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Research Article

Differential Disposition of Soluble and Liposome-Formulated Human Recombinant Interleukin-7: Effects on Blood Lymphocyte Population in Guinea Pigs

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The effects of liposome formulation on interleukin-7 (IL-7)-dependent lymphopoietic activity was investigated based on the pharmacokinetics and tissue distribution profile of soluble and liposome-formulated recombinant human IL-7. Using ^{125}I-IL-7, we determined the role of liposome formulation on *in vivo* IL-7 disposition by analyzing injection site, blood, tissue, and urinary kinetics. Following a 30- to 40-μg subcutaneous dose of soluble IL-7, most of the IL-7 was eliminated through urinary excretion within 24 hr. An equivalent subcutaneous dose of liposome-encapsulated IL-7 resulted in a peak level less than one-tenth that seen with soluble drug but produced sustained blood and urinary levels for 5 days. The bioavailability of liposome-encapsulated IL-7 was comparable to that of soluble IL-7, as determined by both blood and urinary data. Kinetic analysis of IL-7 at the subcutaneous injection site indicated that liposome encapsulation significantly reduced the rate of disappearance at the injection site. Studies with a mixture of 40% liposome-encapsulated and 60% soluble IL-7 gave an intermediate response between that of soluble IL-7 and that of liposome-encapsulated IL-7. Characterization of blood cells from IL-7-treated animals indicated that treatment with two weekly doses of mixed IL-7 liposomes (40% liposome encapsulated IL-7) significantly increased the total numbers of lymphocytes by day 14. In contrast, animals treated with soluble IL-7 on an identical dose and schedule did not produce any effect on blood lymphocytes. Collectively, liposome formulation provided a lower, but significantly sustained blood IL-7 level that enhanced IL-7 effects on blood lymphocyte numbers.

KEY WORDS: cytokine; interleukin-7; sustained release; liposome; lymphopoietic; pharmacokinetics; drug delivery.

INTRODUCTION

Although many cytokines have been genetically cloned and expressed, only a few are used clinically. This is due partly to their relatively short half-life, which requires either continuous infusion or frequent injections to produce a therapeutic effect (for a review, see ref. 1). Rapid clearance is most pronounced in those cytokines with a molecular weight less than 30–40 kd, due to the rapid renal excretion rate exhibited by the small macromolecules. Therefore, a sustained-release formulation of these cytokines that prolongs concentrations in blood while retaining biologic activity *in vivo* will add significantly to the potential utility of the proteins as therapeutic agents.

Interleukin-7 (IL-7), a 25-kd cytokine originally derived from the stromal element of a bone marrow cell line, has been shown to stimulate B-cell progenitors (2,3). Recently, murine and human IL-7 have been cloned, and a recombinant product of IL-7 is now available for *in vivo* studies. Human IL-7 (exhibiting 60% homology to the murine form) can stimulate both human and murine bone marrow cells (4). In addition, results from *in vitro* experiments indicate that IL-7 can also stimulate the growth of mature T and B cells (3–13).

Administration of murine IL-7 to normal mice increases mainly precursor and mature B lymphocytes, while having a modest effect on T lymphocytes in the spleen and lymph nodes (14,15). Recombinant human IL-7 (henceforth referred to as IL-7) has also been shown to accelerate the recovery of either cyclophosphamide-treated or sublethally irradiated immune-suppressed mice (15–17). Recently, we observed that IL-7 adjuvant activities enhance guinea pig immune responses against herpes simplex virus (HSV) infection using a recombinant HSV envelope protein gD in a vaccination protocol (18).

The present studies were undertaken to characterize the differences in pharmacokinetic and tissue disposition of soluble and liposome formulated IL-7. In addition, we examined whether the use of liposome-formulated IL-7 as a sus-

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tained-release formulation to reduce the frequency of IL-7 administration would achieve hematologic and lymphocytic effects comparable to those obtained with soluble IL-7. Our results show that subcutaneously administered liposome-formulated IL-7 exhibits a sustained release rate with a lower peak blood level than the soluble form and, therefore, significantly reduces the rate of urinary IL-7 excretion. The results of lymphopoietic studies suggest that liposome-formulated IL-7 may enable a more practical frequency of weekly dosing intervals for treating patients with leukopenia or lymphopenia.

MATERIALS AND METHODS

Materials

Highly purified human recombinant IL-7 was provided by the Sterling Winthrop Pharmaceuticals Research Division (Collegeville, PA) and was produced in *Escherichia coli* and purified by Immunex Corporation (Seattle, WA). The purified IL-7 (lot Nos. 2116-5-4 and 2664-6-1) contained 3.4×10^7 and 4×10^7 U/mg, respectively; endotoxin contamination was less than 1 U/mg protein. All other reagents were of analytical grade.

Radiolabeling IL-7 with PIB Reagent

IL-7 was labeled at ϵ amino groups using a two-step iodination procedure as described previously (19) to obtain radioiodinated protein with a significantly reduced rate of *in vivo* deiodination [$t_{1/2} \geq 8$ days (19)]. Briefly, *N*-succinimidyl 4-tri-*n*-butyl-stannylbenzoate (3×10^{-2} mmol) was mixed with 100 μCi of $Na^{125}I$ (NEN), and the iodination reaction was initiated with 60 μL of the *N*-chlorosuccinimide solution. After 10 min at room temperature, the reaction was stopped with the addition of $NaHSO_3$. The mixture was dried by evaporation, and 500 μL (0.92 mg) of IL-7 was added in phosphate-buffered saline (PBS), supplemented with 50 m*M* $NaHCO_3$, pH 9.0. The conjugation reaction was allowed to proceed for 10 min at room temperature to label ϵ amino groups of the protein. The labeled ^{125}I-IL-7 was purified using Sephadex G-25 spun-column chromatography. The protein concentration of purified ^{125}I-IL-7 was determined by Lowry protein assay (20). Biologic activity of ^{125}I-IL-7 was determined to be 3.2×10^7 U/mg, compared with 3.4×10^7 U/mg (for the stock IL-7), indicating that iodination of IL-7 using this procedure did not modify the biologic activity. Using this procedure, we obtained specific radioactivity of IL-7 ranging from 4 to 10×10^5 cpm/μg protein.

IL-7 Liposome Preparation

Detailed procedures for liposome preparation have been described (21). Briefly, 1 mg of IL-7 was mixed with 7.2 mg of sonicated unilamellar liposome suspension containing egg phosphatidylcholine and cholesterol (8:2, w/w) in a 1.5-mL final volume. The mixture was frozen in an ethanol–dry ice bath, lyophilized, and stored in lyophilized form at 4°C. Immediately before use, 0.5 mL of USP water for injection was added to each vial to hydrate the liposomes at room temperature for 15 min with slight agitation. Then 6 mL of USP normal saline for injection was added to obtain the multilamellar liposome suspension containing 1.2 mg/mL of lipid and 0.167 mg/mL of IL-7. About 40 ± 5.2% of the IL-7 in the suspension was liposome-associated. This value was unchanged after 4 days of storage at 4°C. The remaining 60% of IL-7 in the IL-7 liposome suspension was further analyzed with a Biogel A-0.5m size-exclusion column chromatography to be free and soluble form. This suspension of IL-7 (referred to as mixed IL-7 liposomes) contained about 60% soluble IL-7. When needed, the soluble IL-7 in the mixed IL-7 liposome preparation was separated from liposome-encapsulated IL-7 by centrifuging the suspension for 30 min at 13,000*g* in a microfuge (Biofuge A). Subsequently, the liposome pellets were washed twice with PBS. The resulting liposome-encapsulated IL-7 preparation contained less than 2% of soluble IL-7, as determined by size-exclusion chromatographic analysis using a Biogel A-0.5m matrix. The mean (±SD) diameter of the liposomes was determined (by negative stained electron microscopy) to be 2.5 ± 2.3 and 2.3 ± 2.1 μm for mixed IL-7 liposomes and liposome-encapsulated IL-7, respectively.

For lymphopoietic studies mixed liposome preparation was carried out as above and the final multilamellar liposome suspension contained 2.4 mg/mL of lipid and 245 μg/mL of IL-7. An approximately 200-μL injection dose (for a 350-g guinea pig) contained 49 μg (140 μg/kg) of IL-7 and 480 μg (1.3 mg/kg) of lipid.

Animal Studies

Hartley female guinea pigs, 250–300 g and approximately 3 months old, were purchased from Tyler Caviary (Bellevue, WA). They were maintained in an isolated animal care facility at the University of Washington, according to NIH guidelines. Guinea pigs were cannulated at the left jugular vein under anesthesia at least 3 days before the experiments. For IL-7 disposition studies, animals were awake and housed unrestrained in metabolic cages throughout the experiments. In addition, the facility was programmed to simulate a normal day–night light cycle.

For lymphopoietic studies, guinea pigs were bled intracardially 1 week prior to the treatments and randomized into placebo or treatment groups of three to seven animals. In the initial experiments, soluble IL-7 was subcutaneously administered twice daily for 7 days at indicated doses (0, 20, or 60 μg/kg) in an approximately 200-μL volume. The placebo group (0 μg/kg) received saline. Soluble IL-7 was diluted from the stock (750 μg/mL) with injection-grade saline immediately before use to minimize the reduction in biologic activity. On day 7, guinea pigs were bled (2 hr after the first injection of soluble IL-7) for analysis of blood-cell distribution. In another set of experiments, two weekly doses of 140 μg/kg soluble IL-7 were given subcutaneously on days 0 and 7. These animals were bled once a week, on days 7, 14, 21, and 35, prior to the administration of soluble IL-7. In another experiment, four weekly doses of soluble IL-7 were given to determine its effects on the platelet concentration in blood.

Blood and Urinary Kinetic Studies of IL-7

^{125}I-IL-7 (30–40 μg) was administered in an approximately 200-μL volume, regardless of the route or the formulation used. The total lipid dose was 216–288 μg for 30–40

μg IL-7 and 540–720 μg for mixed IL-7 liposomes and liposome-encapsulated IL-7, respectively. Blood samples of 100 μL were obtained from the chronic jugular cannula and analyzed for ^{125}I-IL-7 radioactivity at the indicated time point. The data were expressed as IL-7 protein concentration based on the known specific activity of the ^{125}I-IL-7. Similarly, urine and feces samples from animals housed in metabolic cages were collected, and IL-7 was determined by counting ^{125}I radioactivity. In preliminary experiments, we found no radioactivity in the feces of guinea pigs treated with ^{125}I-IL-7, regardless of the formulation or the route of administration. Hence, no further analysis of feces IL-7 was performed. Data are expressed as the mean ± SD of IL-7 for five animals in each group.

Injection-Site Kinetic Studies

The disappearance of ^{125}I-IL-7 from the subcutaneous site was monitored with a Geiger counter. Animals were shaved to expose a 6-cm circular site behind the neck. These animals were injected with 39 μg ^{125}I-IL-7 in 200 μL and immediately examined for ^{125}I radioactivity by placing the probe directly against the skin. The group that received mixed IL-7 liposome (280 μg lipid) as well as the group that received liposome-encapsulated IL-7 (702 μg lipid) was also injected with 39 μg of IL-7 in 200 μL. The total amount of IL-7 at the injection site was determined after correcting for the efficiency of the Geiger counter to detect ^{125}I radioactivity. Data are expressed as mean ± SD for three animals in each group.

Tissue Distribution Studies

Guinea pigs housed in metabolic cages were injected with 39 μg of ^{125}I-IL-7 in either soluble or liposome-encapsulated form. Approximately 200 μL of the drug suspension was injected subcutaneously, and the indicated tissue samples were collected at 1.5 and 24 hr. Blood, urine, feces, kidney, liver, spleen, and injection site (muscle and skin) were weighed and counted for ^{125}I-IL-7 radioactivity. Data were analyzed as the concentration of ^{125}I-IL-7 (ng IL-7/g tissue) and are presented as tissue-to-blood IL-7 concentration ratios to demonstrate tissue association. All the data are expressed as the mean ± SD of three animals for each treatment group.

Pharmacokinetic Analysis

Pharmacokinetic parameters for the time course of IL-7 in the blood were estimated using standard methods. The area under the time curve (AUC_{0-t}) was determined by the trapezoidal rule (22) using the data collected over 0–7200 min. Elimination half-life $t_{1/2\beta}$ was determined from the terminal slope of first-order decay in the blood IL-7 concentration. Bioavailability was determined as the ratio of AUC_{0-7200} to the AUC_{0-7200} of an equivalent intravenous (iv) IL-7 administration.

The total amount of urinary ^{125}I-IL-7, A_u, was determined for 0–7200 min. Bioavailability based on the urine data was analyzed in a manner similar to that described for blood data. The fraction of subcutaneous dose absorbed was further assessed by plotting the cumulative excretion of IL-7 in urine following the subcutaneous administration of each IL-7 formulation. The time required to excrete half the maximum amount of IL-7 in urine was calculated from the plot.

Peripheral Blood Cell Analyses

Blood samples collected in EDTA-treated tubes were analyzed within 2 hr of collection. About 0.5 mL of blood was used to determine the number of white blood cells (WBC), red blood cells (RBC), and platelets using a Coulter cell counter (Hialeah, FL). Although the hemoglobin concentration and hematocrit of the blood samples were determined, no detectable differences were seen among the experimental groups, and hence, these values were not presented. Differential analyses were performed on Wright–Giemsa-stained blood smears to determine basophils, eosinophils, polymorphonuclear cells (PMN), lymphocytes, and monocytes. All the assays described were performed blind by an investigator at Phoenix Central Laboratory (Seattle, WA). For each treatment group, blood from three to seven animals was analyzed, and data are expressed as the mean ± SD of each treatment group.

RESULTS

Effect of Formulation on IL-7 Blood Concentration–Time Course

To compare the effects of formulation on the IL-7 time course in blood, guinea pigs were subcutaneously injected with ^{125}I-IL-7, and blood IL-7 was determined based on its ^{125}I radioactivity. This method was validated by the observation that the specific activity of IL-7 in the blood samples recovered 90 min following iv ^{125}I-IL-7 administration was $3.1 \pm 0.3 \times 10^7$ U/mg, a value comparable to that of the injected sample (sp Act = $3.2 \pm 0.6 \times 10^7$ U/mg). Size-exclusion chromatography using the Biogel A-0.5m matrix, demonstrated that after subcutaneous administration of liposome-encapsulated IL-7, IL-7 detected in blood was free, not associated with liposomes.

A time course comparison for route and formulation effects of IL-7 administration is shown in Fig. 1, and pharmacokinetic parameters are summarized in Table I. After intravenous injection of soluble IL-7 the decline in ^{125}I-IL-7 in blood was biexponential, with an initial half-life of approximately 2 min, followed by a terminal phase with a half-life of 116 min. Following subcutaneous injection of soluble suspension, the peak concentration was observed at approximately 10 min, followed by a monoexponential decline with an apparent half-life of 115 min. In contrast, subcutaneous injection of liposome-encapsulated IL-7 resulted in a peak concentration (2.6 vs 49.7 ng/mL) less than one-tenth that observed after subcutaneous injection of soluble IL-7 and a prolonged apparent-monoexponential time course ($t_{1/2\beta}$ = 3018 min). Because of the extremely slow elimination rate, the half-life of subcutaneously administered liposome-encapsulated IL-7 was difficult to estimate accurately. Following injection of liposome-encapsulated ^{125}I-IL-7, ^{125}I was detectable in blood up to 7000 min, while after subcutaneous injection of soluble IL-7, the concentration had declined below the limit of detection by 400 min. Subcutaneous administration of mixed IL-7 liposomes (containing 40% encapsulated and 60% free IL-7) exhibited a peak concentration of

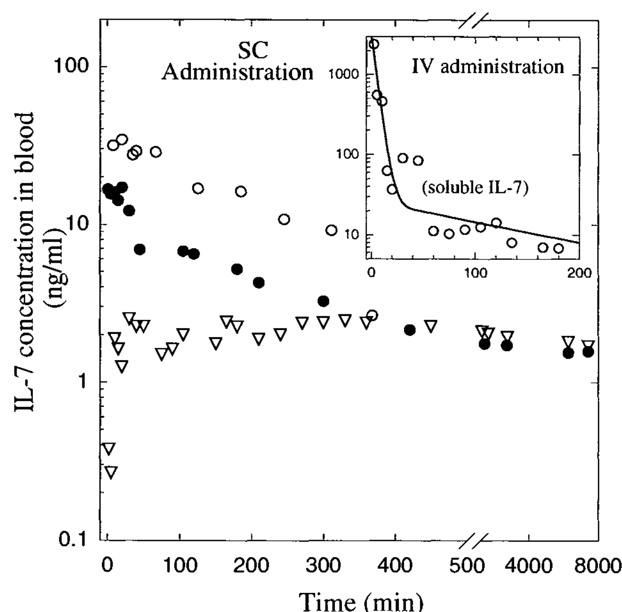

Fig. 1. Time course of blood IL-7 concentration. Guinea pigs were subcutaneously injected with 39 μg of soluble IL-7 (○), mixed IL-7 liposomes (●), and liposome-encapsulated IL-7 (▽). Blood IL-7 concentration was determined at the indicated time intervals. Data expressed are representative of one animal from each treatment group. In parallel, one group of animals was given intravenous, soluble IL-7 (○) at a 42 μg/dose, and the data are presented in the inset.

20.7 ng/mL and an initial half-life comparable to that observed after subcutaneous administration of soluble IL-7 (10 min $< t <$ 200 min), followed by a prolonged phase comparable to that observed after administration of liposome-encapsulated IL-7 (300 min $< t$) (Fig. 1).

The time course of cumulative ^{125}I-IL-7 excretion in urine is shown in Fig. 2. These data are consistent with the blood data, in that the time for eventual excretion of 50% of the IL-7 in the urine was shortest for subcutaneous soluble IL-7 (166 min), longest for liposome-encapsulated IL-7 (3961 min), and intermediate for mixed IL-7 liposomes (591 min). Bioavailability was assessed both from blood AUC_{0-7200} and urine data and is shown in Table I. Mean bioavailability relative to IV IL-7 was 68.6 ± 1.7% for subcutaneous soluble IL-7. Both mixed-IL-7 liposomes and liposome-encapsulated IL-7 formulation exhibit a similar bioavailability (63.6 ± 13 and 64.4 ± 24.9%). These results were replicated with more than two batches of liposomes. These bioavailability values were comparable and were less variable when calculated from the urine data (A_u; Table I).

Collectively, liposome encapsulation significantly reduced peak blood concentration and prolonged blood levels of IL-7 without compromising the bioavailability of subcutaneously administered IL-7.

IL-7 at the Injection Site

The effect of liposome formulation on the rate of ^{125}I-IL-7 disappearance from the injection site is illustrated in Fig. 3. The very slow loss of ^{125}I when a liposome formulation was used precluded accurate estimation of an absorption half-life under these conditions. However, substantially lower rates of IL-7 removal from the injection site were observed with the mixed IL-7 liposomes and liposome-encapsulated IL-7, compared with soluble IL-7. The prolonged time course of liposome-entrapped IL-7 in blood and its slow appearance in urine are probably due to a slow rate of release from the site of injection.

Distribution of IL-7 into Tissues

To determine the tissue distribution of IL-7, guinea pigs were subcutaneously injected with soluble IL-7 or liposome-encapsulated IL-7. Accumulation of IL-7 in the tissue was determined at 1.5 and 24 hr. As shown in Table II, the highest tissue level of subcutaneously injected soluble IL-7 was detected in the kidney and liver, followed by the spleen. The mean concentration of IL-7 in these tissues at 1.5 hr was significantly higher than those found in blood (Table II). About 80% of soluble IL-7 was cleared from the injection site, and 25% of the injected dose was recovered in the urine at 1.5 hr (Fig. 4, inset). In fact, a majority (64%) of the injected soluble IL-7 is found in the urine within 24 hr after injection. For the same time frame, most of the injected IL-7 (83% of injected dose) remained at the injected sites for animals treated with liposome-encapsulated IL-7, and less than 1% of the injected dose was found in the urine. Except for the kidney, animals treated with liposome-encapsulated IL-7 exhibited minimum IL-7 accumulation in the tissues (liver, spleen, lung, and heart) either at the 1.5-hr or the 24-hr time point. At 24 hr postinjection, animals treated with liposome-encapsulated IL-7 showed significant amounts (30%) of IL-7 remained in the injected site, and only 10% of the injected IL-7 dose was recovered in the urine (Fig. 4). The effect of liposome encapsulation on the IL-7 localization remained apparent even after the tissue IL-7 concentrations were corrected for their differential blood concentration (Fig. 4).

To determine whether IL-7 detected in the blood was associated with the blood cells, the plasma was separated from the blood cells, and the distribution between the two components was determined. We found that IL-7 did not bind significantly to blood cells, regardless of the test formulation used (data not shown).

Liposome encapsulation significantly reduces the concentration of subcutaneously injected IL-7 in the liver, kidney, and spleen, while retarding its disappearance from the injection site over the time course of our experiments.

Effect of Twice-Daily Injections of Soluble IL-7 on Guinea Pig Blood-Cell Distribution

To determine the lymphopoietic effects of IL-7, normal outbred Hartley guinea pigs were treated twice daily with soluble IL-7 for 7 days, then peripheral blood-cell distribution was determined. As shown in Table III, administration of IL-7 increased the WBC concentration in a dose-dependent manner, with a larger increase (7.3 ± 1.3 × 10^3 cells/mm^3; $P < 0.05$) seen with 60 μg/kg IL-7 and a smaller increase (5.1 ± 1.2 × 10^3 cells/mm^3; $P < 0.05$) seen with 20 μg/kg IL-7 (threefold lower dose) compared with the placebo group (3.9 ± 0.7 × 10^3 cells/mm^3). Analysis of RBC indicated no significant change due to the therapy. We also found that the IL-7 had no significant effect on hematocrit or blood hemoglobin concentration (data not shown). However, at a

Table I. Selected Pharmacokinetic Parameters of IL-7 Administration[a]

Treatment	Route	Dose (μg)	Peak blood concentration (ng/mL)[b]	Half-life β (min)	AUC_{0-t}/dose (min/mL)[c]	A_u (% injected dose)	Bioavailability (% of iv)	
							AUC_{0-t}[d]	A_u[e]
Soluble IL-7	iv	42	2402 ± 331	116 ± 10	15.6 ± 0.7	93.9 ± 10.2		
Soluble IL-7	sc	39	49.7 ± 8.5	115 ± 12	9.9 ± 0.3	60.6 ± 4.2	68.6 ± 1.7	72.2 ± 5
Mixed IL-7 Liposomes	sc	39	20.7 ± 3.1	1516 ± 366	9.2 ± 0.2	54.6 ± 6.1	63.6 ± 13	65.1 ± 7.3
Liposome-encapsulated IL-7	sc	39	2.6 ± 0.4	3018 ± 897	9.4 ± 3.9	57.3 ± 0.4	64.4 ± 24.9	73.6 ± 0.5

[a] Guinea pigs were injected with ^{125}I-IL-7 at the indicated dose and route. Blood samples were collected, and the pharmacokinetic parameters were determined based on the time course of blood IL-7 profile, as described under Materials and Methods.
[b] Mean ± SD of the peak blood IL-7 data was normalized by the injected dose of IL-7.
[c] Blood AUC was determined from the 0- to 7200-min time–blood concentration curve, corrected for the dose.
[d] Bioavailability determined from blood AUC, corrected for dose.
[e] Bioavailability determined for cumulative amount excreted in urine (A_u) after correcting for dose.

higher dose of soluble IL-7 (60 μg/kg), about a 50% reduction in platelets was detected (220 ± 83 × 10^3 cells/mm^3 for IL-7 vs 450 ± 34 × 10^3 cells/mm^3 for placebo; $P < 0.05$). Differential analysis indicated that the increased WBC concentration was contributed mainly by an IL-7 dose-dependent increase in the lymphocytes. No significant difference in basophil, eosinophil, or PMN among the treatment groups indicated the selectivity of soluble IL-7 on guinea pig lymphocytes (Table III). Although soluble IL-7-treated animals did exhibit an increase in monocyte number, the data were not statistically significant. Therefore, *in vivo* administration of soluble IL-7 increased guinea pig white blood cell concentration, specifically the lymphocyte population.

Liposome-Formulated IL-7 as a Sustained-Release Vehicle to Reduce the Required Frequency of Repeated Soluble IL-7 Administration

Subcutaneously administered mixed IL-7 liposomes (with about 40% liposome-associated and 60% in soluble form) provided detectable blood IL-7 levels for more than 7 days (MRT = 7.9 days), while soluble IL-7 was cleared within 4 hr (MRT = 4 hrs) (Table I and Fig. 1). Therefore, we administered mixed IL-7 liposomes on a weekly schedule

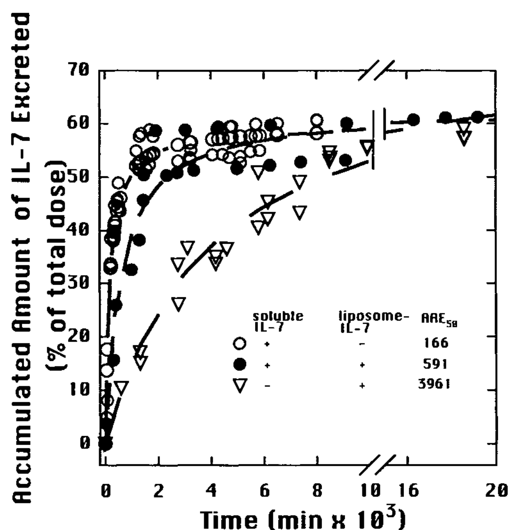

Fig. 2. Time course of IL-7 excreted in urine. The accumulated amount of urine IL-7 excreted is expressed as percentage of total injected dose vs time for the animals subcutaneously injected with 39 μg of IL-7. Animals were injected with soluble IL-7 (○), mixed IL-7 liposomes (●), or liposome-encapsulated IL-7 (▽). The accumulated amount of IL-7 excreted in the urine was presented as a scatter plot for each animal in the groups, at the indicated time intervals.

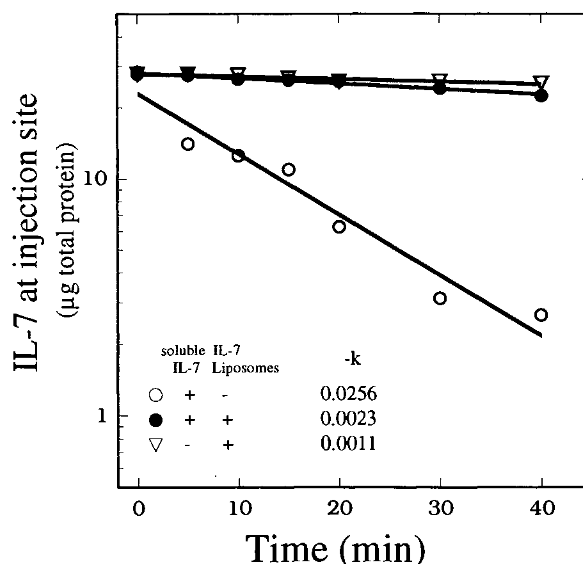

Fig. 3. Disappearance of IL-7 at the subcutaneous injection site. The amount of ^{125}I-IL-7 at the subcutaneous site of injection with soluble IL-7 (○), mixed IL-7 liposomes (●), or liposome-encapsulated IL-7 (▽) was determined at the indicated time intervals. The disappearance rate constant k was determined from the slope of the fitted curve.

Table II. Tissue IL-7 Concentration Following Subcutaneous Administration[a]

Tissue	Soluble IL-7		Liposome-encapsulated IL-7	
	1.5 hr	24 hr	1.5 hr	24 hr
Liver	67.7 ± 16.5	6.9 ± 1.6	3.4 ± 0.3	3.9 ± 0.8
Kidney	104 ± 26	18.2 ± 3.4	18.3 ± 0.2	12.8 ± 0.1
Blood	9.2 ± 4.3	2.6 ± 0.8	1.7 ± 0.3	2.1 ± 0.6
Spleen	26.1 ± 0.5	0.4 ± 0.1	2.1 ± 0.4	2.4 ± 0.1
Lung	5.4 ± 2.0	3.8 ± 1.6	0.8 ± 0.3	1.6 ± 0.2
Heart	4.8 ± 2.0	2.6 ± 0.6	0.6 ± 0.2	1.6 ± 0.2

[a] Tissue IL-7 concentrations as ng IL-7/g were determined at 1.5 and 24 hr for guinea pigs administered with 39 μg of either soluble or liposome-encapsulated IL-7. Data are presented as mean ± SD.

and determined its effects in guinea pigs. As shown in Table IV, two weekly doses of liposome-formulated 140 μg/kg IL-7 subcutaneous (given on days 0 and 7) also increased the guinea pig WBC concentration by day 14, 7 days after the second dose of IL-7. Again, increased WBC concentration was due mainly to the increased lymphocyte numbers, with no significant effect on RBC, platelet, basophil, eosinophil, PMN, or monocyte concentrations. Identical weekly treatments with either soluble IL-7 or placebo did not produce an increase in total WBC or lymphocytes (Table IV), indicating the effect of liposome formulation on IL-7 response in guinea pig cells. The total amount of liposome-formulated IL-7 administered to each animal was identical for those animals that received twice-daily dosing of soluble IL-7 (injection of 20 μg/kg for 7 days: 7 × 2 × 20 μg/kg = 280 μg/kg) (Table III) and for the animals that received two weekly doses (2 × 140 μg/kg = 280 μg/kg) (Table IV). Taken together, when an identical total dose of IL-7 was administered, the two weekly doses of liposome-formulated IL-7, but not soluble IL-7, provided a lymphopoietic response in guinea pigs similar to that with the twice-daily dosing of IL-7 for 7 days.

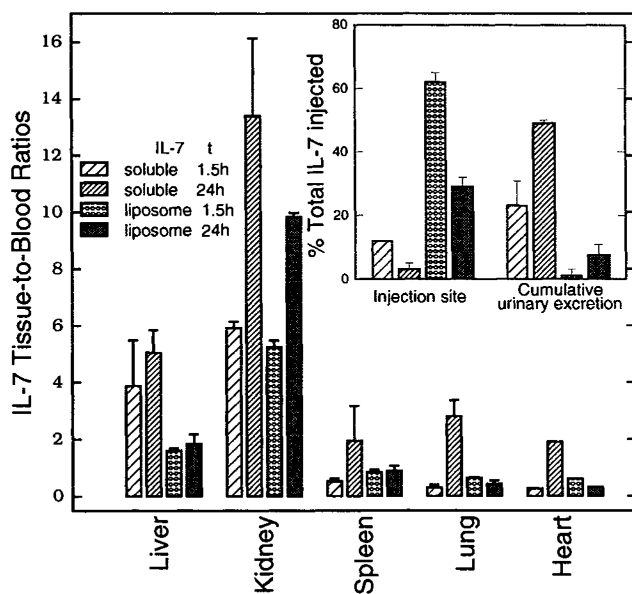

Fig. 4. Tissue-to-blood ratios of IL-7 in guinea pigs. Guinea pigs were subcutaneously injected with 39 μg of soluble or liposome-encapsulated IL-7, and the respective tissue samples were collected at 1.5 or 24 hr postinjection. These data were normalized against the blood IL-7 concentration and are expressed as the mean ± SD of three animals in each group. The inset shows the total amounts of IL-7 found at the injection site and in urine for the same sets of animals.

Effects of Multiple Liposome-Formulated IL-7 Dosing on Guinea Pig Platelet Concentration

Although two weekly administrations of 140 μg/kg IL-7 did not affect the platelet concentration in treated guinea pigs (Table IV), it is possible that increasing the number of doses may have an effect. In Table III we show that twice-daily injections of 60 μg/kg (a 2.3-fold lower amount of IL-7/dose than in the 140 μg/kg liposome IL-7 dose) for 7 days reduced the platelet concentration by about half in treated guinea pigs. Therefore, we administered four doses of liposome-formulated IL-7 on days 0. 7, 14, and 21 and determined the platelet concentration at weekly intervals on days 7, 14, 21, and 28. The results are summarized in Table V. Repeated weekly administration of a liposome-formulated form of IL-7 (given at more than twice the dose than that which had exhibited platelet reduction in a twice-daily schedule) did not affect the guinea pig platelet concentration. After four weekly doses of liposome-formulated 140 μg/kg dose of IL-7, we found no significant reduction in platelet concentration. This indicated that liposome formulations requiring less frequent administration also eliminated the IL-7-mediated reduction in platelet concentration in guinea pigs.

DISCUSSION

IL-7, which has been shown to induce bone marrow and T and B lymphocytes *in vivo,* requires frequent administration. Most often a twice-daily injection at a relatively high dose (14,15,17) [i.e., 0.5–3 μg/mouse b.i.d. (17)] is needed to produce an *in vivo* effect. Administration of soluble IL-7 twice a day for 7 days to guinea pigs increased total WBC, particularly lymphocyte concentrations. The IL-7-induced lymphopoietic effect was dose dependent, in that a 20 μg/kg dose induced a 38% increase, while a threefold higher (60 μg/kg) dose induced a 139% increase in total lymphocyte concentration, compared with the placebo dose (Table III). The need for twice-daily IL-7 administration to increase lymphocyte numbers can be reduced to a weekly injection using a mixed IL-7 liposome suspension containing 40% encapsulated and 60% soluble IL-7 (Table IV). Using these mixed IL-7 liposome suspensions, two weekly doses of IL-7, administered in a total dose equivalent to the twice-daily dosing (at the 60 μg/kg level), also yielded about a 45% increase in lymphocyte concentration over that with the placebo treatment (Table IV). We also characterized the pharmacokinetics and tissue disposition of IL-7 in soluble form, liposome-encapsulated form, and a mixture of the two physical forms. Our results show that formulation of IL-7 in liposomes provides a sustained blood IL-7 level and extends the IL-7 residence time in the body (Fig. 1, Table I).

It is likely that subcutaneously administered liposome,

Table III. Effect of Twice-Daily Injections of Soluble IL-7 on Guinea Pig Blood-Cell Distribution[a]

	IL-7 dose (μg/kg)		
	0	20	60
WBC[b]	3900 ± 700	5100 ± 1200*	7300 ± 1300*
Basophil	20 ± 24	40 ± 43	16 ± 32
Eosinophil	226 ± 89	511 ± 302	311 ± 173
PMN	1089 ± 407	851 ± 355	735 ± 274
Lymphocytes	2584 ± 408	3560 ± 727*	6170 ± 1192*
Monocytes	21 ± 31	117 ± 148	107 ± 78
RBC[c]	4.8 ± 0.0	5.2 ± 0.3	4.8 ± 0.2
Platelet[d]	450 ± 34	400 ± 65	220 ± 83*

[a] Guinea pigs ($n = 5$) were treated subcutaneously with IL-7 twice a day for 7 days and were bled on day 8. Data are the mean ± SD of each group.
[b] WBC, basophil, eosinophil, PMN, lymphocytes, and monocytes are expressed as cells/mm^3.
[c] Expressed as 10^6/mm^3.
[d] Counts expressed as 10^3/mm^3.
* $P < 0.05$ compared with placebo group.

localized mainly at the injection site, results in the retention of liposome-encapsulated IL-7 with a sustained release from the liposome. This hypothesis is supported by the fact that there was a lower disappearance rate at the injection site (Fig. 3) and a sustained blood IL-7 level (Table I) detected for IL-7 liposome formulation compared with soluble IL-7 administration. IL-7 liposomes may also have accumulated in the local draining lymph nodes, which would also provide a similar sustained rate of release into the systemic circulation. Because of their relatively large particulate structure, these multilamellar liposomes are probably not permeable to the vasculature (23,24). Size-exclusion chromatography established that IL-7 in the circulation was free, not associated with liposomes. Regardless of the IL-7 liposome localization, the protein detected in blood was probably in the form of soluble IL-7 and available for its biologic actions.

Liposome-formulated IL-7 reduced the frequency of injections required to achieve a lymphopoietic effect comparable to the more frequent injections of soluble IL-7. Also, the liposome formulation appeared to minimize the reduction of blood platelet concentration observed with soluble IL-7-treated animals. Administration of 60 μg/kg in a twice-daily regimen suppressed platelet numbers after day 7 of therapy (Table III), while up to four weekly injections of 140 μg/kg (about a two- to threefold higher amount/dose) in liposome formulation had no effect on platelet numbers (Table IV). It is possible that a lower total dose of IL-7 given in a liposome formulation (four injections of liposome-formulated IL-7 = 560 μg/kg vs 7 days × 2 injections/day of IL-7 = 840 μg/kg total dose) may also contribute to the observed effect. Reductions in the frequency of IL-7 injections may be partly responsible for the abrogation of toxicity to platelet counts, since two weekly doses of soluble IL-7 also did not affect guinea pig platelets. Alternatively, a sus-

Table IV. Effect of Liposomes on Weekly Administration of IL-7 to Modify Blood-Cell Populations[a]

	Placebo	Mixed IL-7 liposomes	Soluble IL-7
WBC[b]	4200 ± 700	5800 ± 1400*	4500 ± 500
Basophil	52 ± 74	0 ± 0	0 ± 0
Eosinophil	123 ± 61	166 ± 109	323 ± 152
PMN	1455 ± 642	1666 ± 1057	1767 ± 503
Lymphocytes	2247 ± 290	3275 ± 338*	2151 ± 75
Monocytes	134 ± 68	51 ± 20	226 ± 75
RBC[c]	4.7 ± 0.2	4.9 ± 0.2	4.2 ± 0.1
Platelet[d]	443 ± 42	470 ± 80	397 ± 37

[a] Guinea pigs ($n = 7$) were given two 140 μg/kg weekly doses of IL-7, and the blood cell population was determined on day 14, 7 days after the administration of second dose. Data are mean ± SD.
[b] WBC, basophil, eosinophil, PMN, lymphocytes, and monocytes are expressed as cells/mm^3.
[c] Expressed as 10^6/mm^3.
[d] Counts expressed as 10^3/mm^3.
* $P < 0.05$ compared with placebo group.

Table V. Effect of Multiple IL-7 Liposome Dosing on Guinea Pig Blood Platelet Concentration[a]

No. of doses	Placebo	Mixed IL-7 liposomes (140 μg/kg dose)
1	413 ± 42	415 ± 49
2	443 ± 42	396 ± 37
3	463 ± 60	496 ± 83
4	410 ± 44	500 ± 44

[a] Guinea pigs (n = 5) treated weekly with liposome-formulated IL-7 on days 0, 7, 14, and 21 were bled on days 7, 14, 21, and 28 to determine their platelet concentration (× $10^3/mm^3$) in the blood. Data are the mean ± SD of each treatment group.

tained-release effect of liposome-formulated IL-7 in maintaining a relatively low blood IL-7 concentration may contribute to these observed effects. When given at the same dosage, IL-7 in the liposome formulation exhibited a twofold lower level of maximum blood-IL-7 concentration (C_{max}) and a significantly longer half-life in the blood (Fig. 1). Liposome-formulated IL-7 administered subcutaneously at 140 μg/kg should remain detectable in the blood for more than 7 days in guinea pigs, while the soluble form administered under identical conditions will be eliminated within 4 hr (Fig. 1 and Table I).

We could not detect a difference in systemic or urinary bioavailability between animals treated with the IL-7 liposome-encapsulated formulation and those treated with the soluble counterpart (68 vs 63–64%). These results suggest that liposome-encapsulated IL-7 was released eventually and became systemically available. This is further apparent as a less variable bioavailability when the urinary data were used to estimate this parameter (Table I; A_u). In addition, a low but sustained blood concentration provided by liposome encapsulation of IL-7 may play a role in reduced IL-7 accumulation in the liver, spleen, and kidney (Table II and Fig. 4). Whether an alteration of the physical properties of liposomes may additionally modify these observed pharmacokinetic parameters is not known. In the present experiments, we used large multilamellar liposomes (several microns in size) that exhibit a heterogeneous size distribution. IL-7 encapsulated in small unilamellar liposomes (of <0.02 μm) may exhibit a significantly different IL-7 release rate *in vivo* and, hence, may modify the observed kinetic parameters. These and other aspects of the sustained-release formulation remain to be studied.

In theory, any polymer or matrix carrier that exhibits a low toxicity and does not inactivate IL-7 biologic activity during the preparation procedure could be used to provide a similar sustained rate of release that extends the residence time of IL-7 in the body. However, the biologic ability of the formulation to induce different subsets of blood cells may depend on the distribution and cellular localization of IL-7, the carrier, or both. Whether other macromolecular carriers that provide similar IL-7 sustained-release kinetics will also produce a similar lymphopoietic response remains to be evaluated.

In summary, we have shown that when given subcutaneously, IL-7 is cleared primarily and rapidly by renal excretion and that liposome association of IL-7 significantly reduces the rate of IL-7 disappearance from the site of injection. This results in a lower peak blood concentration and a sustained blood IL-7 level that is probably within a therapeutically useful range; it also minimizes accumulation of IL-7 in the liver and spleen, organs that may involve in inactivation of IL-7 biologic activity. Therefore, the requirement for frequent administration of soluble IL-7 can be significantly reduced by using liposome-formulated IL-7 to achieve a comparable lymphopoietic response.

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