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BIODISTRIBUTION AND TARGETING POTENTIAL OF POLY (ETHYLENE GLYCOL)-MODIFIED GELATIN NANOPARTICLES IN SUBCUTANEOUS MURINE TUMOR MODEL

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Abstract

Purpose—In order to develop a safe and effective systemically-administered biodegradable nanoparticle delivery system for solid tumors, the comparative biodistribution profiles of gelatin and poly(ethylene-glycol)-modified (PEGylated) gelatin nanoparticles was examined in subcutaneous Lewis lung carcinoma (LLC)-bearing female C57BL/6J mice.

Methods—Type B gelatin and PEGylated gelatin nanoparticles were radiolabeled (^{125}I) for the *in vivo* biodistribution studies after intravenous (i.v.) administration through the tail vein in LLC-bearing mice. At various time intervals, the mice were sacrificed and blood, tumor, and major organs harvested for analysis of radioactivity corresponding to the localization of the nanoparticles. Percent recovered dose was determined and normalized to the weight of the fluid or tissue sample. Non-compartmental pharmacokinetic analysis was performed to determine the long-circulating property and preferential tumor targeting potential of PEGylated gelatin nanoparticles *in vivo*.

Results—From the radioactivity in plasma and various organs collected, it was evident that the majority of PEGylated nanoparticles were present either in the blood pool or taken up by the tumor mass and liver. For instance, after 3 hours, the PEGylated gelatin nanoparticles were almost 2-fold higher in the blood pool than the control gelatin nanoparticles. PEGylated gelatin nanoparticles remained in the blood pool for a longer period of time due to the steric repulsion effect of the PEG chains as compared to the gelatin nanoparticles. In addition, approximately 4–5% of the recovered dose of PEGylated gelatin nanoparticles was present in the tumor mass for up to 12 hours. The plasma and the tumor half-lives, the mean residence time, and the area-under-the-curve of the PEGylated gelatin nanoparticles were significantly higher than those for the gelatin nanoparticles.

Conclusions—The results of this study show that PEGylated gelatin nanoparticles do possess long circulating properties and can prefentially distribute in the tumor mass after systemic delivery.

Keywords

PEG-modified gelatin nanoparticles; biodistribution; tumor targeting; Lewis lung carcinoma; non-compartmental pharmacokinetic analysis

INTRODUCTION

For efficient systemic delivery of drugs and genes in solid tumor therapy, they must be able to traverse through the circulatory system and reach the tumor mass in sufficient concentration, get transported across the microvessels, and diffuse into the interstitial space [1].

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Unfortunately, the irregular blood supply, high interstitial pressure, low pH and hypoxia, and lack of lymphatic system contribute to inefficient drug uptake and distribution in the tumor mass after systemic administration [1]. The problem of optimum delivery to tumors is further compounded by newer generation of pharmaceuticals, developed through advances in molecular biology and genetic engineering, which are hydrophilic macromolecules based on protein and nucleic acid chemistry, that inherently have very poor diffusional properties.

The tumor vasculature consists of blood vessels formed by secreted pro-angiogenic factors such, as vascular endothelial growth factor (VEGF), from the tumor cells and those recruited from the pre-existing network of the host vasculature [2]. The resulting blood capillaries around the tumor become dense, highly disorganized, tortuous, unpredictable in both structure and function, and leaky. Hydrostatic pressure inside the tumor mass is significantly higher than in the vasculature [2,3]. Due to the pressure gradient, drugs administered in the systemic circulation cannot diffuse evenly within the tumor mass. In addition, the tumor metabolic profile is different due to poor perfusion resulting in elevated levels of lactic acid, and hence, a reduction in pH from 7.4 to about 6.5 [4]. Finally, in most normal tissues, the extravasated drug is taken up by the lymphatic system and returned back into the circulation. Due to lack of functional lymphatic system in tumors, the drug oozing out of the tumor mass will be diluted in the tissue space that surrounds the tumor, thereby reducing the efficacy of the agent against the tumor [5].

Due to the hyperpermeability of the tumor vasculature and the lack of lymphatic drainage, blood-borne polymeric conjugate and nanoparticles are preferentially distributed in the tumor due to the *enhanced permeability and retention (*EPR) effect [6]. The concentrations of polymer-drug conjugates in tumor tissues can reach levels of 10–100 times higher than would be seen after administration of the free drug [6,7]. The enhanced vascular permeability of the tumor, developed through secretion of vascular permeability factors such as bradykinin, VEGF, and nitric oxide, allows for preferential uptake and increased residence time for polymeric drugs and colloidal systems in the vicinity of the tumor mass [8]. Using poly(ethylene glycol) (PEG)-modified liposomes, Jain's group has shown that the effective vascular pore size of most peripheral human tumors range from 200 nm to 600 nm in diameter, with a mean of about 400 nm [1,2].

Systemically-administered liposomes and nanoparticles are rapidly cleared from the circulation by a process of opsonization, which is initiated by complement activation and preferential uptake of the nanoparticles by the organs of reticuloendothelial system (RES) [9–15]. For delivery to solid tumor, surface modification of nanoparticles with water soluble polymers, such as PEG affords long circulation time and passive targeting potential to the tumor mass [16]. Surface-bound PEG chains prevents protein binding, complement activation, and preferential uptake by the RES through steric repulsion mechanism [16]. In a review article Moghimi *et al.*, [17] have extensively discussed the development and applications of long-circulating and target-specific nanoparticles.

In a previous publication, we have described the preparation and characterization of PEG-modified (PEGylated) gelatin nanoparticles as a hydrophilic, biocompatible, biodegradable matrix for delivery of hydrophilic molecules, such as plasmid DNA, to solid tumors [10]. Type B gelatin was modified with PEG-epoxide and nanoparticles were formed, under controlled temperature and pH, by solvent displacement using water-ethanol mixture. Further studies showed that these nanoparticles could efficiently encapsulate plasmid DNA and protected the payload, were internalized in cells by non-specific endocytosis and transported to peri-nuclear region within 12 hours, and resulted in high transgene expression efficiency in NIH-3T3 mouse fibroblast cells [18].

To determine the fate of intravenously (i.v.) administered radiolabeled PEGylated gelatin nanoparticles in solid tumor model, in the present study, we have investigated the long-circulating property in plasma and the biodistribution profile in Lewis lung carcinoma (LLC)-bearing female C57BL/6J mice.

MATERIALS AND METHODS

1. Materials

Type-B gelatin (225 bloom strength) with 100–115 millimoles of free carboxylic acid per 100 g of protein, an isoelectric point of 4.7–5.2, and an average molecular weight 40,000–50,000 daltons was purchased from Sigma Chemical Company (St Louis, MO). Monomethoxy-poly (ethylene glycol) (PEG) with a molecular weight of 5,000 daltons was obtained from Fluka Chemika/Biochemika (Ronkonkoma, NY). Carrier free sodium 125-iodide radionuclide ($Na^{125}I$) used for the iodination of control gelatin and PEGylated gelatin nanoparticles was purchased from Perkin Elmer Life Sciences, PEL (Boston, MA). IODO-Gen® (1,3,4,6-tetrachloro-3α-6α-diphenylglycouril), pre-coated tubes were ordered from Pierce Biotechnology (Rockford, IL). All aqueous solutions were prepared exclusively in deionized distilled water (Nanopure II, Barnstead/Thermolyne, Dubuque, IA).

2. Preparation and Characterization of Nanoparticles

PEG-modified gelatin was synthesized by reacting Type-B gelatin with PEG-epoxide as previously described [10]. Nanoparticles of the unmodified gelatin and PEGylated gelatin derivative were prepared by the ethanol precipitation method under controlled conditions of temperature and pH [10]. Briefly, two-hundred mg of either gelatin or PEGylated gelatin derivative (with 30% of the available amine groups modified) was dissolved in 20 ml of deionized distilled water at 37°C until a clear solution was obtained. The pH of the resulting solution was adjusted to 7.00 with 0.2 M sodium hydroxide. Nanoparticles were formed by gradual displacement of the water with ethanol under controlled stirring conditions. In the final hydroalcoholic mixture of 100 ml, the ethanol to water volume ratio was maintained at 65:35. The formed gelatin or PEGylated gelatin nanoparticles were crosslinked with 1.0 ml of 40% (w/v) glyoxal at room temperature for 10 minutes. The amount of crosslinking agent was optimized based on prevention of the dissolution of the control and PEGylated gelatin nanoparticles in phosphate buffered saline (PBS, pH 7.4) at 37°C for at least 2 hours. Any unreacted aldehyde groups of glyoxal were quenched with aqueous 12% (w/v) sodium metabisulfite and the nanoparticle precipitate was centrifuged at 14,000 rpm for 90 minutes, washed twice with deionized distilled water, and were lyophilized. The nanoparticles were characterized by Coulter particle size analysis as previously described [10].

3. Radiolabeling of Nanoparticles with ^{125}Iodine

Ten-mg of the control and PEGylated gelatin nanoparticles were suspended in a small volume (1.5 ml) of alkaline borate buffer (pH 8.5) sufficiently to hydrate the sample. We intentionally kept the aqueous buffer volume at a minimum to increase the efficiency of radioiodination of the nanoparticles. The suspension was then added to a precoated IODO-gen® tube and mixed by votexing to insure that the nanoparticles had uniformly contacted the tube surface. $Na\text{-}^{125}I$ (2.5 mCi) was added to the nanoparticle suspension at 37°C and the system was mixed by vortexing. The radioiodination reaction proceeded for 30 minutes at room temperature with periodic mixing. At the completion of the reaction, 50 μl of tyrosine solution (10 mg/ml) was added as a scavenger of free ^{125}I. In addition, the nanoparticles were centrifuged at 5,000 rpm for 10 minutes and washed with a 65:35 ethanol-water mixture. Each wash was analyzed for radioactivity the radioiodine was considered bound to the nanoparticles when the supernatant radioactivity had reached a minimum value. The remaining radioactivity on the nanoparticles was assumed to be tightly bound. The nanoparticles were dried under inert nitrogen gas stream

overnight in the hood. The specific activity of the final gelatin and PEGylated gelatin nanoparticles was 0.40 μCi per mg and 0.28 μCi per mg respectively. In addition, the stability of ^{125}I label bound to the nanoparticles at pH 7.4 was verified by incubating the radiolabeled nanoparticles in PBS at 37°C for up to 12 hours and periodically removing the supernatant for detection of free label.

4. Biodistribution Studies in Lewis Lung Carcinoma Model

Cell Culture Conditions—Lewis lung carcinoma (LLC) was selected as the model cell line for growing tumors in mice because it is a relatively faster growing tumor cell line and does not cause secondary tumors and metastasis in animals. Lewis lung carcinoma cells, obtained from American Type Culture Collection (ATCC, Rockville, MD), were maintained through culture in Dubelco's modified Eagle's medium (DMEM) supplemented with glucose, L-glutamine, HEPES buffer, Pen-strep and fetal bovine serum at 37°C and 5% CO_2. Based on the supporting literature supplied by ATCC, the tumor cell lines were confirmed to be free of mouse bacteria or viral pathogens.

Animals—The animal experiments described here were approved by the Northeastern University's Institutional Animal Care and Use Committee. Female C57BL/6J (5–6 weeks old, ~20 g), purchased from Charles River Laboratories (Wilmington, MA), were used as the animal model for the nanoparticle biodistribution studies. LLC model in C57BL/6J was selected based as our previous experience that allowed for reproducible induction of tumors in these animals [10]. In addition, Torchilin's group has shown that the effective pore size of the blood vessels in LLC-bearing mice is significant smaller than other tumor models [19]. As such, LLC-bearing mice model provided a challenge in formulation design that could be optimized based on the vascular permeability cutt-off size.

Development of the Tumor Model—To initiate subcutaneous tumors, the left hind flank region of each mouse was shaved and disinfected. Approximately 30,000 LLC cells in Hank's balanced salt solution (HBSS) were injected in 100–200 μl subcutaneously in ether-anesthetized C57BL/6J mice. The animals were monitored daily following LLC cells inoculation until subcutaneous tumors were palpable and were approximately 10–15 mm in diameter in approximately one week following inoculation. During the time of tumor development and subsequent administration of control and test nanoparticle formulations, the health of tumor-bearing mice was monitored regularly by measuring their body weight.

In Vivo Biodistribution Studies—Biodistribution studies were performed to determine the differences in the localization between control gelatin and PEGylated gelatin nanoparticles after i.v. administration in tumor-bearing mice. During the study, animals were housed under normal conditions with 12 hours of light and dark cycles and were given access to food and water *ad libitum*. The tumor-bearing mice were divided in groups of 4 for the administration of radiolabeled gelatin or PEGylated gelatin nanoparticles suspension in sterile water. After induction of light anesthesia by inhalation of isoflurane at a dose of 0.3 ml for approximately 15 seconds, the animal received an i.v. dose, via the tail vein, of 0.5 μCi of the gelatin and PEGylated gelatin nanoparticles in 100 μl volume using a 25 gauge, 1 inch butterfly needle fitted to a syringe. After 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 10 hours and 12 hours post-administration of the gelatin and PEGylated gelatin nanoparticle formulations, the mice were sacrificed by CO_2 inhalation. Blood sample was collected from the animals from the retrobulbar plexus and plasma was separated by centrifugation at 2,000 rpm for 20 minutes. The volume of obtained plasma was measured. The tumor mass, heart, lung, liver, kidneys, spleen, and brain were surgically excised from the animals and immediately weighed.

Measurements of Radioactivity in Plasma, Tumor, and Tissues—Radioactivity from the plasma, tumor mass, heart, lung, liver, kidney, spleen, and brain, as well as, the carcass was determined using a Wallac 1470 Wizard™ automatic gamma counter (Wallac Inc., Gaithersberg, MD). The radioactivity, in disintegrations-per-minute (dpm), was converted into μCi using a calibration standard. The total recovered radioactivity from the blood, tumor mass, all the organs, and the carcass was calculated. From the measured radioactivity, the nanoparticle concentrations in plasma, tumor mass, and the excised organs were reported as percent of recovered dose per gram of fluid or tissue. Percent recovered dose was calculated as follows:

$$\% \text{ Dose Recovered} = \frac{\text{Activity Recovered / Gram of Organ} \times 100}{\text{Total Administered Radioactivity Dose}}$$

The total radioactivity recovered from all of the tissues, fluid, and carcass of the animals was accounted for based on the amount of radioactivity injected.

5. Data Analysis

Analysis of the experimental results was performed using a student *t* test with Microsoft Excel® (Redmond, WA) software on Windows® NT computer platform. Non-compartmental analysis of the pharmacokinetic data was performed with WinNonLin® version 1.0 software (Scientific Consulting, Inc., Apex, NC). Statistical significance was evaluated at 95% confidence interval ($p < 0.05$). Mean and standard deviation of the results are reported.

RESULTS AND DISCUSSIONS

1. Properties of the Administered Nanoparticles

Gelatin and PEGylated gelatin nanoparticles of approximately 200 nm in diameter with spherical shape, as shown by scanning electron microscopy [10], were prepared for encapsulation and delivery of plasmid DNA to solid tumors. In addition, previous *in vitro* studies showed that both gelatin and PEGylated gelatin nanoparticles could encapsulate plasmid DNA (EGFP-N1, Clontech) at around 98% efficiency at 0.5% (w/w) loading levels [10]. In addition, the control and DNA-encapsulated nanoparticles had a slight negative surface charge to indicate that the DNA was encapsulated, rather than complexed, with either gelatin or PEGylated gelatin nanoparticles. Electron spectroscopy for chemical analysis confirmed the surface presence of PEG chains in PEGylated gelatin nanoparticles [10]. In addition, *in vitro* release profile of a hydrophilic macromolecular fluorescent dye, rhodamine-dextran (10 kDa), from PEGylated gelatin nanoparticles was significantly altered especially in the presence of the enzyme protease (0.2 mg/ml) in the medium due to steric repulsion by the surface bound PEG chains [10]. Intracellular uptake and trafficking studies of gelatin and PEGylated gelatin nanoparticles in NIH-3T3 fibroblast cells showed that the nanoparticles entered the cells by non-specific endocytosis. After about 12 hours, the PEGylated gelatin nanoparticles were predominantly found intact in the peri-nuclear region of the cells. Lastly, green fluorescence protein (GFP) expression in NIH-3T3 fibroblast cells by plasmid DNA-containing gelatin and PEGylated gelatin nanoparticles was observed under fluorescence confocal microscopy and by flow cytometry. GFP expression was evident after 12 hours of exposure of the DNA-containing nanoparticles and remained stable for up to 96 hours post-transfection. In addition, the percentage cells transfected by PEGylated gelatin nanoparticles increase significantly with time, probably due to the ability of the PEGylated gelatin nanoparticles to improve the intracellular stability of DNA in the presence of DNAse and other endosomal/lysosomal or cytosolic degrading enzymes [18].

For delivery of hydrophilic macromolecules, such as plasmid DNA, to solid tumor, in the present study, we have investigated the biodistribution and tumor targeting potential of PEGylated gelatin nanoparticles in LLC murine model. The gelatin and PEGylated gelatin

nanoparticles used in this study had the same properties, such as particle size and size distribution, shape, and surface charge, as those made previously, with and without plasmid DNA.

In addition, we examined the stability ^{125}I label on the gelatin and PEGylated gelatin nanoparticles in order to insure that the free label was not released *in vivo* after administration in tumor-bearing mice. After washing the nanoparticles to remove any unbound label, the remaining ^{125}I bound to either gelatin or PEGylated gelatin nanoparticles remained stable at pH 7.4 and 37°C.

2. Biodistribution Studies in Tumor-Bearing Mice

Plasma Concentration Versus Time Profile—The plasma concentration, expressed as percent recovered dose normalized to the weight of the fluid sample, versus time profile of the two formulations injected *in vivo* is shown in Figure 1. The main differences in the profiles of the fall in the radiolabel versus time for the two formulations lay in the significantly greater dose recovery in the plasma for the PEGylated gelatin nanoparticles over the control unmodified formulation. This increase in the dose recovered for the PEGylated formulation was seen for up to a total period of 3 hours, after which there was no differences in the plasma concentrations the two formulations. The greater recovery of the dose in the plasma with the PEG-modified nanoparticles as compared to the control was indicative of the long-circulating property of the PEGylated nanoparticles. The PEG-modified nanoparticles were probably capable of avoiding both complement activation and subsequent recognition and uptake by the components of RES. Surface PEGylation was found to prevent RES uptake for as long as 3 hours after which, the nanoparticles probably were taken up by the RES or underwent changes in biodistribution patterns as evident by their tumor and liver profiles. It is seen from the two profiles that at 0.5 hour timepoint, approximately 100% of the radiolabel was recovered from the plasma for the PEG-modified nanoparticles, compared to the 40% recovery for the unmodified nanoparticles. This is also indicative of immediate opsonization of the control unmodified nanoparticles on i.v. administration and immediate uptake by the RES system into organs such as the liver. After 3 hours, the PEGylated nanoparticles probably were digested by serum proteases and the steric repulsion effect by surface PEG chains was not significant.

Tumor Concentration Versus Time Profle— Figure 2 shows the concentrations of ^{125}I-labeled gelatin and PEGylated gelatin nanoparticles in the tumor mass, as expressed by percent recovered dose normalized to the weight the tumor, as a function of time. From this data, it is clear that the PEGylated nanoparticles were found to localize in the tumor at significantly higher concentrations than the gelatin nanoparticles for all of measured the time points. Approximately 4% of the recovered dose of PEGylated gelatin nanoparticles was present in the tumor mass for up to 12 hours post-administration. In contrast, only about 1% of the recovered dose of gelatin nanoparticles was found in the tumor for 12 hours. The ability of the PEGylated gelatin nanoparticles to target the tumor mass in significantly higher concentration can be explained on basis of the passive targeting ability of this formulation. The surface PEGylation of the PEG-modified gelatin formulation allows for longer-circulation time in the plasma when compared to the control gelatin nanoparticles. The long-circulating property allows PEGylated gelatin nanoparticles to preferentially distribute in the tumor mass and retention by the EPR effect of the vasculature.

Liver Concentration Versus Time Profile—The concentrations of gelatin and PEGylated gelatin nanoparticles in liver as a function of time are shown in Figure 3. For the gelatin nanoparticles, the profile indicates almost 50% of the radiolabel localization in the liver as early as the 0.5 hour following i.v. administration. This is indicative of the longer residence time of the PEGylated gelatin nanoparticles in the tumor, probably because of the increase in

hydrodynamic diameter of the nanoparticles because of the surface PEGylation and thus enhanced retention in the tumor compared to that of the control unmodified nanoparticles which were not as effectively retained in the tumor. This enhanced retention in the tumor of the PEGylated nanoparticles is also seen as increased expression in the tumor and decreased liver expression. The gelatin nanoparticles are not retained to the same extent as the PEGylated nanoparticles and thus show comparatively less expression in the tumor and a greater liver expression.

3. Non-Compartmental Pharmacokinetic Analysis of Plasma and Tumor Data

Pharmacokinetic Analysis of Plasma Data—Non-compartmental pharmacokinetic analysis of the plasma concentration versus time profiles was carried out and the parameters such as the elimination rate constant, half life, mean residence time, volume of distribution, total body clearance and area-under-the-curve for the two formulations in plasma are reported in Table I. Notable in these results are the approximately 6 fold increase in the area-under the curve for the PEGylated formulation over the control gelatin formulation. This along with the relatively smaller elimination rate constant corresponds to a greater bioavailability of the PEG-modified formulation over the control formulation. This coupled with the observation that the PEGylated formulation has a greater mean residence time in the plasma (36.2 h compared to gelatin nanoparticle's 29.7 h), is a confirmation of the stealth nature of the formulation that allows it to circulate for longer time in the plasma, by avoiding RES uptake.

Pharmacokinetic Analysis of Tumor Data—Non-compartmental pharmacokinetic analysis of the tumor concentrations as a function of time results, shown in Table II. There is an approximately 6.3 fold increase (120 h as compared to the 18.8 h) in the tumor-half life of the PEGylated gelatin nanoparticles over the control formulation and a 5 fold increase in the area-under the curve. These results are again indicative of the enhanced retention of the PEGylated gelatin nanoparticles in the tumor compared to the control. This can be explained on the basis of the EPR effect coupled with the protective steric effect of the PEG chains on the surface that allowed these surface modified nanoparticles to be not only retained better in the tumor because of an increase in the hydrodynamic diameter of the particle that precludes it exit through tumor vasculature but also protects it in the degradative tumor environment.

4. Nanoparticle Biodistribution Profiles in Major Organs

The percent recovered dose of the gelatin and PEGylated gelatin nanoparticles in the spleen, lungs, kidneys, heart, and brain were determined as a function of time. Results from 2 hours, 4 hours, 10 hours, and 12 hours post-administration are shown in Figure 4. Besides plasma, liver, and tumor, the remaining injected dose was predominantly found in the spleen. The concentrations of gelatin and PEGylated gelatin nanoparticles in the lungs, kidneys, heart, or brain was negligible. It is evident from Figure 4 that the gelatin and PEGylated gelatin nanoparticles reach a maximum of about 3.5% of recovered dose per gram of tissue in the spleen in 12 hours. In the case of gelatin nanoparticles, the concentration in the spleen reached 3.5% after 4 hours and remained constant for up to 12 hours. For PEGylated gelatin nanoparticles, on the other hand, the average concentration in the spleen was 2.5% at 4 hour time point and increased to 3.4% after 12 hours. Uptake of nanoparticles in the spleen is also mediated through the RES mechanism and from these results; it seems that the PEG chains were effective in preventing splenic uptake during the earlier time points.

CONCLUSIONS

In the present study, we have examined the biodistribution profiles following intravenous administration of gelatin and PEGylated gelatin nanoparticles to subcutaneous Lewis lung carcinoma-bearing C57BL/6J mice. The results show that PEGylated gelatin nanoparticles

remained in the blood pool for a longer period of time as compared to the gelatin nanoparticles. In addition higher tumor accumulation of PEGylated gelatin nanoparticles was observed, which remained constant for up to 12 hours post-administration. Unmodified gelatin nanoparticles were rapidly removed from the circulation by the RES mechanism that resulted in higher concentrations in the liver and spleen within a few hours of administration. Overall, these results show that PEGylated gelatin nanoparticles were able to remain the circulation and could be effective in passive targeting hydrophilic macromolecules, such as proteins and DNA, to solid tumors.

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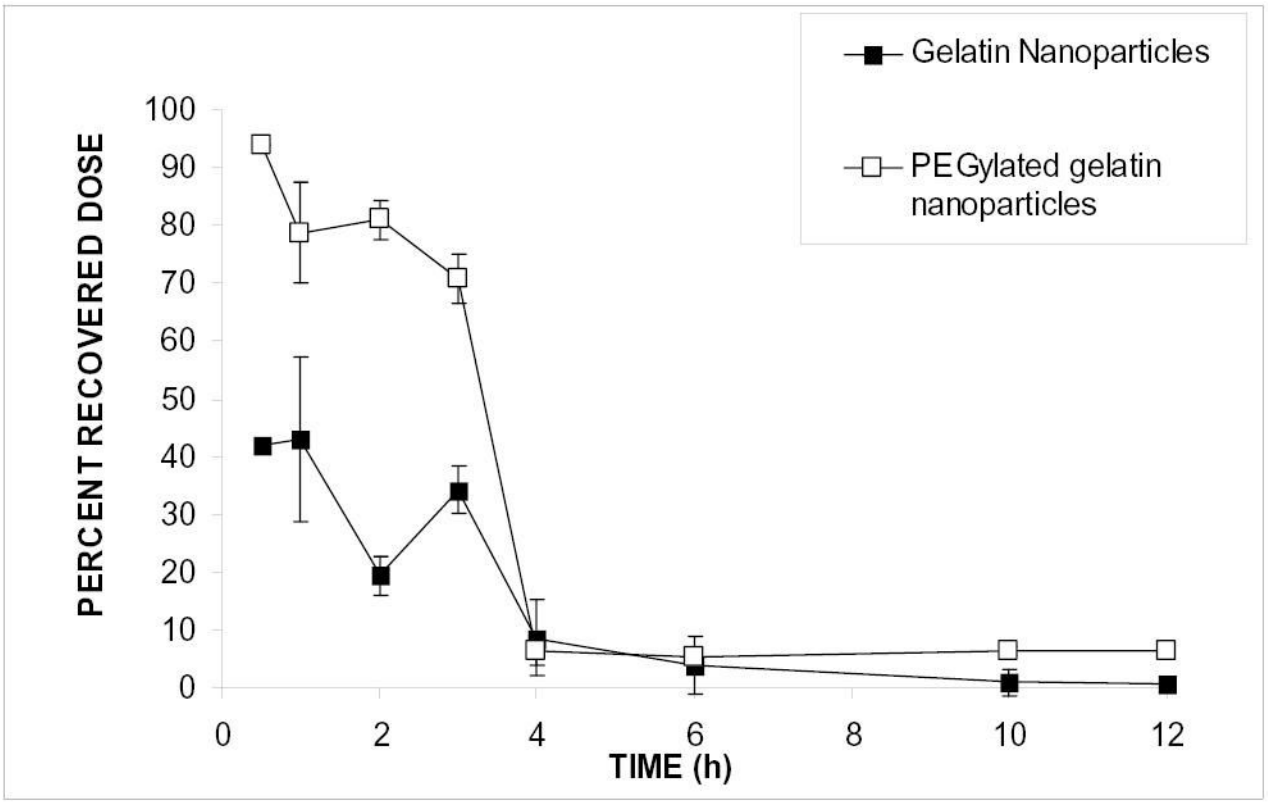

Figure 1.
Percent recovered dose in the plasma per gram as a function of time for the radiolabeled [^{125}I-labeled] gelatin (▪) and PEGylated gelatin (€) nanoparticles administered intravenously in Lewis lung carcinoma-bearing female C57BL/6J mice. Results are expressed as mean ± S.D. (n = 4).

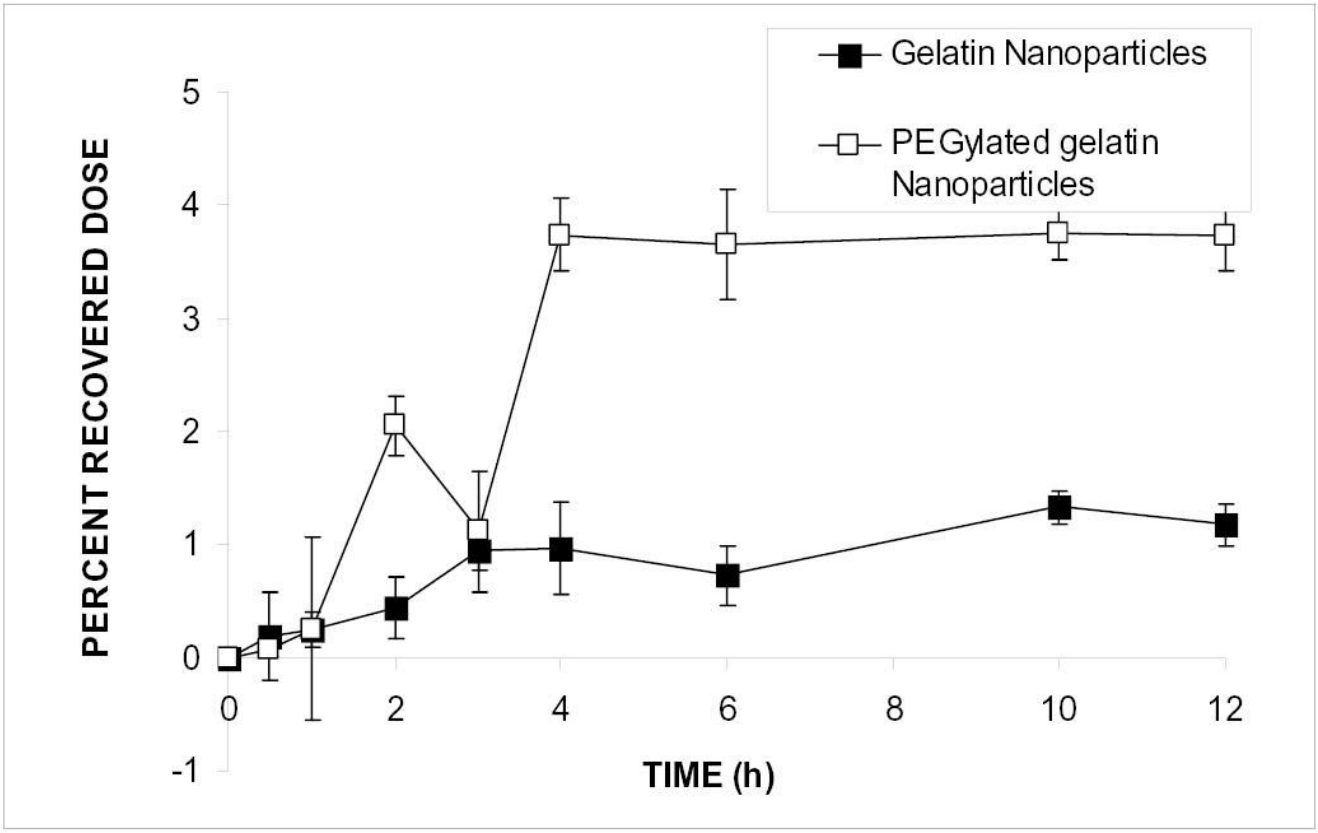

Figure 2.
Percent recovered dose in the tumor per gram as a function of time for the radiolabeled [^{125}I-labeled] gelatin (▪) and PEGylated gelatin (€) nanoparticles administered intravenously in Lewis lung carcinoma-bearing female C57BL/6J mice. Results are expressed as mean ± S.D. (n = 4).

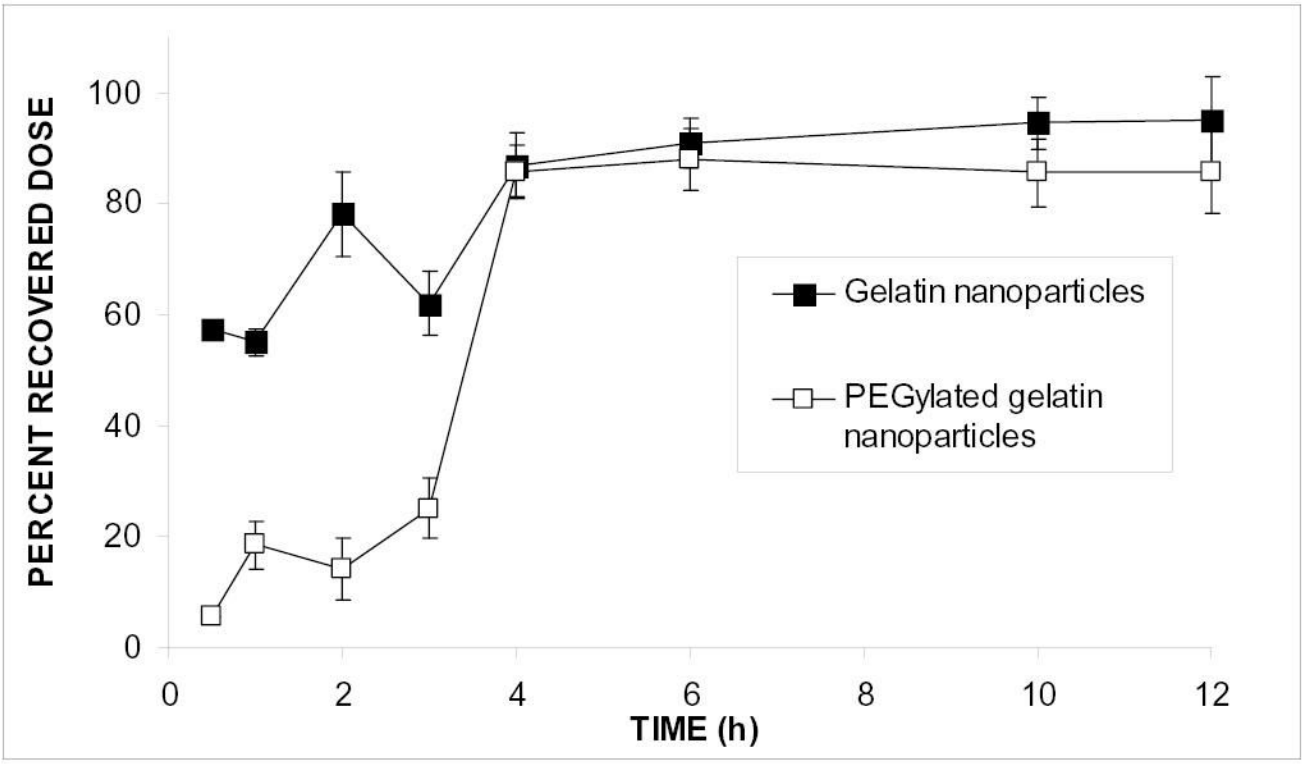

Figure 3.
Percent recovered dose in the liver per gram as a function of time for the radiolabeled [^{125}I-labeled] gelatin (▪) and PEGylated gelatin (€) nanoparticles administered intravenously in Lewis lung carcinoma-bearing female C57BL/6J mice. Results are expressed as mean ± S.D. (n = 4).

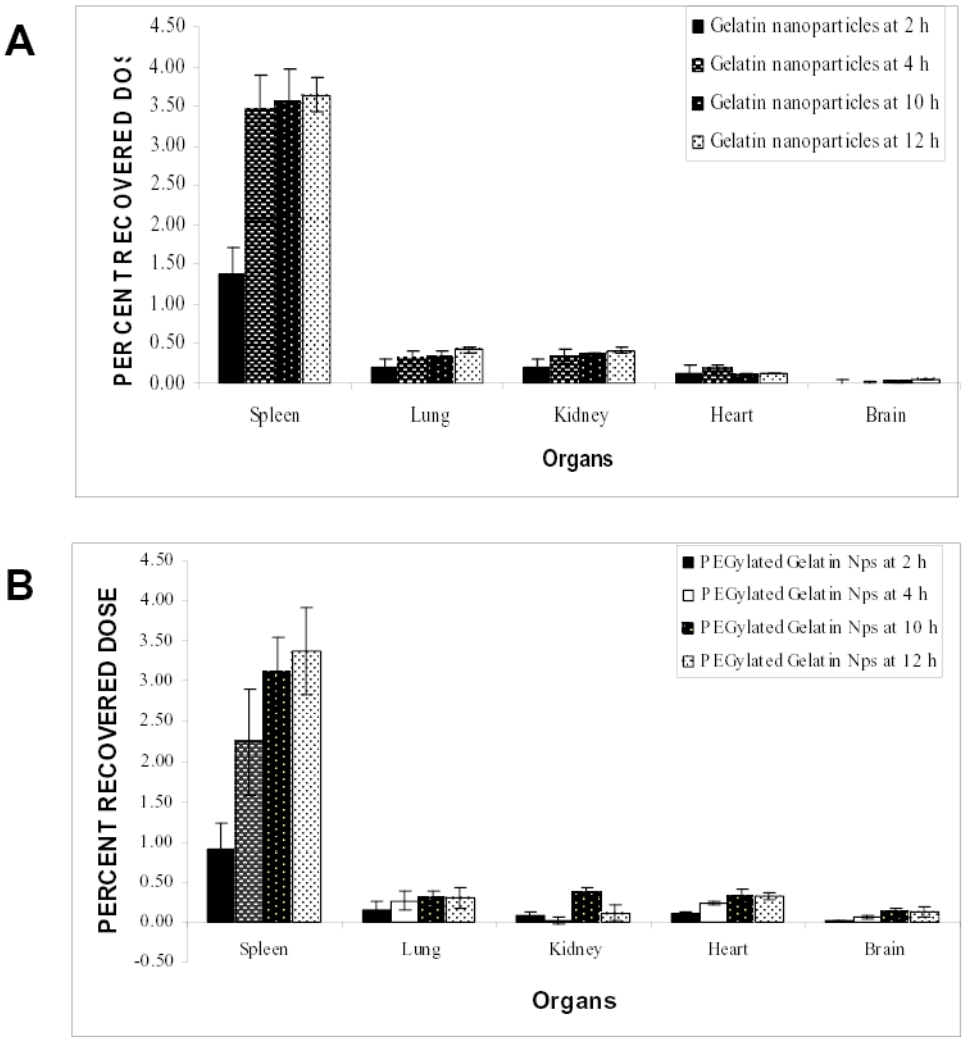

Figure 4.

Percent recovered dose in major organs per gram as a function of time for the radiolabeled [^{125}I-labeled] gelatin (▪) and PEGylated gelatin (€) nanoparticles administered intravenously in Lewis lung carcinoma-bearing female C57BL/6J mice. Results are expressed as mean ± S.D. (n = 4).

Table I

Non-Compartmental Pharmacokinetic Parameters from Plasma for Gelatin and Poly(Ethylene Glycol)-Modified Gelatin Nanoparticles in Lewis Lung Carcinoma-Bearing Mice

Pharmacokinetic Parameter	Gelatin Nanoparticles	PEGylated Gelatin Nanoparticles
Elimination Rate Constant (h^{-1})	0.041 ± 0.006*	0.028 ± 0.003
Half-Life (h)	17.1 ± 1.47	25.1 ± 2.24
Mean Residence Time (h)	29.7 ± 2.14	36.2 ± 6.56
Volume of Distribution (g)	2.44 ± 0.12	0.55 ± 0.01
Total Body Clearance (g/h)	0.099 ± 0.004	0.015 ± 0.006
Area-Under-the-Curve (μCi.h/g)	4.05 ± 0.24	26.2 ± 3.27

* Mean ± S.D. (n = 4)

Table II

Non-Compartmental Pharmacokinetic Parameters from Tumor for Gelatin and Poly(Ethylene Glycol)-Modified Gelatin Nanoparticles in Lewis Lung Carcinoma-Bearing Mice

Pharmacokinetic Parameter	Gelatin Nanoparticles	PEGylated Gelatin Nanoparticles
Elimination Rate Constant (h^{-1})	0.037 ± 0.001*	0.006 ± 0.001
Half-Life (h)	18.8 ± 3.60	120.5 ± 11.7
Mean Residence Time (h)	27.2 ± 4.13	173.9 ± 14.9
Volume of Distribution (g)	10.7 ± 2.24	13.4 ± 0.28
Total Body Clearance (g/h)	0.39 ± 0.12	0.077 ± 0.003
Area-Under-the-Curve (μCi.h/g)	1.02 ± 0.02	5.19 ± 1.10

* Mean ± S.D. (n = 4)