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DRUG DELIVERY USING ANTIBODY-LIPOSOME CONJUGATES

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ABSTRACT

Liposomes bearing surface-attached antibody (L-Ab) were prepared to deliver dideoxyinosine triphosphate (ddITP) to human monocyte/macrophages. A mouse monoclonal antibody (IgG_{2a}) was modified using succinimidyl pyridyl dithiopropionate (SPDP) as a heterobifunctional reagent in order to conjugate the Ab to liposomes through a covalent (thioether) bond. SPDP-modified antibody was incubated with liposomes containing 5 mol% of maleimido phenyl butyrate phosphatidyl ethanolamine (MPB-PE) at room temperature for 8 hr. L-Ab was separated from free and aggregated antibodies by density gradient technique using metrizamide. Uptake of L-Ab by human monocyte/ macrophages was measured as a function of time and compared to liposomes prepared with and without MPB-PE and free ddITP. The uptake increased with time with all samples except for free ddITP after 4 hr. This could be explained by dephosphorylation of ddITP. Uptake of MPB-PE liposomes was 2.7 times higher than those without MPB-PE after 48 hr. However, the uptake of L-Ab was 7 times higher than MPB-PE liposomes, 19 times higher than liposomes without MPB-PE, and 80 times higher than free ddITP after 48 hr. It can be concluded that the delivery of ddITP can be increased by surface attached antibody.

INTRODUCTION

Targeted liposome transport to predefined cells and tissues is one of the main goals in liposome drug delivery. Liposomes containing chemotherapeutic

agents provide the possibility of specific drug delivery and selective cytotoxicity[1]. The conjugation of cell-specific antibodies to liposomes containing chemotherapeutic agents has been shown to increase cytotoxicity selectively in several murine tumor cell lines[2,3]. However, there are only a few reports in the literature examining the application of liposome-bearing antibodies to human cell lines[4]. Closer consideration of drug transport, metabolism and action reveals that liposome dependency is important for targeted drug delivery in general.

Dideoxynucleosides have shown the ability to suppress the replication of human immunodeficiency virus (HIV) which causes the acquired immunodeficiency syndrome (AIDS) by inhibiting the reverse transcriptase of viral RNA. Among the dideoxynucleosides, dideoxyinosine (ddI) has shown significant activity following oral administration. The activity is shown by increased CD4 cell count and reduced p24 antigen[5]. However, ddI therapy can be associated with severe toxicities including peripheral neuropathy, hepatitis and life threatening pancreatitis[6].

The active metabolite of ddI dideoxyinosine triphosphate (ddITP), is unstable in the blood. Encapsulation of ddITP into liposomes can protect the drug from degradation. The amount of ddITP that can be transported to HIV infected cells may be greatly increased if the antibody is bound to liposomes into which the drug is encapsulated instead of it being linked directly to ddITP. This is because liposomes can contain many more drug molecules than can be bound to an antibody molecule without interfering with its immunological reactivity. ddITP does not need any chemical manipulation for liposomal encapsulation and is protected by the liposomal membrane during transit. Furthermore, the altered pharmacokinetic and metabolic properties of ddITP encapsulated in liposomes may be therapeutically advantageous.

EXPERIMENTAL

MATERIALS

Egg phosphatidylcholine (EPC), bovine phosphatidylserine (PS), and maleimido phenyl butyrate phosphatidylethanolamine (MPB-PE) were obtained from Avanti polar lipids, Birmingham, AL. Cholesterol (CHOL), dideoxyinosine triphosphate (ddITP), dithiothreitol (DTT) and Protein A-sepharose were purchased from Sigma Chemical Co., St. Louis, MO. ^{3}H-ddITP was purchased from Moravek Biochemicals (Brea, CA). ^{14}C-Cholesteryl oleate was obtained from Amersham (Arlington Heights, IL). Succinimidyl pyridyl dithio propionate (SPDP) was purchased from Pierce (Rockford, IL). Glass triple distilled water was used in the preparation of all aqueous solutions. All other chemicals and solvents were reagent grade.

METHODS

Preparation and characterization of liposomes: Large, unilamellar liposomes containing ddITP were prepared by modification of the method of Cullis et al[7,8] as follows. Lipids were mixed in chloroform at molar ratios of EPC : PS : Chol : MPB-PE : [^{14}C]Chol oleate (32 : 18 : 45 : 5 : 10^{-5}). 0.125 μCi [^{14}C]Chol oleate was used as a nonexchangeable marker. The lipids were deposited on the walls of glass vials by evaporating the solvent using a nitrogen stream. The vials were stored under vacuum overnight. Liposomes were formed by adding 1 ml of 40 mM ddITP + 1μCi [^{3}H]ddITP to the dry lipid film containing 200 μmoles lipid and vortex mixing until all the lipids were dispersed in the phosphate buffer (approx. 10 minutes). The dispersion was subjected to five freeze-thaw cycles in liquid nitrogen and warm water, and then to eight cycles of pressurized extrusion (at 500-600 psi) through 0.4 μm pore size polycarbonate filters (Nucleopore, Pleasanton, CA). The latter process was done in an

extruder™ (Lipex Biomembranes Inc., Vancouver, B.C. Canada). Unencapsulated solute was removed by four sequential cycles of centrifugal gel filtration[9]. This method provided >93% recovery of lipids with <3% free drug.

Antibody: Mouse monoclonal IgG_{2a} antibody (H-2-K^k) was prepared and purified as described by Heath et al[10]. Briefly, 2.0 ml of ascites diluted with 2.0 ml of phosphate buffered saline (PBS, pH 7.4) was applied to a protein A column equilibrated with PBS (pH 7.4). The column was then incubated at room temperature for 30 minutes and washed with PBS (pH 7.4) until the peak at 280 nm returned to baseline. Bound protein was eluted with 0.1 M sodium citrate/citric acid buffered saline (pH 4.0). Fractions were collected in 0.5 ml of 1 M Tris-HCl buffer (pH 9.0). The fractions eluted with sodium citrate/citric acid buffer (pH 4.0) were dialyzed against PBS (pH 7.4) overnight in cold room. The purified antibody was stored in PBS at -20° C.

Modification of antibody by SPDP: To 1 ml HEPES-buffered saline containing 1 mg antibody in a glass tube was added 1.6 µl of 20 mM SPDP. This gives a molar ratio of 5 mol SPDP per mole of mouse IgG. The reaction mixture was incubated for 30 minutes at room temperature. Unreacted SPDP was separated from labeled antibody by column chromatography using a Sephadex G 25 column equilibrated with acetate buffer (pH 4.5). Fractions were followed by UV spectrophotometer. The SPDP was reduced to the thiol form by addition of DTT to a final concentration of 50 mM. After 20 minutes at room temperature, the antibody was separated from DTT by passage on a Sephadex G 25 column pre-equilibrated with 10 mM HEPES-buffered saline, pH 8.0.

Conjugation of modified antibody to liposomes: The antibody (100 µg/ml) modified with SPDP was mixed with 50 µl of liposomes and incubated at room temperature for 24 hours with constant stirring.

Separation of liposomes from noncoupled antibodies: The metrizamide density gradient was used to separate L-Ab from unreacted antibodies. Briefly, 2.5 ml of 40% w/v solution of metrizamide was placed over the reaction mixture in a centrifuge tube followed by 1.5 ml of 20% metrizamide solution and 0.5 ml of PBS on top. The gradient was centrifuged for 16 hours at 143, 000 x g at 5° C.

Cellular Uptake: Monocyte/macrophages were incubated with antibody-bearing liposomes or plain liposomes in 6-well Costar plates (5×10^6 cells/well). Uptake of plain liposomes and antibody-bearing liposomes was estimated by counting cell-associated ddITP radioactivity after washing the cells 4 times with ice-cold saline.

RESULTS AND DISCUSSION

The results of cellular uptake of ddITP are shown in Fig. 1. It was found that uptake of ddITP by monocyte/macrophages increased with time for all samples except free ddITP after 4 hr. The lack of increase of ddITP uptake after 4 hr. may be due to dephosphorylation of the attached phosphate groups. Delivery of ddITP was enhanced by encapsulation into all three types of liposomes. This enhancement of uptake is expected because of the natural tendency of monocyte/macrophages to phagocytize liposomes. The uptake of MPB-PE liposomes was 2.7 times higher than those without MPB-PE after 48 hr. This may be due to binding of unreacted groups of MPB-PE on liposomes to cellular membrane proteins. The conjugation of monoclonal antibodies to liposomes increased the uptake of L-Ab by 7 times compared to MPB-PE liposomes, 19 times compared to liposomes without MPB-PE and 80 times compared to free ddITP after 48 hr. This increase in uptake is consistent with the suggestion that uptake is mediated by Fc-receptors on the cell surface[11]. In our

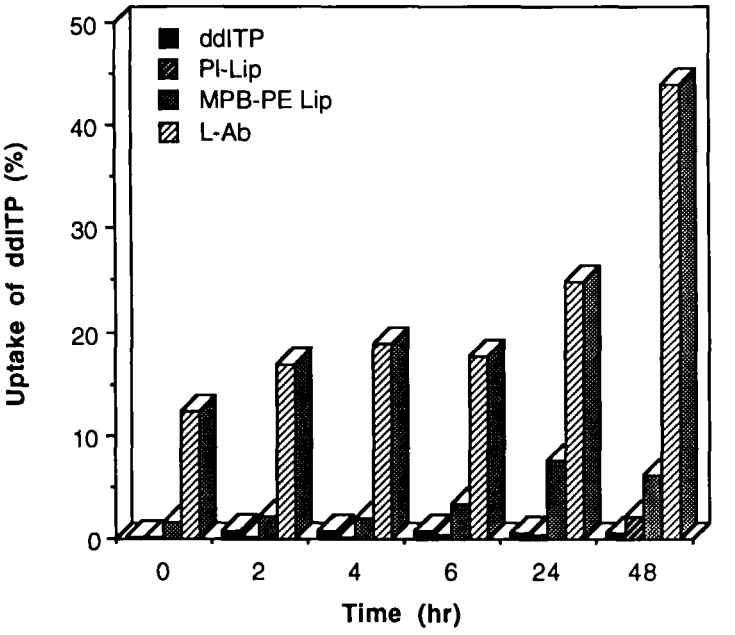

FIGURE 1

Cellular uptake of free ddITP, and ddITP from plain liposomes, MPB-PE liposomes and antibody-bearing liposomes.

previous report, we have shown that antibody-bearing liposomes increased the delivery of ddCTP to human monocyte/macrophages[12]. However, the extent of delivery was less than that noted in this study. This may be due to the difference in the antibody (16-1-11N) employed in this study or the extent of antibody conjugation to liposomes.

Early investigations of the interaction of liposomes with a variety of phagocytic cells indicated that the majority of liposomes that become cell-associated are internalized by the cells[13,14]. The extent of liposome binding and subsequent ingestion by macrophages is greatly influenced by the type of liposome employed (Multilamellar versus unilamellar). However, the success of antibody-liposome mediated targeting depends on efficient, stable and

irreversible conjugation of the antibody with the liposome. It would appear, therefore, that the most direct approach towards targeting liposomes to macrophages would be to use specific anti-macrophage antibodies.

The results of this study suggest that encapsulation of ddITP into liposomes stabilizes ddITP. It can be concluded that the delivery of ddITP to monocyte/macrophages can be increased by surface attached antibody. This enhancement of delivery of ddITP may have great clinical significance in the treatment of AIDS.

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