate system can protect the drug or antigen from acidic and enzymatic degradation, yet still allow the drug or antigen to be administered orally, where they are taken up by the specialized uptake systems, and release the entrapped material in a sustained manner or are processed by phagocytic cells such as macrophages. When the entrapped material is a drug, elimination of the first-pass effect (metabolism by the liver) is highly advantageous.

2.2. Liposomes

Conventional liposomes have been proposed for use as an oral drug delivery system, for example, by Patel and Ryman, *FEBS Letters* 62(1), 60–63 (1976). Liposomes are typically less than 10 microns in diameter, and, if they were stable to passage through the G-I tract, may be absorbed through Peyer's patches (Aramaki, Y., H. Tomizawa, T. Hara, K. Yachi, H. Kikuchi, and S. Tsuchiya, 1993 Stability of liposomes in vitro and their uptake by rat Peyer's patches following oral administration. *Pharm. Res.* 10:1338, 1331; Childers, N., F. R. Donya, N. F. Magoo, and S. M. Michalek 1990. Ultrastructural study of liposome uptake by M cells of rat Peyer's patch: an oral vaccine system for delivery of purified antigen. *Regional Immunology* 3:8–16). Liposomes also have some features that should be advantageous for a particulate system for oral drug or antigen delivery. The phospholipid bilayer membrane of liposomes separates and protects entrapped materials in the inner aqueous core from the outside. Both water-soluble and -insoluble substances can be entrapped in different compartments, the aqueous core and bilayer membrane, respectively, of the same liposome. Chemical and physical interaction of these substances can be eliminated because the substances are in these different compartments. Further, liposomes are easy to prepare. However, liposomes are physically and chemically unstable, and rapidly leak entrapped material and degrade the vesicle structure. Without fortifying the liposomes, they are not good candidates for oral drug or antigen delivery. Thus, despite the early proposal for use of conventional liposomes in oral drug delivery, their use has still not been accepted.

Several methods have been tried to fortify liposomes. Some methods involve intercalating cholesterol into the bilayer membrane or generating the liposomes using phospholipids with high melting temperature or physically stabilizing preformed liposomes with excipients such as simple sugars or polysaccharides. Generally, these methods are not believed to be sufficient in making liposomes for oral delivery since during oral delivery liposomes are exposed to an acidic Ph in the stomach and bile salts and phospholipases in the intestine. These conditions typically dissolve the characteristic liposomal bilayer membrane and contents are released and degraded.

2.3. Polymerized Liposomes

Polymerization of liposomes has been shown in vitro to be an effective means of stabilizing the liposomes and