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Liposomes as carriers of the antiretroviral agent dideoxycytidine-5′-triphosphate

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Abstract

The presence and replication of the human immunodeficiency virus (HIV) in cells of the mononuclear phagocyte system (MPS) together with the preferential uptake of liposomes in macrophages suggest that liposomes can become a valuable carrier of anti-HIV agents. Moreover, liposomes reduce toxicity of encapsulated drugs and protect encapsulated drugs against rapid degradation in the blood circulation. To overcome problems associated with the administration of free nucleosides and to improve targeting to the MPS, dideoxycytidine-5′-triphosphate (ddCTP) was encapsulated in liposomes. Liposomes were stable with regard to retention of the entrapped drug, particle size and chemical stability of ddCTP. Results obtained with liposome encapsulated ddCTP in the murine acquired immunodeficiency syndrome (MAIDS) model indicate that ddCTP encapsulated in liposomes can reduce proviral DNA in cells of the mononuclear phagocyte system (MPS) in both spleen and bone marrow.

Keywords: Liposomes; HIV; ddCTP; MAIDS

1. Introduction

Several antiretroviral nucleoside analogues have been developed for the treatment of patients suffering from the acquired immunodeficiency syndrome (AIDS). These antiviral agents are able to combat replication of the human immunodeficiency virus (HIV) by inhibiting reverse transcriptase and, thereby, viral DNA synthesis. A major limitation associated with the clinical use of these antiviral agents is their dose-limiting toxicity. The entrapment of the drugs into particulate carrier

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systems, such as liposomes, represents a potential approach for overcoming the toxicity of nucleoside analogues (Philips, 1992). Moreover, the preferential uptake of particulate carriers by cells of the mononuclear phagocyte system (MPS) (Senior, 1987) makes such carriers particularly attractive to control the progression of AIDS since macrophages play a central role in AIDS pathogenesis by acting as reservoirs and propagators of HIV throughout the immune system (Embretson et al., 1993; Pantaleo et al., 1993)

To be pharmacologically useful, dideoxynucleosides, such as dideoxycytidine (ddC) must be phosphorylated into 5′-triphosphates by cellular kinases (Mitsuya et al., 1990, 1991). As some cell types have a low ability to phosphorylate these compounds (Balzarini et al., 1988), administration of the phosphorylated form, i.e. the nucleotide would be most appropriate. However, administration of the nucleotides is not feasible as cell membranes are impermeable to the phosphorylated form and phosphatases present in body fluids rapidly hydrolyse the nucleotides into the corresponding nucleosides (Cohen, 1975; Rossi et al., 1993; Magnani et al., 1994). In an attempt to overcome the problems of nucleoside toxicity, phosphorylation inefficiency, and instability in the biological environment ddC was encapsulated in the active phosphorylated form (dideoxycytidine-5′-triphosphate, ddCTP) in liposomes. An additional reason to encapsulate ddCTP instead of ddC is based on the observation made by Szebeni et al. (1990) that ddC is not effectively retained by liposomes.

In the present paper results on the preparation of a stable liposome formulation containing ddCTP for the therapy of retroviral infection are presented. To this end the stability of ddCTP in aqueous solutions was studied and a method for the analysis for ddCTP in liposomes was developed. Liposomes containing ddCTP were prepared and characterised regarding encapsulation efficiency, chemical stability, retention of the entrapped drug during storage and size distribution. The antiretroviral activity of liposomal ddCTP was studied in a murine AIDS (MAIDS) model which shows pathological similarity with HIV-induced AIDS. In particular the effect on cells of the mononuclear phagocyte system (MPS) was evaluated.

2. Materials and methods

2.1. Chemicals

Egg-phosphatidylcholine (EPC) and egg-phosphatidylglycerol (EPG) were a gift from Lipoid GmbH (Ludwigshafen, FRG). Cholesterol (Chol) was obtained from Sigma (St. Louis, MO). 2′,3′-Dideoxycytidine 5′-triphosphate (lithium salt) (ddCTP) was obtained from Boehringer Mannheim GmbH (Mannheim, FRG). All other reagents were of analytical grade. Demineralised water was used throughout the study.

2.2. Stability experiments

The chemical stability of ddCTP was studied in the following aqueous media: (a) 0.01 M $HClO_4$ pH 2; (b) Hepes/NaCl buffer pH 7.4, consisting of 10 mM Hepes, 155 mM NaCl, 0.01 mM EDTA; (c) phosphate buffered saline (PBS) pH 7.4, consisting of 3.6 mM KH_2PO_4, 6.3 mM Na_2HPO_4, 155 mM NaCl, 0.01 mM EDTA and d) 0.01 M NaOH pH 12.

Free ddCTP in aqueous media and ddCTP encapsulated in liposomes was filled into ampoules and incubated at a constant temperature of 4 or 37°C. After appropriate time intervals samples were taken. The free ddCTP samples were immediately stored at −20°C until high performance liquid chromatography (HPLC) analysis. The ddCTP-liposome samples were immediately ultracentrifuged (30 min, $155\,000 \times g$), the pellet was reconstituted in an appropriate amount of water and stored at −20°C until further analysis.

2.3. Preparation and characterisation of ddCTP-containing liposomes

Negatively charged liposomes composed of EPC:EPG:Chol (10:1:4, molar ratio) were prepared by the thin film-extrusion method (Olson et al., 1979). In previous studies this lipid composition has been shown to be stable in the intraperi-

toneal cavity against rapid loss of encapsulated hydrophilic markers (Nässander, 1991; Nässander et al., 1992). Lipids were dissolved in a mixture of chloroform/methanol (4:1 v/v) and evaporated to dryness by rotation under reduced pressure at 35°C. The film was hydrated in sterile Hepes/NaCl-buffer pH 7.4 at a ratio of 17 μg ddCTP per μmol total lipid (TL) at a concentration of 120 mM TL. The resulting liposome dispersion was extruded five times through a 0.4 μm polycarbonate membrane filter (Nuclepore; Costar, Cambridge, MA) under nitrogen pressure. Removal of non-entrapped ddCTP and concentration of liposomes was performed by ultracentrifugation (45 min at $225\,000 \times g$ at 4°C, Beckman L70; Beckman Instrument, Palo Alto, CA) and subsequent removal of the supernatant. The pelleted liposomes were redispersed in sterile Hepes/NaCl buffer pH 7.4. Liposome-encapsulated ddCTP was determined after disrupting 10 μl of liposome dispersion with 30 μl methanol. After adding 260 μl of a 1 M KH_2PO_4 solution pH 7.0, 20 μl of the diluted sample was directly injected into the HPLC system.

Phosphate concentration in the final liposome dispersion was determined by the colorimetric method of Rouser et al. (1970). The final lipid phosphate concentration was calculated by correcting for phosphate originating from ddCTP. Mean particle size was determined by dynamic light scattering at 25°C with a Malvern 4700 system using a 25 mW He–Ne laser (NEC, Tokyo, Japan) and the automeasure version 3.2 software (Malvern Ltd., Malvern, UK). As a measure of particle size distribution the system reports a polydispersity index (pd). This index ranges from 0.0 for an entirely homogeneous up to 1.0 for a complete heterogeneous size distribution.

2.4. HPLC system

The HPLC system for the measurement of ddCTP consisted of a solvent delivery system (M6000, Waters Ass., Milford, MA), a Rheodyne injector unit, an absorbance detector (Model 440, Waters Ass.) operating at 254 nm and a microscribe recorder (Houston Instruments, USA). A Lichrosphere 100 RP-18 (5 μm) column (Merck, Darmstadt, FRG) was eluted at room temperature with a mobile phase, consisting of an aqueous solution of 10% methanol in 50 mM KH_2PO_4 and 5 mM tetrabutylammoniumbromide (TBABr) and adjusted to pH 7.0 with 2.0 M NaOH. The flow rate was 1.0 ml/min. Samples (20 μl) were injected into the system.

2.5. Animals and virus

C57BL/6 female mice were bred at Nossan (Milan, Italy). The animals were housed at about 22°C, with 12 h light/dark cycle, about 60% humidity and 12 air changes/h and had free access to water and standard laboratory chow. At the start of the experiment the animals were 4 weeks of age. LP-BM5 virus was kindly provided by Dr R. Yetter (Veterans Administration Hospital, Baltimore, MD) and was maintained in a persistently infected SC-I cell line, as described earlier (Mosier et al., 1987).

Mice were infected with two single i.p. injections of the virus stock containing 6×10^6 cpm of reverse transcriptase, on day 1 and day 2 prior to treatment. Treatment of the mice (ten animals in each group) was started 24 h after the last infection with a single i.p. injection of empty liposomes (70 μl containing 85–110 μmol TL/ml) or liposomes containing encapsulated ddCTP at a dose of 32 μg ddCTP in a volume of 70 μl (85–110 μmol TL/ml). Injections were repeated once every 10 days for a period of 3 months. This dose and schedule were selected on the basis of previous results obtained with ddCTP erythrocytes (Magnani et al., 1992). After 45 days of treatment a blood sample was taken from each mouse in order to determine plasma hypergammaglobulinemia. Three months after the start of the treatment the animals were sacrificed and blood, bone marrow, lymph nodes (mediastinal, brachial, lumbar, mesenteric and inguinal) and spleen were collected.

2.6. Determination of antiretroviral activity

Total cellular DNA was isolated from lymph nodes, bone marrow and spleen by lysis with 8 M urea, 0.3 M NaCl and 10 mM Tris–HCl pH 7.5

for 60 min at 37°C. Extraction was performed with phenol–chloroform–isoamyl alcohol (25:24:1) and then with chloroformisoamyl alcohol (24:1). The DNAs were precipitated with ethanol and stored at −20°C until use in the polymerase chain reaction (PCR).

Probe D30, which was derived from the 137-bp *Smal-Xhol p12 gag* fragment of the defective virus genome (DUSH) (Aziz et al., 1989) was kindly provided by Dr P. Jolicoeur (Montreal, Canada). The probe G6PD represented the 203-bp of the murine genome. The glucose-6-phosphate dehydrogenase (G6PD) gene probe was obtained by PCR using the following oligonucleotide primers: 5′primer, 5′-TGTTCTTCAACCCCGAGGAGT-3′ (sense) and 3′primer, 5′-AAGACGTCCAGGATGAGGTGATC-3′ (antisense) (Kurdi-Haidar et al., 1990).

PCR analysis of LP-BM5 proviral DNA was performed with the following oligonucleotide primers used for the amplification of defective virus genome (BM5d): 5′primer, 5′-AACCTTCCTCCTCTGCCA-3′ (sense) corresponding to nucleotides 1456–1473 in the viral sequence and 3′primer, 5′-ACCACCTCTCGGGCTTTC-3′ (antisense) corresponding to nucleotides 1579–1596 of the BM5d genome (Chattopadhyay et al., 1991). A second pair of oligonucleotide primers was used for amplification of 203-bp of the mouse G6PD gene. This amplification served as an internal control (endogenous standard) for evaluation of relative BM5d integration in the mouse genome. The nucleotide primers for this amplification were as follows: 5′primer, 5′-TGTTCTTCAACCCCGAGGAGT-3′ (sense) and 3′primer, 5′-AAGACGTCCAGGATGAGGTGATC-3′ (antisense). PCR was performed in a Perkin-Elmer thermocycler (Perkin-Elmer, Norwalk, CT) in a 25 μl final volume containing 1 μg of genomic DNA, 50 mM KCl, 10 mM Tris–HCl, pH 8.3, 1 mM $MgCl_2$, 0.005% NP-40, 0.001% gelatine, 150 μM each of the four deoxyribonucleoside triphosphates, 20 pmol of each primer and 2.5 U of Replitherm DNA polymerase (Epicentre, UK). The reaction mixtures were subjected to 37 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 30 s followed by final extensions at 72°C for 10 min. The PCR products were analysed by electrophoresis on 2.5% agarose gel, transferred onto nylon membrane and hybridised with either the ^{32}P-labeled D30 or G6PD probe. Labeling of the DNA probes was achieved using the random primer DNA-labeling kit from Bio-Rad (Richmond, CA). Detection was with a ^{32}P-labeled D30 probe for BM5d or a specific G6PD probe. Values were obtained by laser scan densitometry of the autoradiografic film.

2.7. Determination of serum IgG

Serum IgG levels were determined by using an enzyme-linked immunosorbent assay (ELISA) technique. Briefly, polyvinylchloride microtitre plates (Sterilin, Hounslow, UK) were coated with serial dilutions of murine serum in 50 mM sodium borate, pH 8.5 and incubated for 48 h at 4°C. The plates were washed four times with 0.05% Tween-20 in phosphate buffered saline (TPBS) and blocked with 1.2% bovine serum albumin (BSA) in TPBS for 30 min at room temperature. After two washings in TPBS, 100 μl of goat anti-mouse IgG horseradish peroxidase (HRP)-conjugate (Bio-Rad) diluted 1:3000 in 0.2% BSA in TPBS, was added. After incubation for 3 h at room temperature and four washes, serum IgG was determined using a color development solution containing 2.2 mM *o*-phenylenediamine. Absorbance was measured at 492 nm on Model 2550 EIA READER (Bio-Rad). Absolute serum IgG concentrations were obtained using known concentrations of standard mouse IgG.

2.8. Hematological parameters

Phenotyping of peripheral blood lymphocytes was obtained by FITC-labeled monoclonal antibodies to CD4, CD8, Thy 1.2 and Ly5 and analysed by flow cytometer (FACScan; Becton-Dickinson, Rutherford, NJ. [^{3}H]Thymidine incorporation upon lymphocytes stimulation was performed as previously described (Rossi et al., 1992). DNA to be used in PCR amplifications was quantified with PicoGreen quantification (Molecular Probes Inc., Eugene, OR).

2.9. Statistics

The effect of the different treatments was compared by a two-tail Student's t-test assuming equal variances with 95% confidence interval. Differences were considered significant when the P-value was less than 0.05.

3. Results

3.1. Characteristics and stability of ddCTP-containing liposomes

An isocratic, reverse phase HPLC system was developed for the determination of ddCTP in liposomes as such a method has not yet been described in the literature. With the developed method, ddCTP was eluted from the column with a retention time of 4 min. Calibration curves in two areas of sensitivity were validated with 20 μl samples containing 1–8 μg ddCTP/ml for the higher and 15–60 μg ddCTP/ml for the lower area of sensitivity. These standard curves showed correlation coefficients of 0.999 ($n=4$) or higher for the higher area of sensitivity and 0.996 ($n=4$) or higher for the lower area of sensitivity. The y-intercepts did not significantly differ from 0 ($P=0.01$). Between-run reproducibility of the analytical method was demonstrated by the coefficient of variation for the slopes of the calibration lines being 1.7% ($n=4$) in the lower and 2.7% ($n=4$) in the higher area of sensitivity. The intraday and interday precisions were high in both areas of sensitivity as demonstrated by the coefficients of variation for repetitive injections which were equal to or lower than 1.5% ($n=5$).

Liposome-encapsulated ddCTP was determined after disrupting the liposomes with methanol. The sample was directly injected into the HPLC system. No difference in the chromatograms was observed between free ddCTP and ddCTP encapsulated in liposomes. The detection limit of ddCTP in liposome dispersions was 4.5 μg/ml.

To select a suitable hydration medium for the preparation of liposomes, experiments were performed to investigate the chemical stability of ddCTP at various pH values. For this purpose Hepes/NaCl buffer pH 7.4, PBS pH 7.4, NaOH solution pH 12 and $HClO_4$ solution pH 2 were used. Degradation was detected by decreasing peak heights of ddCTP and increasing peak heights of the degradation product. Degradation of ddCTP did not occur after 4 days of storage in either Hepes/NaC1 buffer pH 7.4, PBS pH 7.4 and NaOH solution pH 12 at 4 or 37°C. Moreover, degradation was negligible in Hepes/NaCl buffer pH 7.4 and PBS pH 7.4 when stored at 4°C for 40 days. Significant degradation of ddCTP was observed when stored in $HClO_4$ solution pH 2 at 37°C. The HPLC method turned out to be stability indicating. Degradation products of ddCTP were observed by two chromatographic peaks at 2 and 3 min retention time with nearly base-line resolution with the parent ddCTP peak

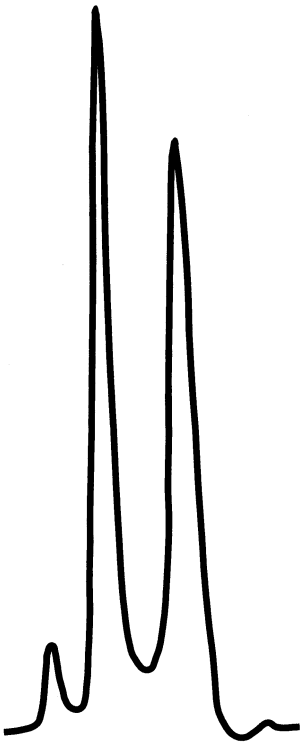

Fig. 1. High performance liquid chromatography (HPLC) analysis of dideoxycytidine-5′-triphosphate (ddCTP) stored in $HClO_4$ solution pH 2 at 37°C for 60 h. ddCTP was eluted from the column with a retention time of 4 min. Degradation products of ddCTP were observed at 2 and 3 min retention time.

(Fig. 1). The exact nature of these compounds is not known yet, however, it is conceivable that they result from a dephosphorilation process. The degradation followed pseudo-first order kinetics. The rate constant for this degradation, k_{obs} was calculated from the slope of the degradation line as 1.4×10^{-6}/s with a half-life of ddCTP of 138 h.

Encapsulation of ddCTP was obtained by hydrating the lipid film with ddCTP dissolved in Hepes/NaCl-buffer pH 7.4. The mean encapsulation efficiency in the final liposome dispersion was relatively high, about 26% which corresponds to 4.5 μg ddCTP/μmol TL. The mean particle size of the liposomes after extrusion was about 0.3 μm ($pd < 0.3$). Encapsulation of ddCTP into liposomes had no effect on the chemical stability of ddCTP. Chemical degradation was negligible after 4 days of storage at 37°C or upon 10 weeks of storage at 4°C both at pH 7.4. Physical stability of liposomes was investigated by studying leakage of the drug from the liposomes and determining size distribution. Leakage of the entrapped drug over 4 days at 37°C or 10 weeks of storage at 4°C was negligible. A 10-fold dilution of the dispersion with Hepes/NaCl buffer did not have a negative influence on the stability with respect to leakage. Changes in size distribution did not occur during storage of liposomes for 10 weeks at 4°C.

3.2. Therapeutic efficacy in the MAIDS model

Mice, infected with the LP-BM5 virus, were treated i.p. with multiple doses of 32 μg of ddCTP encapsulated in liposomes. The first injection was given 1 day after infection. Subsequent injections were given at 10-day intervals over a period of 3 months. The selected dose, route and frequency of administration were based on results previously obtained with ddCTP-loaded erythrocytes in the same animal model (Magnani et al., 1992). Two groups of ten mice served as controls: one group included mice which were non-infected and not treated, the other group included infected mice treated with empty liposomes with the same lipid dose and treatment schedule.

Proviral DNA content was determined in spleen, bone marrow and lymph nodes at the end of the 3 month treatment period by a semiquantitative PCR assay that selectively detects BM5d (the defective virus responsible for MAIDS) (Aziz et al., 1989; Chattopadhyay et al., 1989; Fraternale et al., 1996). As shown in Fig. 2, administration of ddCTP-liposomes reduces the proviral DNA load in both spleen and bone marrow (about 32 and 26%, respectively). Apparently, liposomes are able to deliver ddCTP in the active form to cells of the MPS in spleen and bone marrow. In contrast, proviral DNA reduction in lymph nodes (mediastinal, brachial, lumbar, mesenteric and inguinal) was negligible.

Activation and proliferation of B-cells in MAIDS results in hypergammaglobulinemia that peaks between 8 and 10 weeks post infection and then declines (Mosier et al., 1985). Hypergammaglobulinemia was evaluated early (45 days) and late (at the end of the 3 month treatment period). As shown in Fig. 3, ddCTP liposomes reduced hypergammaglobulinemia by 35% at 45 days (Fig. 3A). However, the late effect on hypergammaglobulinemia was negligible (Fig. 3B).

Several other blood parameters, such as red blood cells count, hematocrit, hemoglobin and counts of $CD4^+$, $CD8^+$, Thy 1.2^+ and $Ly5^+$ cells, were not affected by administration of ddCTP liposomes as compared to animals treated with empty liposomes. Additionally, administration of ddCTP encapsulated in liposomes had no significant effect on both lymphadenopathy and splenomegaly and on T- and B-cell response (results not shown).

Results obtained from infected mice treated with empty liposomes were similar to effects observed in previous experiments in non-treated infected mice (results not shown).

4. Discussion

The multifaceted immunopathology of HIV infection will likely preclude the majority, if not all, of the single therapeutic approaches that are currently being developed. In this study the option that liposome-based drug delivery can offer a

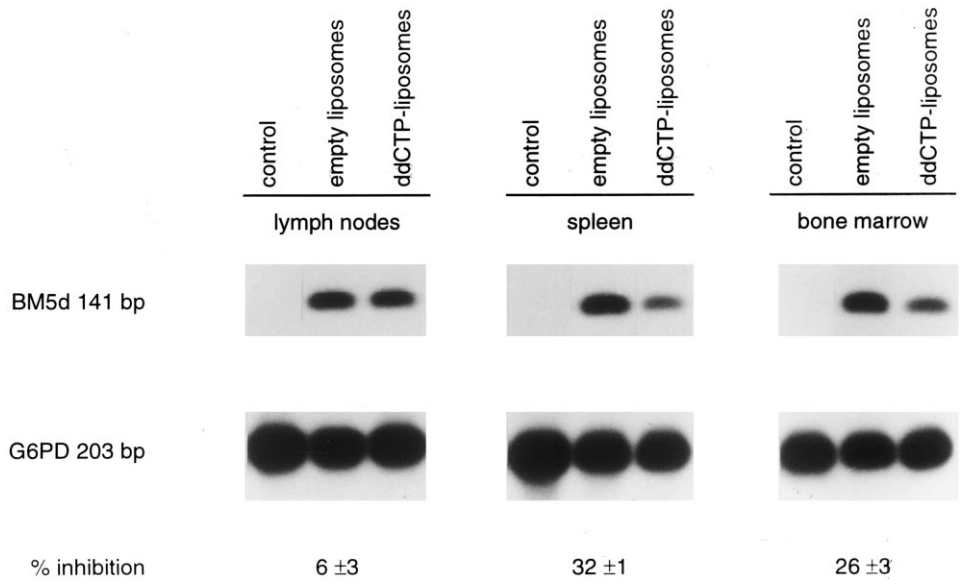

Fig. 2. Polymerase chain reaction (PCR) analysis of BM5d proviral DNA in spleen, lymph nodes and bone marrow of mice infected with LP-BMS and control (not infected, not treated) mice. Infected mice were treated with empty liposomes or liposome-encapsulated dideoxycytidine-5′-triphosphate (ddCTP) for 3 months. The results of the PCR analysis were corrected for the differing amounts of G6PD. Values represent the mean ± S.D. of ten animals.

valuable contribution to the therapeutic strategies currently followed in the treatment of HIV infection was investigated.

Cells of the MPS are important hosts for HIV and play a key role in the pathogenesis of AIDS by providing long term reservoirs for the virus (Gartner et al., 1986; Fauci, 1988; Meltzer et al., 1990; Embretson et al., 1993). As liposomes are preferentially taken up by cells of the MPS, their use as carriers of antiviral agents represents an interesting approach for targeting of antiviral agents to cells of the MPS. Enhanced uptake of ddC incorporated into liposomes as compared to the free drug by cells of the MPS in vitro has been demonstrated previously (Makabi-Panzu et al., 1994). This study evaluated the feasibility of using ddCTP containing liposomes to reduce proviral DNA in cells belonging to the MPS. First ddCTP-liposomes were prepared and characterised with regard to chemical and physical stability. Then the antiretroviral efficacy of these liposomes was evaluated in vivo after i.p. administration. A control experiment with free ddCTP was not performed as water-soluble drugs, such as ddCTP are generally rapidly absorbed into the bloodstream after i.p. administration and hydrolysed in body fluids (Cohen, 1975; Rossi et al., 1993; Magnani et al., 1994). Therefore, no substantial therapeutic effect is anticipated following ip administration of free, non-encapsulated ddCTP.

Liposomes prepared by the thin-film hydration method were found to be stable with regard to the chemical stability of ddCTP, leakage of ddCTP and size. The relatively high encapsulation efficiency of ddCTP (26%) is likely to be the result of the high lipid concentration during hydration (Talsma, 1991). The data derived from the stability experiments performed with free ddCTP indicate that Hepes/NaCl buffer pH 7.4, that is often used in the preparation of liposome dispersions, is also a suitable hydration medium for the preparation of ddCTP-containing liposomes.

The in vivo antiretroviral efficacy of ddCTP encapsulated in liposomes was evaluated in mice infected with the LP-BM5 murine leukemia virus. Infection of C57BL/6 mice with the LPBM5 retroviral complex causes an immunodeficiency syndrome known as MAIDS, resembling the im-

munodeficiency syndrome in humans infected with HIV (Jolicoeur, 1991). This animal model of AIDS has been used before in several preclinical studies of antiviral drugs (Gangemi et al., 1989; Ohnota et al., 1990; Basham et al., 1991; Hersh et al., 1991). Infection with the virus results in acute lymphoproliferative disease and profound immunosuppression, including lymphadenopathy, hypergammaglobulinemia and immunosuppression of cellular and humoral immunity (Mosier et al., 1985).

The amount of proviral DNA was selected as the main parameter of infectivity as the level of proviral BM5d increases with the progression of the disease (Pozsgay et al., 1993). The observed reduction of proviral DNA in spleen and bone marrow indicates that liposomes can selectively deliver ddCTP to cells of the MPS in vivo which are the main natural target cells for liposomes. Considering the fact that ddCTP encapsulated in liposomes did not affect the level of proviral DNA in lymph nodes, it should be taken into account that transfer of circulating liposomes from the blood to the lymphatic system is limited, therefore no effect of the i.p. administered ddCTP-containing liposomes in lymph nodes not involved in drainage of the peritoneal cavity should be anticipated. Additionally, other important target cells of crucial importance in the HIV pathogenesis, like $CD4^+$ T-lymphocytes, are not reached efficiently by the liposomes. As infection of T-cells is the process that primarily leads to T-cell destruction in AIDS, effects on these cells are required. Positive effects of ddCTP-containing liposomes towards $CD4^+$ T-lymphocytes, might be accomplished by selective targeting of surface modified liposomes. Monoclonal antibody directed against the $CD4^+$ receptor of the target cells can be attached to the liposomal surface (immunoliposomes) (Debs et al., 1987; Peeters et al., 1987; Crommelin et al., 1992; Vingerhoeds et al., 1994) and direct the liposomal carrier specifically to the $CD4^+$ target cell. Additionally, attaching poly(ethyleneglycol) chains to the surface of such immunoliposomes is known to increase circulation time of immunoliposomes in vivo, resulting in improved target binding of the immunoliposomes (Torchilin et al., 1992; Blume et al., 1993; Allen, 1994)

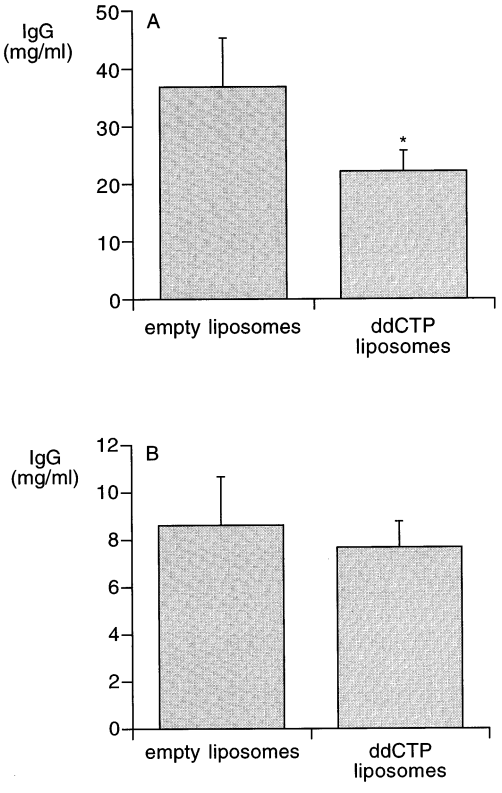

Fig. 3. Inhibition of hypergammaglobulinemia by liposome-encapsulated dideoxycytidine-5′-triphosphate (ddCTP) in mice infected with LP-BMS and control mice (not infected, not treated). Infected mice were treated with empty liposomes or liposome-encapsulated ddCTP for 3 months. (A) Serum IgG levels 45 days after infection. (B) Serum IgG levels 3 months after infection. Values represent the mean $\pm$ S.D. of ten animals. Note the differences in the y-axis scale. * $P = 0.05$.

In conclusion, ddCTP was formulated in stable liposomes containing ddCTP. The findings in the present paper indicate that ddCTP encapsulated in liposomes can reduce proviral DNA in cells of the MPS in both spleen and bone marrow in the MAIDS model. Future experiments will focus on a full evaluation of liposomal ddCTP in the MAIDS model, including assessment of dose-response relationship. In addition, the use of surface-modified liposomes with a homing device directed against the $CD4^+$ receptor on HIV host cells other than cells of the MPS might be considered.

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