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RESEARCH PAPER

Sustained-Release Interleukin-12 Microspheres in the Treatment of Cancer

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ABSTRACT

Interleukin-12 (IL-12) is a recently discovered cytokine with tremendous antitumor potential. It has been shown to boost the host immune response against experimental cancers in animal models. However, most studies have utilized IL-12 in the solution form, necessitating frequent dosing with higher doses, consequently leading to issues of toxicity. The only attempts at sustaining release have been in the production and use of genetically engineered cells that can secrete IL-12 constantly. These attempts are cost prohibitive and involve extensive labor. This study demonstrates the use of biodegradable albumin microspheres to sustain the release of IL-12. In vitro release of IL-12 from the microspheres was found to fit Higuchi's square-root-of-time model, suggesting diffusion-mediated release. About 46% of the theoretical IL-12 content was released slowly over a period of 24 hr. When administered intraperitoneally to C57BL/6 mice bearing subcutaneous melanomas, the microspheres significantly prolonged the survival when administered at half the weekly dose of the solution formulation. The microsphere dosage form also resulted in generally lower levels of liver and kidney function enzymes, suggesting lower toxicity.

Key Words: *Cancer; Drug delivery; Interleukin-12; Melanoma; Microspheres; Sustained release.*

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INTRODUCTION

Sustained release of therapeutic agents for the treatment of cancer is appealing considering the fact that therapy is usually long term. It offers the possibility of using lower doses to achieve similar therapeutic effects as conventional nonsustained dosage forms. With the advent of biotechnology and the advances in the techniques of molecular biology, our antitumor arsenal has expanded rapidly to include protein drugs, peptides, and cytokines. These new weapons, although potent, still need suitable delivery systems. Being protein in nature, these agents may be targets of enzymes in the blood. As a result, injecting these agents requires very high doses, which are not only cost prohibitive, but also potentially dangerous. Interleukin-12 (IL-12) is a recently discovered heterodimeric cytokine (1–3). It has been shown in various animal models of cancers to have tremendous antitumor potential (4–8). Using genetically engineered fibroblasts, it has been demonstrated that the sustained presence of lower concentrations of IL-12 produces the same antitumor effects as larger concentrations that are not sustained (9,10). However, it is not easy to produce genetically engineered cells, and it is even more difficult to adapt the production for mass therapy in general due to the considerations of cost and the amount of labor involved. Better alternatives exist in the form of particulate drug delivery systems such as microspheres, which not only shield such protein drugs from the enzymes in the blood, but also can sustain their release. Microspheres also have the added advantage of large-scale production, in addition to being amenable to preparation using a wide variety of biodegradable polymers (11–13).

In this study, we have prepared bovine serum albumin (BSA) microspheres containing IL-12 and evaluated them for sustained release in vitro and for antitumor efficacy in vivo using C57BL/6 mice bearing subcutaneous melanomas.

MATERIALS

The BSA was purchased from Sigma Chemical Company (St. Louis, MO). The IL-12 was a generous donation from Genetics Institute (Cambridge, MA). B16 cells were obtained from ATCC (Rockville, MD). Cell culture supplies were purchased from Life Technologies (Grand Island, NY). All other reagents used were purchased from Fisher Scientific Company (Norcross, GA).

Male C57BL/6 mice were purchased from Harlan Sprague Dawley, Incorporated (Indianapolis, IN). The animals were housed in groups of 5 with controlled temperature, humidity and light-dark cycles. The animals were allowed free access to food and water.

METHODS

Preparation of Microspheres

The BSA microspheres were prepared by a modified suspension cross-linking method using glutaraldehyde as the cross-linking agent (13). An aqueous phase (1–5 ml) containing 20 μg IL-12 per milliliter in 100 mg/ml BSA was added to 100 times its volume of olive oil (100 to 500 ml) maintained at 4°C. A fine water-in-oil emulsion was then formed by mixing the solution at high speed using a biohomogenizer. Samples were withdrawn periodically to check the particle size of the dispersed phase under an optical microscope. When the particle size of the dispersed phase was reduced to about 10 μm (in about 10 min), 0.5 ml of glutaraldehyde (25% v/v) per milliliter of the aqueous phase was added to cross-link the albumin droplets at the interface. The emulsion was then maintained at 4°C and constantly stirred with a magnetic stirrer for 2 hr under a low setting to allow time for cross-linking to complete and to prevent the microspheres from settling during this process. The microspheres were then separated from the oil by centrifugation at 1500 *g* for 5 min. The remaining traces of oil were removed by washing the microspheres three times with ether.

Enzyme-Linked Immunosorbent Assay for Interleukin-12 Quantitation

The enzyme-linked immunosorbent assay (ELISA) for IL-12 quantitation was carried out at room temperature. All the solutions were brought to room temperature except as noted. Wells of a 96-well ELISA plate were first coated with 100 μl of IL-12 standards (0 to 1000 ng/ml prepared in Dulbecco's phosphate buffered saline [DPBS]) or samples containing the IL-12 from the dissolution study (see below) prepared in DPBS. The plate was then placed on a plate shaker (Ika Labs, model MTS-4) for 2 hr and shaken at 400 shakes per minute. After the 2-hr period, a 2% BSA solution was added to block the unreacted sites on the plate. The plate was then washed with PBS containing 0.05% Tween 20 using an automated plate washer (Biorad, model 1550) set for four washes with a 64-sec soak time between washes. Following this, 100 μl of IL-12 polyclonal antibody (500 μg/ml sheep antimurine, supplied by Genetics Institute) at a 1:4000 dilution in DPBS were added to all the wells.

After the 2 hr, the plate was washed, and 100 μl of rabbit antisheep-HRP conjugated antibody (1 mg/ml) at a dilution of 1:250 were added to each well. Color was developed using 100 μl of a 1 mg/ml 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution with 0.03% peroxide. After the blue color developed (30 min), 50 μl of 2 N sulfuric acid were added to each well. The plate was read immediately on an ELISA plate reader (Biorad, model 3550) at a wavelength of 405 nm.

Cell Culture of B16 Cells

B16 cells are a murine melanoma cell line. They were grown as monolayers of attached cells in 75-cm^2 cell culture flasks in RPMI-1640 medium supplemented with 10% fetal calf serum, 2 mmol glutamine, 2.2 g sodium bicarbonate, 10,000 units penicillin, 10 mg streptomycin, and 25 μg fungizone per liter. The flasks were incubated in a sterile incubator at 37°C with 5% CO_2 and 100% relative humidity. The cells in the log phase were then detached gently using 4 ml of Ca^{++} and Mg^{++} free trypsin-EDTA solution (0.25% porcine trypsin + 1 mM EDTA.4Na, Life Technologies). They were then pipetted vigorously to break up the clumps and to obtain a single-cell suspension. Cells were always used within 10-cell culture cycles of being passed through a live animal.

Bioassay for Interleukin-12

The bioassay was based on the observation that splenocytes from mice with B16 melanoma could kill the tumor cells in vitro in the presence of IL-12 in a concentration-dependent manner. For each bioassay, a pool of spleens from three mice was used. The assays were carried out in triplicate using 96-well cell culture plates. Mice were first injected with 10^4 B16 cells by tail vein to lodge the cells in the lungs. This subtumorigenic dose was boosted by another injection of 10^4 cells after 4 days by the same route. After another 4 days, a midline incision was made in the abdomens of the mice under deep anesthesia, and their spleens were removed aseptically. Following this, the mice were euthanized. The spleens were placed in sterile cold RPMI medium and were teased apart gently with the aid of forceps. The pieces were then vigorously pipetted to break up the clumps. The supernatant cell suspension was then centrifuged in sterile 50-ml centrifuge tubes at 200 *g* for 5 min at 4°C to collect the splenocytes. Red blood cells (RBCs) were destroyed by subjecting the cells to osmotic shock three times by incubating the cells in hypotonic saline for 1 min, followed by centrifuging and resuspending in normal saline. This procedure routinely yielded approximately 20×10^7 splenocytes per spleen. These splenocytes were used as a source of lymphocytes for all the experiments without further enrichment.

Meanwhile, B16 cells were cultured in vitro in 96-well cell culture plates. Prior to confluency and 24 hr before harvesting the splenocytes, 2500 B16 cells/100 μl of RPMI were transferred to the wells of the plates. They were allowed to incubate for 24 hr, during which they adhered to the surface of the wells. The splenocytes (5×10^5 cells/50 μl) were then added to the wells. Following this, different concentrations of IL-12 standard solutions and the samples from the dissolution study containing unknown concentrations of IL-12 (50 μl each) were added to these cells. The cells were then incubated at 37°C in 5% CO_2 for 4 days. The cytotoxicity of the splenocytes to the B16 cells was then measured using a standard MTS colorimetric assay as follows using appropriate controls: Briefly, a reagent solution consisting of MTS (2 mg/ml) and phenazine methosulfate (PMS; 0.92 mg/ml) in RPMI was added to the wells of the microplates containing the cells so that the final ratio of the added reagent solution to the contents of the wells was 6:1. The absorbances of the resulting solutions were measured using an automated microplate reader (Biorad, model 3550) at 595 nm using 655 nm as a reference. Controls representing absorbances due to splenocytes and the blank media were subtracted to arrive at the corrected absorbances representing only the surviving B16 cells.

Content Analysis of Microspheres

Albumin microspheres (2.5 mg) containing IL-12 were crushed in a mortar and pestle with 0.5 ml of DPBS. They were then diluted to 10 ml, transferred to a beaker, and placed on a shaker for 24 hr in a cold water bath. After 24 hr, the solution was filtered using a 0.22-μm membrane. The filtered solution was then assayed for the concentration of the intact dimer by the ELISA assay and bioassay. The concentration of IL-12 in the microspheres was then determined from the standard plot for each of the assays. The content analysis determination was made in triplicate.

Dissolution Study

Albumin microspheres (2.5 mg) containing IL-12 were suspended in 10 ml of sterile DPBS in sterile 50-ml centrifuge tubes ($n = 6$) at room temperature and kept on a shaker for 7 days. The tubes were capped to prevent evaporation. At various time points (from 0 hr to 7 days),

400-μl samples were withdrawn aseptically and centrifuged. The supernatants were then collected and stored at 4°C until analysis. These supernatants were then assayed for IL-12 concentrations using the bioassay and the ELISA assays described above. The dissolution studies were repeated a total of three times.

Development of Experimental Tumors in C57BL/6 Mice

The B16 cells were grown in cell culture flasks as monolayers of attached cells. They were collected by detaching them using trypsin-EDTA solution prior to reaching confluency, and 10^6 cells suspended in 0.2 ml of RPMI medium were then injected with a 22-gauge needle subcutaneously into C57BL/6 mice on the back just below the neck. The animals were then monitored daily for visible signs of tumor growth. The mice were allowed free access to food and water and were housed in an approved animal facility in rooms with 12-hr light and dark cycles and controlled temperature and humidity.

Determination of Interleukin-12 Dose and Dosing Frequency

For determination of IL-12 dose and dosing frequency, 72 male C57BL/6 mice were each injected with 10^6 B16 melanoma cells to create the subcutaneous melanoma tumors. On day 7, the mice were divided randomly into three groups according to the frequency of dosing. The three groups received treatment once a week, twice a week, or every other day for a period of 7 weeks each. The animals were then monitored for 12 weeks or until they died, whichever occurred earlier. Each group was further divided into four subgroups of 6 mice each based on the following treatments (*a*) plain DPBS (controls), (*b*) 50 ng IL-12, (*c*) 200 ng IL-12, or (*d*) 500 ng IL-12, each administered as 0.2 ml i.p. injections. Tumor progress was monitored by measuring the diameter of the tumor using a vernier scale. Also, the survival time of the animals in each group was noted.

Evaluation of Interleukin-12 Microspheres and Solutions in C57BL/6 Mice

The 40 C57BL/6 mice were injected with 10^6 B16 cells as described before to create the subcutaneous melanoma tumors. The tumors were visible by day 7. On day 7, the mice were divided randomly into four groups of 10 mice each. The mice were then treated by injecting the solutions intraperitoneally as described in Table 1 for a period of 7 weeks. The animals were to be monitored for 12 weeks or until they died, whichever occurred earlier. The solutions were administered as a 0.2-ml injection in DPBS vehicle twice every week. The microspheres, either blank or corresponding to an IL-12 dose of 500 ng, were suspended in DPBS containing 40% polyethylene glycol 400 (PEG-400) as a suspending agent. They were administered in a volume of 0.2 ml intraperitoneally once every week. The tumor progress was evaluated by measuring the diameter of the tumor mass on the skin using a vernier caliper scale. The animals were also monitored for their body weight and for visual signs of fatigue or distress. Finally, the survival of the animals was also noted.

Assessment of Hepatic and Muscle Toxicity

Blood samples (100 μl) were obtained from the tail veins of mice on days 0 (baseline), 10, 20, 40, and 60. The serum was analyzed for serum glutamic-oxaloacetic transaminase (SGOT) and serum creatinine (SCr) using kits purchased from Sigma Chemical Company.

Statistical Analysis of Data

Data obtained for survival, tumor size, weight change, and serum enzyme levels in the different groups of ani-

Table 1

Description of Treatment Groups

Group	Description	Treatment	*N*
I	Solution control	Vehicle (DPBS) alone	10
II	IL-12 solution	IL-12 (500 ng per injection)	10
III	Microsphere control	Blank albumin microspheres	10
IV	IL-12 microspheres	IL-12 albumin microspheres (500 ng IL-12 per injection)	10

mals were statistically analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keul's post hoc test. The confidence level was fixed at 95%.

RESULTS AND DISCUSSION

Content Analysis of Albumin Microspheres

The IL-12 content of albumin microspheres measured by the ELISA assay and bioassay was found to be 44.09% ± 1.17% (w/w) and 46.43% ± 1.59% (w/w), respectively, based on the theoretical amount of IL-12 that was incorporated into the microspheres. This translated into a content of 8.82 ± 0.24 μg and 9.75 ± 0.32 μg of IL-12 per 100 mg of albumin microspheres when assayed by each of the assays, respectively. The general agreement between the results from the ELISA assay and the bioassay suggests that the entire amount of IL-12 detected by the assays consists of the intact and bioactive IL-12 dimer.

Dissolution Study

Figure 1 shows the cumulative release of IL-12 from the microspheres. Although the duration of the dissolution study was 7 days, almost all of the IL-12 release occurred during the first 24 hr. About 45% of the theoretical amount of IL-12 that was encapsulated, which represented almost all of the actual content of IL-12, was released during the first 24 hr, as indicated by the ELISA assay. After 24 hr, essentially no more IL-12 was released. These results were confirmed by measuring the IL-12 concentrations by the bioassay. A burst effect was seen for the first hour, during which about 16% of the theoretical amount of IL-12 was released. This was followed by a sustained-release period until 24 hr, during which all of the remaining IL-12 was released.

An important fact to be considered when evaluating release for proteins and particularly for a dimeric cytokine like IL-12 after its release from the microspheres is the retention of its biologic activity. The bioassay was developed to check for this. Although the ELISA was designed to measure the concentration of the intact dimer, it did not guarantee that the IL-12 would still be active after its release. The bioassay indicated that about 46% of the theoretical amount of IL-12 was released in an active form, and this suggests that all of the IL-12 detected by the ELISA was indeed in a biologically active form. This amount represents the entire content of IL-12 present in the microspheres as determined by content analysis study.

The post–burst effect dissolution data for release of IL-12 (between 1 and 24 hr) from the microspheres, when plotted against the square root of time, showed a good linear fit, with a correlation coefficient of 0.977, suggesting a good fit to the Higuchi model of drug release (Fig. 2). This suggests that the release of IL-12 from the albumin matrix in vitro is primarily diffusion mediated.

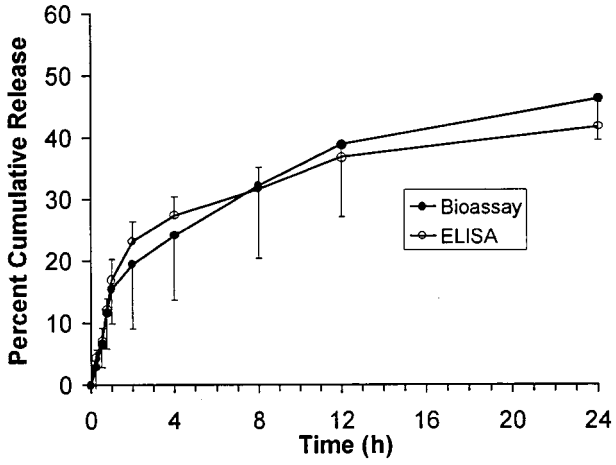

Figure 1. Percentage cumulative release of IL-12 from the microspheres evaluated by ELISA and bioassay based on the theoretical content of 20 μg IL-12 per 100 mg of microspheres. The graph shown is for IL-12 release during the first 24 hr only because no further release was observed after 24 hr. Data represent a mean of six readings (±SD). Experiment was performed 3 times with similar results. Results are shown for one study.

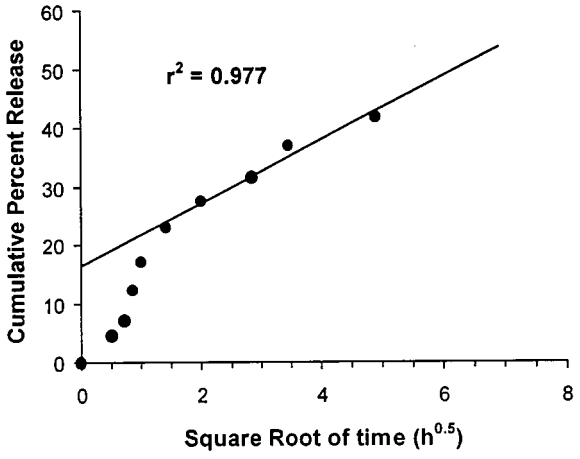

Figure 2. Higuchi square-root-of-time fit for the dissolution of IL-12 from albumin microspheres between 2 and 24 hr.

Determination of Interleukin-12 Dose and Dosing Frequency

The mice in the group dosed once per week did not show any significant increase in survival with an increase in IL-12 dose compared to the control group (Fig. 3). Animals in the twice-per-week group and the group that was dosed every other day showed a significantly improved survival at IL-2 doses of 50, 200, and 500 ng compared to their respective controls ($p < .05$). However, there was no significant increase in survival in the group of mice that was dosed every other day over the group dosed twice per week that received similar IL-12 doses.

IL-12 is a potent cytokine drug, but in many tumors, it only produces temporary suppression or slowing of tumor growth, but no cure. Also, frequently such tumors resume their original rapid growth rate once IL-12 therapy ceases. It has often been demonstrated that the sustained presence of IL-12 either further slows the tumor progress or in some situations effects a cure (14). We were interested in determining if sustained release of IL-12 from albumin microspheres could cause any of these beneficial effects in B16 tumors, in which IL-12 does not readily promote a cure.

From Fig. 3, it is evident that dosing once a week at any of the four IL-12 doses (0 to 500 ng) did not improve survival. Considering the fact that the half-life of IL-12 in rodents is about 3 hr (6), the once-a-week dosing does not allow enough time for the IL-12 to be in the system long enough to activate the immune effector cells. Thus, after 18 hr (6 half-lives), the IL-12 is essentially eliminated from the body. By contrast, the groups dosed twice a week or every alternate day showed a significantly improved survival at all the doses administered (50 to 500 ng) when compared to their respective controls. Since dosing every other day did not improve the survival further when compared to dosing twice a week, the latter was selected as the dosing interval for further studies.

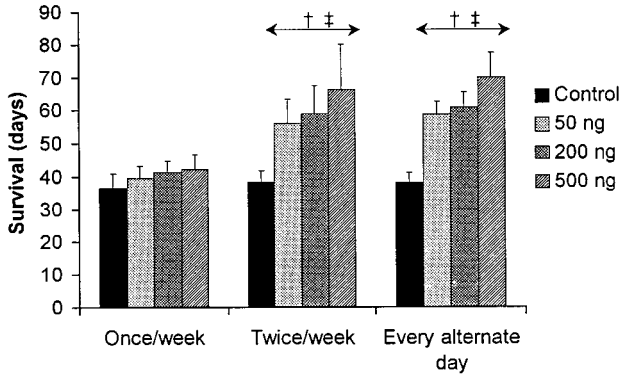

Figure 3. Survival of C57BL/6 mice treated with IL-12 solutions (±SD). Data represent a mean of 6 mice for each group. †, $p < .05$ compared to the respective controls. ‡, $p < .05$ compared to the corresponding subgroups in the once-per-week group.

Figures 4A, 4B, and 4C show the increase in tumor size with time for the three groups. The animals in all the control groups were dead by day 42. There was no significant difference in the tumor sizes of mice receiving 0, 50, 200, or 500 ng IL-12 solutions in the group dosed once per week ($p < .05$).

Mice treated with the 500 ng IL-12 solution that were dosed twice per week or every other day showed a significantly smaller tumor size from days 21 to 56 when compared to the 50 ng or the 200 ng IL-12 solution groups ($p < .05$). After day 56, the statistical differences between the groups disappeared. In fact, the tumors were in general smaller in size from the second week until the animals died, but the differences were statistically significant only between days 21 and 56. There was no difference in tumor sizes in mice treated twice a week when compared to those treated every other day at the same doses ($p < .05$). Hence, based on the results of survival and tumor size, a dose of 500 ng IL-12 administered twice a week was selected as the regimen for further studies.

Evaluation of Interleukin-12 Microspheres and Solutions in C57BL/6 Mice

Figure 5 shows the survival of the mice from the four groups. Both IL-12 solutions (twice a week, 500 ng each) and IL-12 microspheres (once a week, 500 ng) showed significantly improved survival in mice (70 days and 69 days, respectively) compared to their respective controls (40 days each, p .05). Mice injected with a single injection per week of IL-12 microspheres (500 ng IL-12) showed a similar improvement in survival as those that were injected twice a week with the IL-12 solution (1000 ng IL-12).

As seen in Fig. 6, animals injected with either the IL-12 microspheres or the IL-12 solutions showed significantly reduced tumor sizes from day 21 until about day 40 when compared to their respective controls, by which time all the animals in the control group had died ($p <$ .05). Animals treated with the microspheres (once a week, 500 ng) showed a similar reduction in tumor size compared to those treated with the solutions (twice a week, 500 ng each).

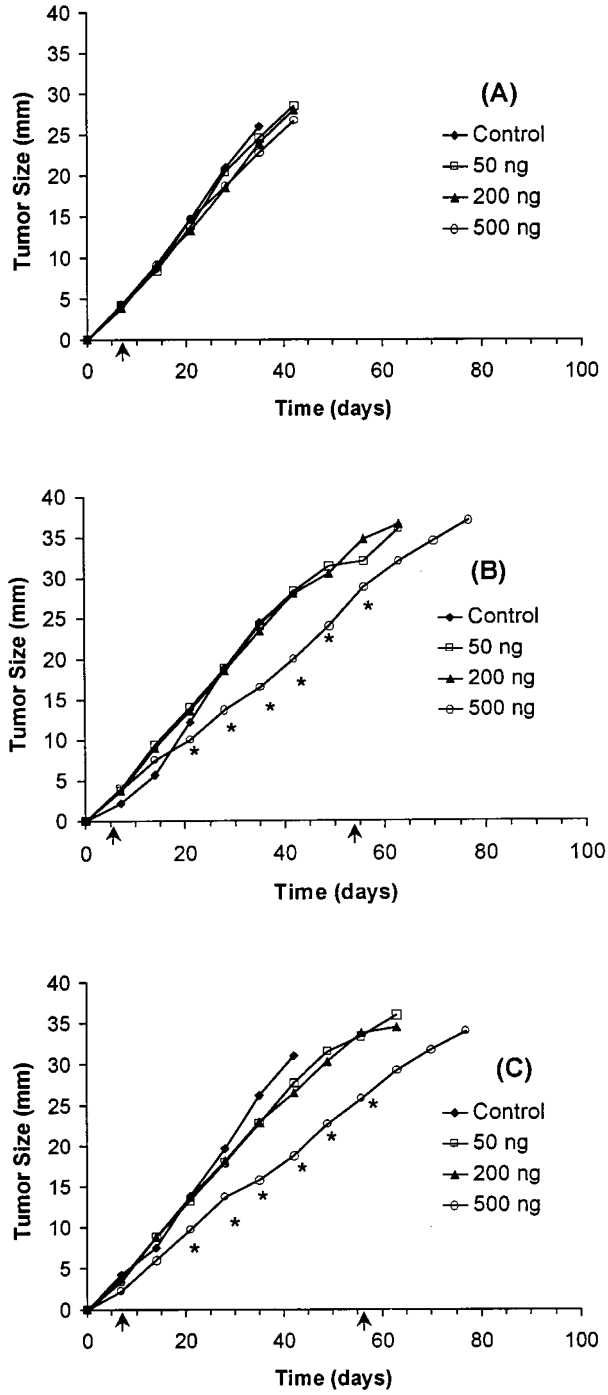

Figure 4. Increase in tumor diameter with time for mice treated with IL-12 solutions injected (A) once a week, (B) twice a week, (C) or every alternate day. *, $p < .05$ compared to all the other groups. Arrows indicate the beginning and end of IL-12 treatments.

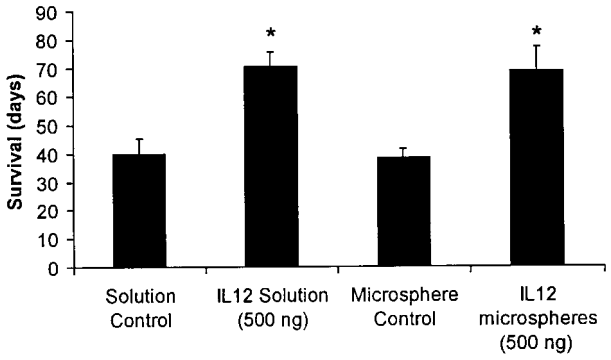

Figure 5. Survival of C57BL/6 mice treated with IL-12 solutions and microspheres (±SD). Data represent a mean of 10 mice per group. *, $p < .05$ compared to the respective controls.

As seen in Fig. 5, IL-12 improved the survival with both solutions and microspheres; however, the microsphere dosage form required administration only once a week, as opposed to twice a week for the solution form. It should be noted that the microspheres administered once a week delivered a total dose of 500 ng of IL-12 per week, while the solution administered twice a week delivered a total IL-12 dose of 1000 ng. This fact, combined with the observation that both the IL-12 groups (solution and microspheres) resulted in significantly reduced tumor sizes (Fig. 6) compared to their respective controls, makes it evident that 500 ng IL-12 delivered via

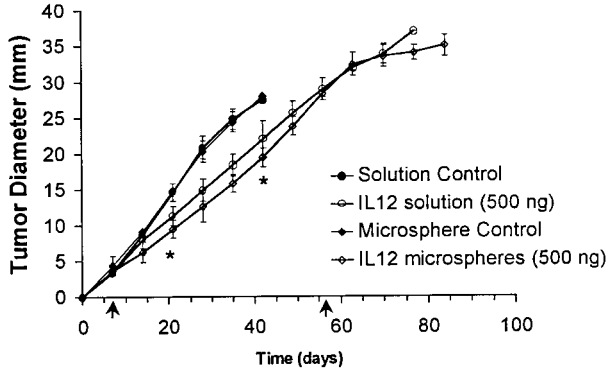

Figure 6. Increase in tumor diameter with time in mice treated with IL-12 solutions and microspheres (±SD). Vehicles for the solution and the microspheres were DPBS and 40% v/v PEG-400 in DPBS, respectively. *, $p < .05$ for both IL-12 solution and microsphere groups compared to their respective controls. Arrows indicate the beginning and end of IL-12 treatments.

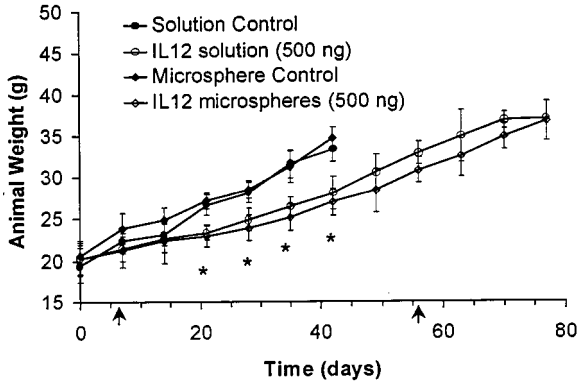

Figure 7. Change in weights of tumor-bearing C57BL/6 mice treated with IL-12 solutions and microspheres (±SD). Data represent a mean of 10 mice per group. *, $p < .05$ for both the IL-12 solution and microsphere groups compared to their respective controls. Arrows indicate the beginning and end of IL-12 treatments.

microspheres once a week are equivalent to 1000 mg IL-12 delivered as solution per week. Further, the tumor sizes for the animals in the group treated with IL-12 microspheres tended to be smaller than those for the animals treated with IL-12 solution until day 63, although this did not achieve statistical significance.

Figure 7 shows that the trend in body weights paralleled the trend seen in Fig. 6 for tumor size. A significant difference was observed in body weights of the mice treated with the IL-12 (solutions and microspheres) when compared to their respective controls ($p < .05$). The body weights of the animals (Fig. 7) reflected an increase in tumor size in all the groups tested. The body weights of the mice in both the control groups increased much faster than in the groups treated with IL-12. However, the body weights of the mice in the treated groups were similar to those of the control groups when the tumor sizes were similar, although this happened at a much later time due to a significantly slowed increase in the tumor size in the treated groups. It is thus evident that the weight change reflects the weight change in the tumor mass.

Assessment of Hepatic and Muscle Toxicity

SGOT is a transaminase enzyme present primarily in the hepatocytes and heart cells. It is released in the blood in larger quantities in cases of heart or liver damage. Increased levels usually are associated with heart attacks or liver disease. Cardiotoxic and hepatotoxic drugs can also result in elevated SGOT levels. Thus, although an elevated SGOT level is not specific for liver disease, it is used primarily to diagnose and monitor the course of liver disease. IL-12 in very large doses has been known to cause an elevation in serum transaminase levels (7). The IL-12 solution group showed significantly elevated levels of the enzyme on days 10, 20, and 40 when compared to the levels on day 0 (Fig. 8). These differences disappeared in the microsphere group, for which the levels were not significantly different on any of the days.

Creatinine is the waste product of muscle metabolism. Its level is a reflection of the body's muscle mass. Low levels are sometimes seen in people with kidney damage, protein starvation, or liver disease. Elevated levels are sometimes seen in those with kidney diseases due to alteration of the kidney's clearance of creatinine, muscle degeneration, and some drugs involved in the impairment of kidney function. In situations in which kidney damage is not present, creatinine levels serve as a measure of muscle mass (7). In this study, serum creatinine levels were measured to assess the degree of muscle degeneration, a well-documented side effect of IL-12 in large doses or during prolonged therapy.

Serum creatinine levels were measured in mice treated with the IL-12 solutions and microspheres (Fig. 9). The levels were significantly elevated in the mice treated with IL-12 solution on days 10, 20, 40, and 60 when compared to the baseline levels measured on day 0 ($p < .05$). By contrast, serum creatinine levels in mice treated with IL-12 microspheres were not elevated compared to their

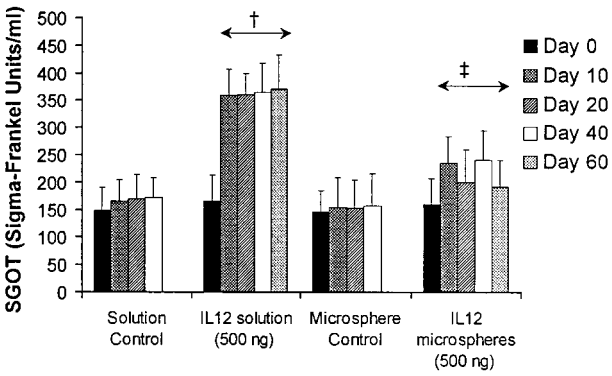

Figure 8. Effect of IL-12 solutions and microspheres on SGOT levels. The animals in the controls were all dead by day 60. Data are represented as means of 10 mice per group (±SD). †, $p < .05$ compared to day 0. ‡, $p < .05$ compared to corresponding days in the IL-12 solution group.

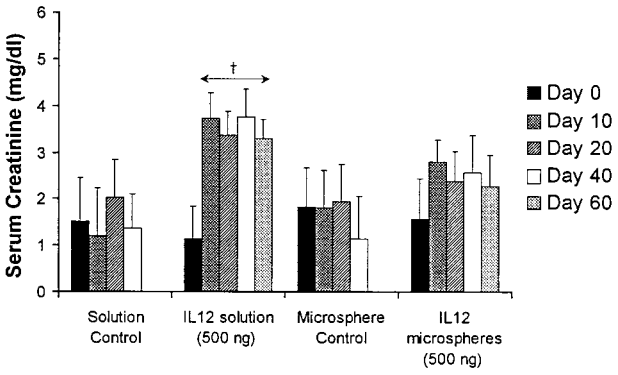

Figure 9. Effect of IL-12 solutions and microspheres on serum creatinine levels. The animals in the control group were all dead by day 60. Data are represented as means of 10 mice per group (±SD). No statistically significant differences were observed.

baseline levels on day 0. Although the enzyme levels in the IL-12 microsphere group did not differ statistically from those in the IL-12 solution group, they appeared to be generally lower on days 10, 20, and 40.

With a reduction in dose, the toxicity is expected to be reduced. Results seen in Figs. 8 and 9 show generally reduced levels of SGOT and serum creatinine with the microsphere dosage form. In general, the elevation observed in these enzyme levels with IL-12 solution is mild and not something of concern. These observations agree with the reports that most toxicities from IL-12 occur at doses far above those used for this study (7). The levels of serum enzymes that were mildly elevated in the solution dosage forms returned to control levels in the microsphere dosage form.

Subcutaneous tumors, as opposed to tumors of vital organs, are not as well perfused and accessible to immune effector cells like the T cells and NK (natural killer) cells, which are critical to the success of IL-12 therapy. Yet, albumin microspheres proved to be partly successful in prolonging survival and keeping the tumor progress slow. These results seem to indicate a very strong potential for the use of albumin microspheres in combination therapy with other agents like chemotherapeutic drugs for such tumors. Since IL-12 microspheres are virtually nontoxic, their combination with these drugs would be no more toxic than the drugs themselves, yet at the same time, the benefits of combination therapy would be realized. On the other hand, IL-12 microspheres by themselves may be enough to produce dramatic results with tumors of well-perfused organs like the lungs and liver. Studies to determine these effects are in progress.

CONCLUSIONS

The IL-12 microsphere formulation proved to be effective in prolonging the survival of the mice at half the dose and dosing frequency of the solution form of IL-12, and it had dose-related reductions in SGOT and serum creatinine levels. The release of IL-12 from the microspheres in vitro was determined to be primarily diffusion mediated and followed Higuchi's square-root-of-time model after the burst effect.

The success seen here in the form of prolonged survival at a reduced IL-12 dose and dosing frequency is a first step toward the formulation of sustained-release dosage forms of IL-12 in lieu of genetically engineered cells that could eventually be used to treat a wide variety of tumors, especially when the constant presence of IL-12 has been demonstrated.

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