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Research paper

In vivo investigation of tolerance and antitumor activity of cisplatin-loaded PLGA-mPEG nanoparticles

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ABSTRACT

The tolerance of BALB/c mice to different doses of blank and cisplatin-loaded PLGA-mPEG nanoparticles and the in vivo anticancer activity of these nanoparticles on SCID mice xenografted with colorectal adenocarcinoma HT 29 cells were investigated. Nanoparticles with an average size of 150–160 nm and approximately 2% w/w cisplatin content were prepared by a modified emulsification and solvent evaporation method. Normal BALB/c mice tolerated three weekly intravenous injections of a relatively high dose of blank PLGA-mPEG nanoparticles (500 mg/kg, equivalent to about 10 mg nanoparticles/mouse) and three weekly intravenous injections of a high dose of nanoparticle-entrapped cisplatin (10 mg/kg). Also, histopathology examination indicated that there were no differences in the kidneys or spleens from animals treated with cisplatin-loaded nanoparticles or blank nanoparticles compared to the untreated control group. A moderate granulation of protoplasm of hepatic cells was observed in the livers from mice treated with cisplatin-loaded nanoparticles and blank nanoparticles, however, both the hepatic lobe and the portal hepatis maintained their normal architecture. The cisplatin-loaded PLGA-mPEG nanoparticles appeared to be effective at delaying tumor growth in HT 29 tumor-bearing SCID mice. The group of mice treated with cisplatin-loaded nanoparticles exhibited higher survival rate compared to the free cisplatin group. The results justify further evaluation of the in vivo antitumor efficacy of the PLGA-mPEG/cisplatin nanoparticles.

1. Introduction

Cisplatin is one of the most potent anticancer agents available today and is widely used in the treatment of many malignancies, including testicular, ovarian, bladder, head and neck, small cell and non-small cell lung cancers [1,2]. However, its use is associated with severe side effects, such as acute nephrotoxicity and chronic neurotoxicity [3]. A more selective administration (targeting) of cisplatin to cancer cells would reduce drug toxicity and enhance its therapeutic potential. Passive targeting of anticancer drugs to tumors could be achieved by attaching them to long-circulating soluble or particulate carriers taking advantage of the "enhanced permeability and retention" (EPR) effect. The EPR effect is a result of leaky capillaries adjacent to solid tumors and a lack of a lymphatic system for the drainage of drugs back to the systemic circulation [4]. The association of drugs with long-circulating carriers alters drug pharmacokinetics and results in increased drug accumulation in tumors, based on the EPR effect. For a more selective delivery to tumors, cisplatin has been administered in the form of soluble drug–polymer conjugates [5–8], or in the form of colloidal carriers, such as pegylated liposomes [9], poly(aspartic) acid-poly(ethylene glycol) micelles [10], and poly(caprolactone)-poly(ethylene glycol) or poly(caprolactone)-poly[2-(*N*,*N*-dimethylamino)ethyl methacrylate] micelles [11].

We have developed long-circulating PLGA-mPEG nanoparticles of cisplatin for the passive targeting of cisplatin to tumors. The PLGA-mPEG nanoparticles were investigated as potential intravenous carriers of cisplatin because they have a number of important characteristics with regard to their application in drug targeting, such as biocompatibility, biodegradability, and persistence in blood after intravenous administration in experimental animals [12–14]. We showed that the intravenous administration of PLGA-mPEG nanoparticles loaded with cisplatin into mice resulted in prolonged cisplatin residence in systemic circulation [15]. We also showed that PLGA-mPEG nanoparticles loaded with cisplatin entered prostate cancer cells and exerted in vitro anticancer activity that was comparable to the activity of free (non-entrapped in

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nanoparticles) cisplatin [16]. In this communication, we report data on the tolerance of BALB/c mice in different doses of blank and cisplatin-loaded PLGA-mPEG nanoparticles and the in vivo anticancer activity of cisplatin-loaded PLGA-mPEG nanoparticles on SCID mice bearing HT29 colon adenocarcinoma tumors.

2. Materials and methods

2.1. Materials

DL-Lactide (LE) and glycolide (GE) were purchased from Boehringer Ingelheim (Germany). They were recrystallized twice from ethyl acetate and dried under high vacuum at room temperature before use. Monomethoxy-poly(ethyleneglycol) (mPEG, molecular weight: 5000) was obtained from Sigma (St. Louis, MO) and dried under high vacuum at room temperature before use. Cisplatin (cis-platinum diamine dichloride), stannous octoate and sodium cholate were also obtained from Sigma. Miscellaneous chemical reagents and solvents, all of analytical grade, were obtained from Sigma (St. Louis, MO) and Merck (Darmstadt, Germany).

2.1.1. Cell lines and animals

Human Caucasian colon adenocarcinoma grade II (HT 29) cells were purchased from the ATCC. HT 29 cells were maintained and cultured in RPMI 1640 containing 10% fetal calf serum in a humidified atmosphere containing 5% CO_2 at 37 °C. BALB/c (male; 20–28 g body weight; 6 weeks old) and SCID mice (male and female; 16–27 g body weight; 6 weeks old) were provided by Hellenic Pasteur Institute.

2.2. Preparation of PLGA-mPEG copolymer

A diblock PLGA(16)-mPEG(5) copolymer (numbers in parentheses indicate the molecular weight of the blocks in kDa) was synthesised by polymerization of DL-lactide and glycolide in the presence of mPEG. Briefly, lactide (5 g), glycolide (1.34 g), and mPEG (2 g) were dissolved in toluene (pre-dried with molecular sieves) in a three-necked polymerization flask. The set-up allowed for solvent refluxing and the continuous flow of dried nitrogen over toluene. The temperature was set at 110 °C. Stannous octoate (0.05% w/w, dissolved in *n*-heptane) was added in the flask and the polymerization was allowed to proceed for 3 h. Then, toluene was evaporated off under a nitrogen stream and the solid residue was dissolved in dichloromethane. This solution was transferred to an excess volume of diethyl ether (dropwise) and the purified copolymer was dried under vacuum at room temperature. The composition of the purified copolymer was determined by ^{1}H NMR. The ratio of lactic, glycolic, and ethylene oxide units in the copolymer was 1:0.35:0.66.

2.3. Preparation and characterization of PLGA-mPEG nanoparticles

PLGA-mPEG nanoparticles loaded with cisplatin were prepared by a modified emulsification and solvent evaporation method. Cisplatin (typically 8 mg) was dissolved in dimethylformamide (500 μl) and the solution was transferred to a PLGA-mPEG solution in dichloromethane (typically 50 mg polymer in 2 ml solvent). The organic phase was transferred to an aqueous solution of sodium cholate (6 ml, 12 mM) and the mixture was probe sonicated at 15 W for 2 min. The o/w emulsion formed was gently stirred at room temperature in a fume hood until the evaporation of the organic solvent was complete. The nanoparticles were purified by centrifugation and reconstitution of the precipitate in fresh water and filtered through a 1.2 μ filter (Millex AP, Millipore). Blank (without drug) nanoparticles were also prepared with the same method.

The size and ζ (zeta) potential of the nanoparticles were determined using non-invasive back scatter technology (NIBS) and microelectrophoresis, respectively, in a Malvern Nano ZS instrument. The ζ potential of the nanoparticles was measured in a phosphate buffered saline (0.01 M, pH 7.4).

The drug content of the nanoparticles was determined using a direct procedure. Samples of nanoparticles (1 ml) were lyophilized and the lyophilized samples were dissolved in dimethylformamide (DMF). The solutions were assayed for drug content by measuring their absorbance at 307.5 nm. The following equation was applied:

$$\% \text{ loading} = W_d/W_{np} \times 100, \quad (1)$$

where W_d is the amount of drug (mg) found in the sample (lyophilized nanoparticles) and W_{np} is the amount (mg) of the sample (lyophilized nanoparticles).

2.4. In vitro cytotoxicity study

The toxicity of cisplatin-loaded PLGA-mPEG nanoparticles and free cisplatin (control to the cisplatin-loaded nanoparticles) against HT29 cells was investigated by the MTT assay [17]. HT29 cells were seeded in 24-well plates at a density of 10,000 cells per well in 500 μl RPMI-1640 supplemented with 10% fetal bovine serum. Twenty four hours after plating, different amounts of a cisplatin solution in water or cisplatin-loaded nanoparticles (suspended in water) were added in the wells. After 24 h of incubation at 37 °C, 50 μl of MTT solution (5 mg/ml in PBS pH 7.4) was added into each well and the plates were incubated at 37 °C for 3 h. The medium was withdrawn and 200 μl acidified isopropanol (0.33 ml HCl in 100 ml isopropanol) was added in each well and agitated thoroughly to dissolve the formazan crystals. The solution was transferred to 96-well plates and immediately read on a microplate reader (Bio-Rad, Hercules, CA, USA), at a wavelength of 490 nm. The experiments were performed in triplicate and repeated three times. Cell viability was calculated from the ratio between the absorbance provided by the cells treated with the different cisplatin formulations and the absorbance provided by non-treated cells (control).

2.5. Evaluation of the tolerance of BALB/c mice to different doses of nanoparticles

Five groups ($n = 3$) of male BALB/c mice were used to evaluate the tolerance of BALB/c mice to different doses of nanoparticles. All groups received three intravenous injections at 7-day intervals. Three groups of mice received cisplatin-loaded nanoparticles with a cisplatin content of 2, 5 or 10 mg cisplatin/kg. The fourth group of mice received blank nanoparticles at a dose of 500 mg/kg, i.e. equivalent to the nanoparticles dose in the group of mice treated with the maximum cisplatin-loaded nanoparticles. The final group of mice ($n = 3$) received 100 μl saline (0.9% NaCl) served as control. The injection volume was 100 μl in all cases. The weight and physical state of the mice were monitored for a 3-week period, which covered the treatment period plus 1 week after the third injection. All animal studies were approved by the Ethics Committee of Hellenic Pasteur Institute.

2.6. Histopathology evaluation

One week after the third injection, mice were sacrificed and the following tissues were dissected for further histopathological analysis: spleen, liver, and kidney. Samples were fixed for 3 h in 4% paraformaldehyde (BDH) and washed extensively with PBS overnight. Tissues were paraffin embedded (BDH) and sections were cut at 4 μm thickness. Sections were de-paraffinised and

dehydrated in xylene and ethanol (Sigma) and stained with hematoxylin and eosin (Sigma) to assess histological alterations using a Leica DMLB microscope.

2.7. In vivo antitumor activity

HT 29 tumor cells (2×10^6 cells per mouse) were injected subcutaneously into the left flank of SCID mice. Fifteen days later, the mice (at groups of 6–8) were injected intravenously via the tail vein five times at weekly intervals with free cisplatin (100 μl of aqueous cisplatin solution) at a dose of 5 mg/kg or cisplatin-loaded nanoparticles at the same dose (100 μl nanoparticles, 5 mg/kg on a cisplatin basis). Control groups in which blank nanoparticles (same polymer amount as in the cisplatin-loaded nanoparticles group, i.e. 250 mg/kg) or saline (100 μl) was administered, were also included in the study. The antitumor activity was evaluated in terms of the tumor size at different times of post-administration, which was estimated by the following equation: $V = (W) \times (0.5L)^2$, where (W) and (L) are the width and the length of the tumor measured by a digital caliper, respectively. The body weight of the mice was measured simultaneously as an indicator of systemic toxicity. The survival rate (percent surviving mice) was also calculated at different times of post-administration. When tumors reached 2.0 cm in any orientation mice were sacrificed and counted as deceased for the purposes of the survival curves [18].

2.8. Statistical analysis of the data

Appropriate statistical procedures (Student t-test for means, Statgraphics plus 3.3 software) were applied in the statistical analysis of the experimental data.

3. Results

3.1. Characteristics of cisplatin-loaded PLGA-mPEG nanoparticles

The basic characteristics of the nanoparticles used in the in vivo experiments are presented in Table 1. The average size of the cisplatin-loaded nanoparticles ranged between 153 and 163 nm and their drug loading lied between 1.94% and 2.17% w/w. The average size of the blank nanoparticles was 174 nm. The size polydispersity index (P.I.) values (Table 1) are indicative of polydisperse nanoparticles samples.

3.2. In vitro cytotoxicity of cisplatin-loaded PLGA-mPEG nanoparticles

The in vitro anticancer cytotoxic activity of free cisplatin and cisplatin-loaded PLGA-mPEG nanoparticles on HT 29 cells, expressed as % cell viability, is shown in Fig. 1. The nanoparticles loaded with cisplatin exhibited in vitro anticancer activity comparable to that of free cisplatin (differences non-significant, $p > 0.05$, at all concentrations tested). The cytotoxic activity of both free and nanoparticle-entrapped cisplatin increased with increasing cisplatin concentration. In the case of the cisplatin-loaded nanoparticles the carrier itself did not contribute to cytotoxicity as blank nanoparticles with a polymer content identical to that used in cisplatin-loaded nanoparticles (Fig. 1) did not reduce cell viability (data not shown).

Table 1
Basic characteristics of nanoparticles used in the in vivo studies

Batch	Average size (Z average, nm)	Size polydispersity index (P.I.)	% Loading with cisplatin (% w/w)
A	153	0.201	2.17
B	163	0.226	1.94
C (blank)	174	0.239	–

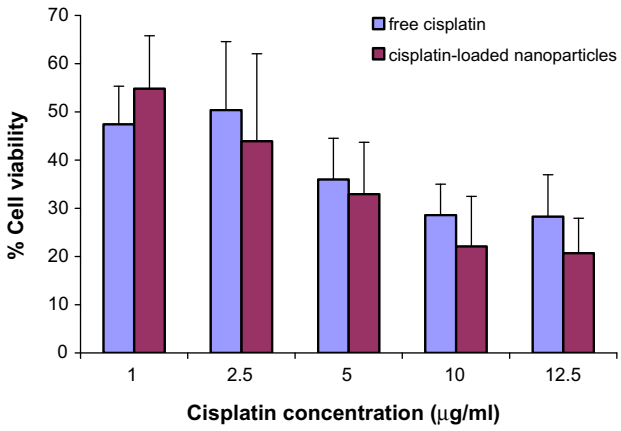

Fig. 1. Cytotoxicity of free cisplatin and cisplatin-loaded PLGA-mPEG nanoparticles on HT 29 cells. Mean values and standard deviations are presented ($n = 9$).

3.3. Evaluation of the tolerance of BALB/c mice to different doses of nanoparticles

In order to assess for potential toxicity, BALB/c mice were intravenously injected with different doses of cisplatin-loaded PLGA-mPEG nanoparticles at 7-day intervals and changes in overall body weight were examined (Fig. 2). In all three dose levels tested (2, 5, and 10 mg/kg on a cisplatin basis), the weight of mice did not change significantly ($p > 0.05$) during a period of 3 weeks. A small reduction of weight (not statistically significant) was observed only in the group of mice treated with the highest dose (10 mg/kg) of cisplatin-loaded nanoparticles. Also, the weight of the control mice treated with 500 mg/kg of blank nanoparticles at 7-day intervals did not change significantly ($p > 0.05$). During the observation period, no deterioration in health was witnessed in mice treated with the cisplatin-loaded nanoparticles or the blank nanoparticles and the overall behaviour was no different to that observed in untreated animals.

One week after the final injection selected organs (liver, kidney, and spleen) were removed from the treated mice for histopathology analysis. A moderate granulation of protoplasm of hepatic cells was observed in the livers from mice treated with both cisplatin-loaded nanoparticles and blank nanoparticles (Fig. 3). This accumulation of exogenous compound/material most probably represents the nanocarrier under examination. However, despite nanoparticles accumulation, both the hepatic lobe and the portal hepatis maintained their normal architecture. There were no differences observed in the kidneys or spleens from animals treated with cisplatin-loaded nanoparticles or blank nanoparticles compared to the control group of mice treated with saline (Fig. 3).

3.4. In vivo antitumor activity of cisplatin-loaded PLGA-mPEG nanoparticles

Free cisplatin (5 mg/kg) and cisplatin-loaded PLGA(16)-mPEG(5) nanoparticles (5 mg/kg on a cisplatin basis, approx. 250 mg nanoparticles/kg) were administered intravenously at 7-day intervals to SCID mice bearing HT29 colon adenocarcinoma tumors. After the fourth dose, the size of tumors in the groups receiving cisplatin-loaded nanoparticles ($n = 8$) were significantly lower than the tumor size observed in the control groups of mice, i.e. mice in which blank (unloaded) nanoparticles ($n = 7$; $p = 0.031$, day 25) or saline ($n = 5$; $p = 0.022$, day 29) had been administered (Fig. 4).

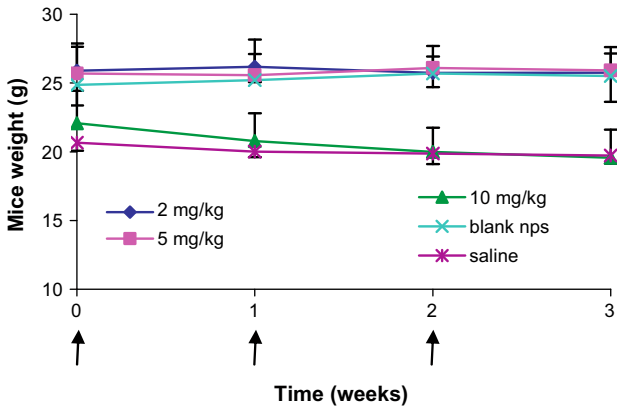

Fig. 2. Body weight with time of BALB/c mice in which different doses of cisplatin-loaded PLGA-mPEG nanoparticles (2, 5, and 10 mg/kg on a cisplatin basis), blank (unloaded) nanoparticles (500 mg/kg) and saline (100 μl) were administered at 7-day intervals (arrows indicate injections times). Mean values and standard deviations are presented ($n = 3$).

However, treatment with free cisplatin ($n = 7$) was more effective at preventing tumor growth (Fig. 4), with tumor reduction being significant at all times after the 17th day when compared to the saline control group ($p < 0.03$). After the fifth dose tumors from treated mice began to ulcerate and were difficult to measure. Therefore, measurement of tumors' size was discontinued on day 29 (last measurement, Fig. 4). The animals treated with free cisplatin or nanoparticles-entrapped cisplatin first began to display ulcerated tumors 25 days after the treatment began, and by the 29th day 2/5 mice treated with blank nanoparticles, 4/5 mice treated with free cisplatin, and 1/7 mice treated with cisplatin-loaded nanoparticles had ulcerated tumors, whereas no ulceration was witnessed in the saline control group (0/4). At this point, the overall health of surviving mice was monitored closely and mice were sacrificed if any deterioration was witnessed. Ulcerated tumors did not continue to grow and a degree of shrinkage was observed so that by the end of the tumor-measurement period (day 29), both the cisplatin-loaded nanoparticles and free cisplatin had caused some shrinkage of tumors in the surviving mice (Fig. 4).

Overall, the group of cisplatin-loaded nanoparticles exhibited higher survival rate compared to the other groups (Fig. 5). At the end of the observation period (day 40), 4/8 mice had survived in the group of cisplatin-loaded nanoparticles, 1/7 in the free cisplatin-treated group, 2/8 of the mice treated with blank nanoparticles and 1/6 for the control (saline-treated) mice (Fig. 5). However, it should be noted that 5/6 of the mice in the saline control group were sacrificed during the course of the experiment because their tumors had reached a size of 2.0 cm or above in at least one dimension.

As an indication of systemic toxicity, the change of body weight during the course of the treatment was recorded (Fig. 6). In the control groups of mice, i.e. the mice treated with saline or blank nanoparticles, there was no significant ($p > 0.05$) change of body weight during the observation period. In the mice treated with free cisplatin and cisplatin-loaded nanoparticles, there was a significant ($p < 0.05$) drop of body weight after the second dose (day 10 onwards) and the fourth dose (day 25 onwards), respectively (Fig. 6).

4. Discussion

We have considered the feasibility of using long-circulating PLGA-mPEG nanoparticles as carriers for a more selective delivery of cisplatin to tumors. We have shown that the intravenous administration of PLGA-mPEG nanoparticles loaded with cisplatin to mice resulted in a prolonged cisplatin residence in systemic circulation [15] and that PLGA-mPEG nanoparticles loaded with cisplatin exerted in vitro anticancer activity against LNCaP prostate cancer cells that was comparable to the activity of free (non-entrapped in nanoparticles) cisplatin [16]. In this work, we investigated the tolerance of BALB/c mice in different doses of blank and cisplatin-loaded PLGA-mPEG nanoparticles and the in vivo

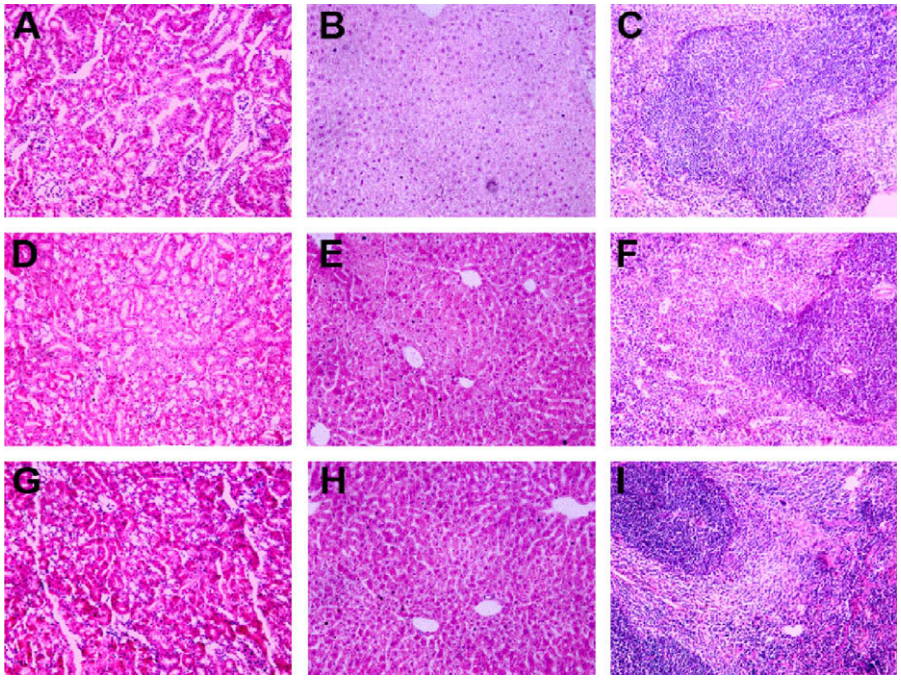

Fig. 3. Histopathology of organs from BALB/c mice treated with blank nanoparticles (500 mg/kg) (A–C), cisplatin-loaded nanoparticles (10 mg/kg on a cisplatin basis) (D–F), or saline (100 μl) (G–I). Liver (A, D, and G), Kidney (B, E, and H) and Spleen (C, F, and I) were removed from treated animals 1 week after the final injection and analysed for abnormalities subsequent to staining with H&E (20× magnification).

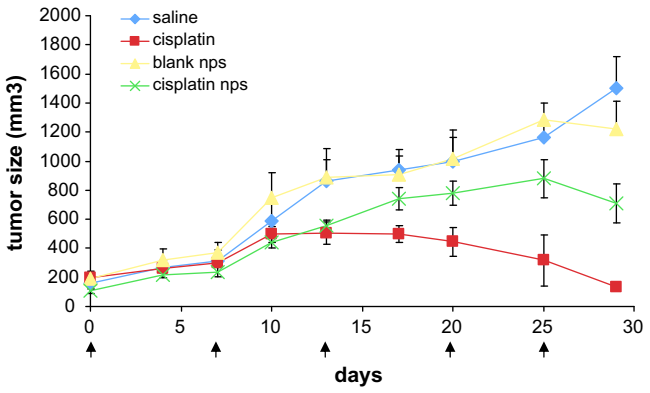

Fig. 4. Effect of free cisplatin (5 mg/kg) and cisplatin-loaded PLGA(16)mPEG(5) nanoparticles (5 mg/kg on a cisplatin basis) on the growth of HT 29 tumors s.c. inoculated to SCID mice. Each formulation was administered five times at 7-day intervals (arrows). Control groups included (a) mice in which blank (unloaded) nanoparticles (250 mg/kg) were administered, and (b) mice in which saline (100 μl) was administered. Mean values and standard errors of the mean are presented (n = 6–8).

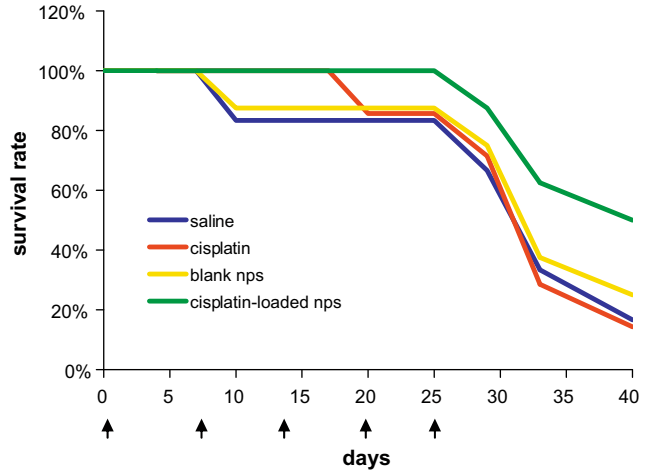

Fig. 5. Survival rates of HT 29 tumor-bearing SCID mice treated with free cisplatin (5 mg/kg), cisplatin-loaded nanoparticles (5 mg/kg on a cisplatin basis), blank nanoparticles (250 mg/kg) and saline (100 μl). The arrows indicate dosing days.

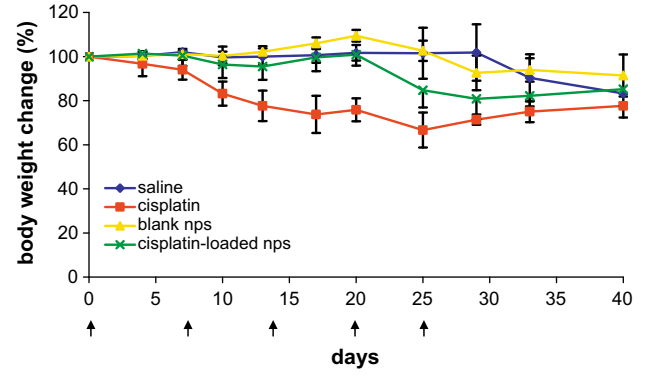

Fig. 6. Body weight change with time of HT 29 tumor-bearing mice treated with free cisplatin (5 mg/kg), cisplatin-loaded nanoparticles (5 mg/kg on a cisplatin basis), blank nanoparticles (250 mg/kg) and saline (100 μl). Each formulation was administered five times at 7-day intervals (arrows). Bars indicate standard deviations.

anticancer activity of cisplatin-loaded PLGA-mPEG nanoparticles in SCID mice bearing HT29 colon adenocarcinoma cell tumors.

Alterations in mouse weight and histopathology were used to evaluate the tolerance of BALB/c mice to different doses of blank and cisplatin-loaded PLGA-mPEG nanoparticles. The mice were injected three times with blank or cisplatin-loaded nanoparticles. In all three dose levels of cisplatin-loaded nanoparticles (2, 5, and 10 mg/kg on a cisplatin basis) examined, and in blank nanoparticles at a dose of 500 mg/kg (corresponding to the polymer dose which was administered to mice when they were injected with the highest dose of cisplatin-loaded nanoparticles, i.e. with 10 mg/kg on a cisplatin basis), the weight of mice did not change significantly. Since no weight loss was observed, the mice appeared to tolerate well three weekly intravenous injections of a relatively high dose of blank PLGA-mPEG nanoparticles (500 mg/kg, equivalent to about 10 mg nanoparticles/mouse) and three weekly intravenous injections of a high dose of nanoparticle-entrapped cisplatin (10 mg/kg). A similar regime (weekly intravenous injections) of 5 mg/kg doses of free cisplatin has been shown to induce systemic toxicity in mice (manifested by weight loss starting from the second dose) [19]. The entrapment of cisplatin in the PLGA-mPEG nanoparticles apparently caused a reduction of cisplatin toxicity. In line with the results from the mice weight measurements, the histopathology data indicated that three weekly intravenous injections of a relatively high dose of blank PLGA-mPEG nanoparticles (500 mg/kg) and three weekly intravenous injections of cisplatin-loaded nanoparticles (at doses of 2, 5, and 10 mg/kg on a cisplatin basis) did not cause alterations in tissue architecture in mouse tissues where cisplatin tends to accumulate at relatively high levels, such as the liver, the spleen and the kidneys (Fig. 3). Based on both the mice weight measurements and the histopathology data it can be deduced that the administration of relatively high doses of blank or cisplatin-loaded PLGA-mPEG nanoparticles were well tolerated by BALB/c mice.

The PLGA-mPEG nanoparticles loaded with cisplatin exhibited in vitro anticancer activity against HT-29 cells comparable to that of free cisplatin (Fig. 1). Enhanced accumulation in tumors of drugs entrapped in long-circulating nanocarriers compared to the free drug has been shown in a number of studies [10,20]. Therefore, having established that the cisplatin-loaded PLGA-mPEG nanoparticles were active against cancer cells in vitro and that they were not toxic to mice at doses containing the necessary amounts of cisplatin to induce a pharmacological response, we proceeded to evaluate the anticancer activity of the cisplatin-loaded PLGA-mPEG nanoparticles in vivo. During the initial phase of the experiment free cisplatin was the most effective agent at preventing tumor growth (Fig. 4). However, the cisplatin-loaded PLGA-mPEG nanoparticles also reduced the growth of tumors when compared to blank nanoparticles and saline controls (Fig. 4). Interestingly, after the fifth dose (day 25) tumors begun to ulcerate and were difficult to measure. Thus reliable tumor size data could only be obtained until day 29 (i.e. 29 days after the first dose). Ulceration of tumors is indicative of necrosis possibly due to a reduction of tumor vascularisation [21].

Although it is one of the most effective anticancer drugs in use today cisplatin confers considerable toxicity to patients. Therefore, it is important that novel technologies are developed to selectively target cisplatin to tumor cells and reduce unwanted side effects caused by its accumulation in healthy tissue. Based on the survival rate (Fig. 5) and the evolution of mice weight during the treatment period (Fig. 6), the cisplatin-loaded PLGA-mPEG nanoparticles appeared to be better tolerated than free cisplatin.

In the SCID mice xenografted with HT29 cells, we chose to administer equal amounts of cisplatin based on a maximum tolerated dose of 5 mg/kg for cisplatin (personal communication T. Boulikas, Regulon A.E.). Given that mice appeared to tolerate cisplatin-loaded nanoparticles better than free cisplatin (as judged by weight and survival), it is important that we further optimise

the dose and injection regime in order to maximise the antitumor efficacy of these cisplatin-loaded nanoparticles. Nonetheless, our initial results indicate that PLGA-mPEG/cisplatin nanoparticles are effective anticancer agents and justify the further study of these agents in vivo.

5. Conclusions

PLGA-mPEG/cisplatin nanoparticles were well tolerated by normal BALB/c mice even when relatively high doses (three weekly injections of 10 mg/kg on a cisplatin basis) were administered to mice. In an in vivo antitumor activity assay, the PLGA-mPEG/cisplatin nanoparticles appeared to reduce tumor growth in SCID mice with HT29 xenografts, and these mice exhibited higher survival rate than free cisplatin. The results justify further evaluation of the potential usefulness of the PLGA-mPEG/cisplatin nanoparticles in cancer treatment.

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