

ments, for example, cancer chemotherapy, may be ultimately limited by the toxicity of drugs to normal tissues. One possible approach to increasing the effectiveness and decreasing the side effects of a drug is to use methods that alter their distribution in the body, such that drug concentrations are increased in target tissue and decreased in normal tissues. Liposomes can affect the therapeutic index by avoiding nontarget tissues that are normally sensitive to the drug. The application of liposome technology as a drug carrier and delivery system involves the targeting of liposomal drugs to specific sites.³ Highly effective targeting of liposomes may be achieved through ligands coupled to the liposome surface.

Hepatocellular carcinoma is one of the major cancers in Taiwan.⁴ Targeting of drugs or therapeutic agents to hepatocytes may efficiently deliver these agents to improve the effectiveness of treatment. The asialoglycoprotein receptor (ASGP-R) has been shown to be present on the plasma membrane of liver parenchyma cells.⁵⁻⁸ The receptor displays high affinity and avidity for its ligands. Novel approaches have been designed to enhance the hepatocyte-specific delivery of liposome-associated substances to the receptors,⁹ leading to preferential hepatocyte uptake or delivery of entrapped drugs or genes. Liposomes carrying asialoglycoproteins can be specifically directed to parenchyma cells, and may facilitate hepatic uptake of liposomally entrapped materials. Our previous study demonstrated that asialofetuin-labeled liposomes (AFLP) significantly improved the intracellular delivery of inulin into hepatoma cells *in vitro*.¹⁰ The purpose of this study was to evaluate the targeting efficiency of AFLP *in vivo*.

The physical characteristics of liposomal vesicles play an important role in the biological fate of liposomes. A study in rats by intravenous (i.v.) injection of differently charged intermediate unilamellar liposomes (IUV) showed that the plasma elimination profile of negatively charged liposomes differed from those of neutral and positively charged liposomes.¹¹ In the present study, asialofetuin was grafted to negatively charged and neutral liposomes, and their tissue distributions were evaluated *in vivo*.

MATERIALS AND METHODS

Materials

Egg yolk phosphatidylcholine (PC), phosphatidic acid (PA), cholesterol (Chol), N-palmitoyl-oxy-succinimide (NPSI), and asialofetuin (AF) were purchased from Sigma (St. Louis, MO, USA). Chloroform was from E. Merck (Darmstadt, Germany). Peanut agglutinin (PNA) was from Vector (Burlingame, CA, USA). ³H-inulin, ¹⁴C-cholesterol and aquasol-2 were from New England Nuclear (Boston, MA, USA). Tris (hydroxymethyl)-aminomethane (Tris) was from Gibco BRL (Rockville, MD, USA). All chemicals used were reagent grade.

Preparation of Liposomes

The fundamental lipid compositions of liposomes (noncharged, (o)LP) were PC/Chol in a 7:3 molar ratio. For anionic liposomes, (-)LP, 2 parts of PC were replaced by PA. Plain liposomes were prepared by the mechanical dispersion method as previously described.¹² Briefly, a chloroform solution of lipid mixture (total lipids 180 μ mol) containing a trace of ¹⁴C-cholesterol was evaporated at reduced pressure under N₂ gas in a rotary evaporator to form a thin lipid film. Four milliliters of Tris-HCL buffer (10 mM, pH 7.4) was added to the dried lipid film and then shaken on a vortex mixer to produce multi-lamellar vesicles (MLVs). Palmitoyl-AF was introduced into the liposome membrane by the detergent-dialysis method as described previously.^{12,13} Briefly, 10 mg of AF was dissolved in 2 ml Tris-HCl buffer (10 mM, pH 7.4) containing NPSI 50 μ g/ml and 2% sodium deoxycholate (DOC), and placed in a shaking incubator (200 rpm) at 37 °C for 12 h to prepare palmitoyl-AF; the palmitoyl moiety served as a hydrophobic anchor. The palmitoyl-AF solution was added to the MLV suspension. The mixture was vortexed for 5 min, and then set at 4 °C for 6 h to allow it to reach equilibrium. DOC was removed by dialysis against Tris-HCl buffer at 4 °C for 48 h. The liposomes were warmed to 37 °C for 10 min and extruded through a 0.2- μ m-pore polycarbonate membrane, and freeze-dried. ³H-inulin was entrapped in liposomes by a freeze-thaw method as follows. The freeze-dried liposomes were mixed vig-