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Antitumor drug Paclitaxel-loaded pH-sensitive nanoparticles targeting tumor extracellular pH

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ABSTRACT

Research efforts have been devoted to demonstrating that the pH-sensitive characteristics of poly NIPAAm/chitosan nanoparticles can be applied to targeting tumors. A copolymer of (NIPAAm) and chitosan (4:1, m/m) was synthesized, and its drug release characteristics investigated. The results revealed that drug-loaded nanoparticles which encapsulation and loading efficiencies were 85.7% and 9.6%, respectively, exhibited pH-sensitive responses to tumor pH. The cumulative release rate was significantly enhanced below pH 6.8 and decreased rapidly above pH 6.9 at 36.5 ± 0.5 °C. MTT assay and fluorescence microscopic study showed that drug release was drastically promoted in tumor surroundings while exerting less effect in normal conditions. For mice treated with nanoparticles, the decrease in body weight was limited, and significant tumor regression was observed with complete regression in more than 50% of the mice. The life span of tumor-bearing mice was significantly increased when they were treated with nanoparticles. Thereby, the super pH-sensitive poly NIPAAm/chitosan nanoparticles may provide outstanding advantages for anti-cancer drug delivery.

1. Introduction

It is well known that most anticarcinogens affect not only rapidly dividing cells such as those in tumors, but also those in highly proliferative normal tissues. This nonspecific drawback has limited the clinical application of most anticarcinogens. For example, as a favorable anticarcinogen, Paclitaxel has not been well utilized due to its side effects, which are induced by nonspecific cytotoxicity (Ayres, Loike, & Lignans, 1990; Rowinsky, Eisenhauer, Chaudhry, Arbuck, & Donehower, 1993; Rowinsky et al., 1991 and Arbuck et al., 1993). Hence, there has been a global quest to develop safe and efficient drug carriers that can deliver anticarcinogen exclusively to the intended site without provoking adverse reactions in disease therapy. To reach the goal, stimuli-sensitive polymers (Kaetsu, Uchida, Sutani, & Sakata, 2000; Qiu & Park, 2001) especially pH- and thermo- sensitive polymers have been extensively studied for drug delivery, because pH and temperature are distinctive environmental factors inside the human body, and some disease states manifest themselves by a change in temperature and/or pH (Ju, Kim, Kim, & Lee, 2002; Martin & Jain, 1994 and Sherwood 1997). The lower extracellular pH in most solid tumors than in the surrounding tissues and blood is now regarded as a phenotype for solid tumors' growth and invasiveness (Conner, Yatvin, & Huang 1984; Greidziak, Bogdanov, Torchilin, & Lasch 1992; Leroux, Roux, Garrec, Hong, & Drummond, 2001; Stubbs, McSheehy, Griffiths, & Bashford, 2000 and Tannock & Rotin, 1989). Consequently, this feature could be used for designing drug delivery systems that facilitate tumor-targeting. Many materials with pH sensitivity have been synthesized to prepare pH-sensitive polymeric nanoparticles or liposomes, and their pH-dependent self-assembly behavior has been extensively studied. However, systems responsive to small fluctuations (6.8–7.2) in pH in physiological conditions are seldom observed. Recently, it was reported that pH-sensitive, self-assembled nanoparticles of pullulan acetate/sulfonamide conjugates might have that possibility in response to tumor extracellular pH (Na & Bae, 2002 and Na, Lee, & Bae, 2003). However, aggregation of these particles might induce cytotoxicity. The cytotoxicity of the pullulan acetate/sulfonamide conjugates to normal cells has not been determined, except for the cytotoxicity of drug-loaded nanoparticles to the breast tumor cell line MCF-7.

In a recent study, we found that a particular ratio in graft copolymerization of N-isopropylacrylamide (NIPAAm) to chitosan could give precise pH-sensitive characteristics in response to tumor extracellular pH, with biocompatibility and nontoxic properties. Poly(N-isopropylacrylamide) (PNIPAAm) is well known to

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have thermal reversibility. It exhibits a lower critical solution temperature (LCST) around 32 °C in aqueous solution (Schild, 1992); that is, it dissolves in water below the LCST and precipitates from water above the LCST. Chitosan and its derivatives are useful polymeric biomaterials in the biomedical area because of their biological properties such as biocompatibility, biodegradability and nontoxicity. Chitosan, in particular, shows pH-sensitive behavior due to the large number of amino groups on its chains (Cerrai, Guerra, & Tricoli, 1996; Chellat et al., 2000; Chenite et al., 2000; Felt et al., 1999 and Hirano, Zhang, Nakagawa, & Miyata, 2000). At present, research and development on the graft copolymer of *N*-isopropylacrylamide (NIPAAm) and chitosan is concentrated on its temperature-sensitive characteristic as a carrier for drug delivery. For instance, Cao et al. reported that poly (N-isopropylacrylamide)-chitosan could be used as a thermosensitive in situ gel-forming system for ocular drug delivery (Cao et al. 2007), and temperature-sensitive hydrogels composed of chitosan and hyaluronic acid as injectable carriers for drug delivery were reported by Fang, Chen, Leu, and Hu (2007). Those gels were also reported by Lee and Yuk (2007) as polymeric delivery systems for protein, based on their thermosensitive characteristics. However, pH-sensitive characteristics of NIPAAm/chitosan copolymer, in response to the extracellular pH of tumor have not yet been reported.

In a previous study, we found that Camptothecin encapsulated in the copolymer system may have super pH-sensitive characteristics in response to the extracellular pH of a tumor, and have the potential to target solid tumors (Li et al., 2008). The present study has been devoted to demonstrating whether Paclitaxel-loaded nanoparticles for targeting tumor cells have the same characteristics. The results show that Paclitaxel-loaded nanoparticles with encapsulation and loading efficiencies 63.7% and 2.4%, respectively, exhibited super pH-sensitive response to tumor pH. MTT assay and fluorescence microscopy confirmed that polyNIPAAm/chitosan nanoparticles drastically promoted drug release in tumor surroundings while exerting little effect on drug release in normal conditions. For mice treated with Paclitaxel-loaded nanoparticles, the decrease in body weight was limited, and significant tumor regression was observed, with complete regression for more than 50% of the mice. In addition, the life span of tumor-bearing mice was significantly increased when they were treated with Paclitaxel-loaded nanoparticles. Hence the super pH-sensitive poly NIPAAm/chitosan nanoparticles may provide unprecedented advantages for anti-cancer drug delivery.

2. Materials and methods

2.1. Materials

NIPAAm (Sigma–Aldrich) was purified by repeated recrystallization from a mixture of toluene and hexane (1:5 v/v), giving spindle-like crystals. Chitosan (medium M_w approximately 75,000 g mol^{-1}, degree of deacetylation: 74%), the crosslinker N,*N*-methylenebisacrylamide (MBA), initiator tert-butyl hydroperoxide (TBHP, 70% solution in water), 1,6-diphenyl-1,3,5-hexatriene (DPH), propidium iodide (PI) and Paclitaxel with purity exceeding 99% were all purchased from Aldrich Chemical Co and used without further purification. Human pulmonary carcinomal cell lines A549 were kindly donated by the Department of Pharmacology, School of Pharmacy, Fourth Military Medical University. All other chemicals and reagents were obtained from Sigma (USA). MTT assay and other biological study reagents were purchased from Promega (USA). Freshly deionized and distilled water was used as the dispersion medium.

2.2. Methods

2.2.1. Preparation of blank and Paclitaxel-loaded polyNIPAAm/chitosan nanoparticles

For a total solution volume of 100 ml, chitosan (0.25 g) was first dissolved in 5 % acetic acid solution. A mixture of purified NIPAAm (1.0 g) and MBA (0.01 g) was added to the chitosan solution and agitated by magnetic stirring under nitrogen for 30 min at 80 °C. Dilute TBHP solution (1.5 ml, 1.0×10^{-2} M) was added dropwise to the mixture, and the solution was stirred at 80 °C for 3 h under nitrogen. The suspension was further purified by introducing it into a dialysis tube with a 100,000 Da molecular weight cut-off (Spectria/Por®CE), and dialyzed against 1 l of water for one week at room temperature with daily changes of water. Dry nanoparticles were obtained by lyophilization and stored at 4 °C for further study.

The procedure for preparing Paclitaxel-loaded poly NIPAAm/chitosan nanoparticles was similar to that of blank nanoparticles with initial addition of Paclitaxel (200 mg) to the solution. The nanoparticles were collected by centrifugation and washed four times with distilled water. Dry nanoparticles were obtained by lyophilization and stored at 4 °C.

2.2.2. Morphological examination of nanoparticles

Transmission electron microscopy (TEM) examination was carried out on a JEM-2010 microscope (Japan) with an electron kinetic energy of 300 keV. The samples were negative stained using phosphotungstic acid, and air-dried at 20 °C for TEM analysis.

Scanning electron microscopy (SEM) was performed using a LEO 1530 (LEO Electron Microscopy Inc., Thornwood, NY) instrument operating between 1 and 3 kV with a filament current of about 0.5 mA. Liquid samples were deposited on vitreous carbon stubs and dried at room temperature. They were then coated with a palladium–platinum layer about 4 nm thick using a Cressington 208HR sputter-coater with a rotary-planetary-tilt stage, equipped with an MTM-20 thickness controller.

Particle sizes of all blank and drug-loaded samples were measured by photon correlation spectroscopy using a Zetasizer 3000 HAS (Malvern Instruments, UK). All samples were diluted with the aqueous phase of the formulation to get optimum kilocounts per second (Kcps) of 50–200 for measurements.

The collected nanoparticles were sealed and deposited in a 4 °C refrigerator or in a desiccator at room temperature for three months. The appearance, morphology and drug content of nanoparticles were used to test the stability of nanoparticles.

2.2.3. Evaluation of encapsulation and loading efficiencies

The drug-loaded nanoparticles were separated from the aqueous suspension medium by ultracentrifugation at 10,000 rpm and 4 °C for 1 h. The amount of free Paclitaxel was measured in the clear supernatant by a reversed-phase (RP) HPLC method. A Shim-Pack CLC-ODS column was used as the stationary phase and methanol:acetonitrile:water (25:40:39) as the mobile phase. Separation was carried out at a flow rate of 1.2 ml min^{-1}. The compound was detected at a wavelength of 227 nm. The standard curve for the quantification for Paclitaxel was linear over the concentration range 50 to 100,000 ng ml^{-1} with correlation coefficient $R^2 = 0.9999$. Loading efficiency and encapsulation efficiency were calculated as follows:

$$\text{Encapsulation efficiency } (\%) = \frac{\text{Weight of drug found loaded}}{\text{Weight of drug input}} \times 100$$

$$\text{Loading efficiency } (\%) = \frac{\text{Weight of drug found loaded}}{\text{Weight of drug} - \text{loaded nanoparticles}} \times 100$$

2.2.4. Evaluation of in vitro Paclitaxel release

Precipitated nanoparticles (0.2 g) were resuspended in 100 ml of phosphate buffered saline solution (PBS; pH 5.0–pH 8.0, with pH intervals 5.0, 6.0, 6.5, 6.9, 7.5, and 8.0) and incubated at 37 °C under light agitation. At hourly intervals, 3 mL aliquots were removed from the release medium and the same volume of fresh PBS at the same temperature was added to the release medium. The samples were assayed for drug content by HPLC according to the standard curve. The results of triplicate measurements were used to calculate cumulative drug release.

2.2.5. Biological evaluation of nanoparticles

Cell culture: The A549 cell cultures were grown using 75 cm^3 flasks in a humidified 5% CO_2/95% atmosphere incubator at 37 °C. For the A549 cells, 500 ml Minimum Essential Medium Eagle, 50 ml fetal bovine serum, 5 ml L-glutamine solution (200 mM) and 0.5 ml gentamicin (50 mg ml^{-1}) were used. The medium was exchanged every 2–3 days.

For the MTT assay, A549 cells were seeded at a density of 1×10^3 cells per well, in 96-well plates in 100 μl of the same medium used for culture in cell culture flasks. The cells were grown at 37 °C in a 5% CO_2 atmosphere for 24 h before use in cell viability assays.

MTT assay: The blank and drug-loaded nanoparticles were assayed for cytotoxicity over 24 and 48 h in A549. Blank nanoparticles were evaluated for cytotoxicity with chitosan as a positive control, and drug-loaded nanoparticles were evaluated with free Paclitaxel as a control. The cells were cultured for 24 h then exposed to blank nanoparticles for another 12 h. After exposure to nanoparticles at various concentrations, the cells were washed three times with RPMI 1640 medium, and 200 μl of RPMI 1640 medium without serum was then added. Cytotoxicity was assessed using MTT to measure the viability of the cells. 50 μl of MTT solution (5 mg mL^{-1}) was added to each well. The plates were incubated for an additional 4 h and then the medium was discarded. 150 μL of DMSO was added to each well, and the solution was vigorously mixed to dissolve the reacted dye. The absorbance of each well was read on a microplate reader (FL600, Bio-Tek, USA) at a test wavelength of 490 nm and reference wavelength 570 nm.

In vitro pH-sensitive cytotoxicity measurement of drug-loaded nanoparticles was done in the same way by initially plating the cells at a density of 1×10^3 cells per well in 96-well tissue culture dishes for 24 h, then exposing them to free Paclitaxel and Paclitaxel-loaded nanoparticles for 48 h at pH 7.4 and 6.8, respectively.

Fluorescence microscopy study: Fluorescent nanoparticles were prepared by adding 10 mg of DPH (0.25% w/v) as fluorescent marker to the reaction solution.

Fluorescent nanoparticles were obtained as described above for drug-loaded nanoparticles. The cellular uptake of nanoparticles was further studied using fluorescence microscopy. A549 cells were grown on coverslips for 24 h in a six-well tissue culture plate at 37 °C. The cells were then incubated for 24 h with the fluorescent nanoparticles at a concentration of 2 mg ml^{-1}. .After rinsing with PBS, the cells were fixed by treatment with 95% ethanol solution for 30 min. The nuclei of the cells were then stained using 5 mg ml^{-1} of PI for 10 min at 37 °C. The stained coverslips were mounted on a glass slide and photographed using a fluorescence microscope (Nikon H660L, Japan). DPH and PI showed blue and orange colors, respectively.

2.2.6. Evaluation of in vivo anticancer activity of nanoparticles

Animals. Female Kunming strain mice (20 ± 2 g) were supplied by the Experimental Animal Center, Fourth Military Medical University, (Xi'an, China). The animals were acclimatized at a temperature of 25 ± 2 °C and relative humidity of 70 ± 5% under natural light/dark conditions for 1 week before dosing.

In vivo anticancer activity was evaluated against S-180-bearing KMmice (female, 6 weeks old, n = 8–10). Mice were inoculated subcutaneously in the armpit with S-180 cells (obtained from the Department of Pharmacology, School of Pharmacy, Fourth Military Medical University, Xi'an, China). After 7 days, Paclitaxel injection and nanoparticle suspensions were given by intravenous injection five times at 2-day intervals, at a dose rate of 20 mg kg^{-1} day^{-1} for 10 days. Group 1 was treated with saline, and groups 2–4 with Paclitaxel injection, Paclitaxel-loaded nanoparticles and blank nanoparticles, respectively. Body weight and tumor volume ([major axis] × [minor axis]^2 × 1/2) were measured at defined time periods. Statistical evaluation of tumor volume was done using Student's t-test.

3. Results and discussion

3.1. Synthesis and characterization of nanoparticles

The nanoparticles were prepared by graft copolymerization of NIPAAm onto chitosan in 80 °C aqueous media. TBHP first interacted with the amino groups on the polymer backbone to form amino and ^{t}BuO radicals. These radicals initiated the graft copolymerization of NIPAAm simultaneously with MBA as crosslinker. The chitosan-*g*-PNIPAAm polymers that were generated acted as surfactants, self-assembling to form nanoparticles. TBHP initiator was used in aqueous solution to incorporate the vinyl monomer (NIPAAm) into the polymer backbone. The most important feature of the oxidation with TBHP is that it proceeds via a single electron transfer with formation of free radicals on the reducing agent. Thus if the reducing agent is a polymeric molecule such as chitosan, and the oxidation is carried out in the presence of a vinyl monomer such as NIPAAm, the free radical produced on the polymeric molecule backbone initiates polymerization to produce a graft copolymer. This method yields substantially pure graft copolymers since the free radicals are formed exclusively on the backbone.

The chemical composition of synthesized nanoparticles was confirmed by FTIR and ^{1}H NMR measurements. Incorporation of NIPAAm was confirmed by the formation of a new peak at a particular wavelength. As shown in Fig. 1A, the typical carbonyl and amino group bands in amide at 1643 and 1548 cm^{-1}, respectively, were observed in the spectrum of the synthesized nanoparticles. Strong evidence for successful incorporation of NIPAAm into nanoparticles was the appearance of characteristic bands of NIPAAm at 1540–1640 cm^{-1} in the FTIR spectra of the synthesized nanoparticles. Comparison of FTIR spectra of polyNIPAAm/chitosan nanoparticles and chitosan

^{1}H NMR spectroscopy measurements were carried out to confirm the graft polymerization of NIPAAm onto chitosan. The spectrum of PNIPAAm (Fig. 1B–a) exhibited two peaks (–CH–CH_2) at 1.00–2.00 ppm, a peak (–NH–CH<) at 3.79 ppm and a strong methyl group peak at 1.10 ppm. The peaks of vinyl protons (5.60–6.20 ppm) disappeared after polymerization of NIPAAm. The spectrum of chitosan (Fig. 1B–b) exhibited typical peaks including the proton on the anomeric carbon (at 4.65 ppm), the methyl protons from partially acetylated chitosan (at 1.99 ppm) and the proton on the carbon bearing amino (partially acetamido) groups (at 3.07 ppm). The spectrum of chitosan-g-NIPAAm copolymer in Fig. 1B–c is similar to that of PNIPAAm except for a weak peak at 1.99 ppm and a proton peak at 3.07 ppm from the carbons

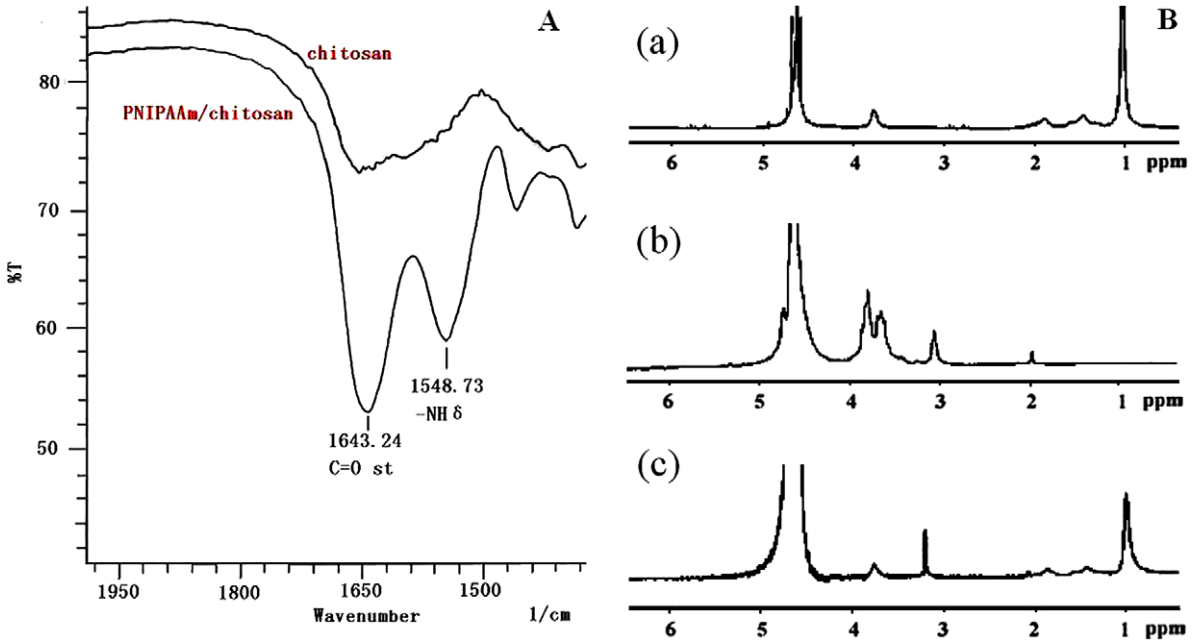

Fig. 1. (A) FTIR spectra of polyNIPAAm/chitosan nanoparticles and chitosan. (B) ^{1}H NMR spectra of (a) PNIPAAm, (b) chitosan, and (c) polyNIPAAm/chitosan nanoparticles.

bearing the amine (partially acetamido) groups of chitosan. It is estimated that a high proportion of PNIPAAm moiety was introduced into the chitosan backbone during the preparation of the water-soluble chitosan copolymer. ^{1}H NMR spectra of (a), PNIPAAm; (b), chitosan; (c), polyNIPAAm/chitosan nanoparticles. Paclitaxel was successfully loaded into poly NIPAAm/chitosan nanoparticles by physical incorporation in the hydrophobic domains of the nanoparticles. Though Paclitaxel contains an ester group in the molecule, it easily degrades and changes into an isomeric form in an alkaline environment. It was established using UV-spectrophotometry and FTIR spectra that after reaction Paclitaxel had not changed its chemical structure in the reaction medium. To increase the drug loading efficiency, 0.5–5% acetic acid was added gradually to meet the need of both the morphology of the nanoparticles and the low solubility of Paclitaxel. Although redundant, acetic acid can improve the solubility of Paclitaxel, possibly by inducing a chemical structure change in chitosan, and thereby changing the characteristics of the material. Moreover, as the removal of acetic acid by dialysis was always accompanied by release of Paclitaxel, dialysis of abundant acetic acid in post-processing could induce decrease of the drug loading efficiency, thus greatly restricting biological applications. During repeated experiments, 5% acetic acid was finally chosen for use, on the basis of repeated test results. HPLC analysis indicated that drug loading efficiency was 9.6% while encapsulation efficiency was 85.7%.

3.2. Morphology of nanoparticles

Spherical nanoparticles with particle diameter in the approximate range 50-200 nm were formed spontaneously on addition of TBHP solution to the reaction solution under magnetic stirring, as observed using TEM (Fig. 2a and b) and SEM (Fig. 2c and d). TEM and SEM micrographs of blank and drug-loaded nanoparticles. (a), TEM of blank nanoparticles; (b), TEM of drug-loaded nanoparticles; (c), SEM of blank nanoparticles; (d), SEM of drug-loaded nanoparticles.

The nanoparticles were stable with good fluidity when sealed and stored under conditions such as refrigeration or at room temperature for three months. The appearance and loading efficiencies of poly NIPAAm/chitosan nanoparticles hardly changed.

3.3. In vitro release studies

Fig. 3 shows the Paclitaxel release profile in 112 h from Paclitaxel-loaded nanoparticles at various pH values (5.0, 6.0, 6.5, 6.9, 7.5 and 8.0) at 36.5 ± 0.5 °C. Whereas the Paclitaxel cumulative release rates showed slight pH dependence (93.32 % to 84.56 %, at pHs from to 6.9, respectively), the cumulative release rates drastically decreased from 84.56% at pH 6.9 to 32.14% at pH 7.5. One of the reasons for that characteristic of pH sensitivity could be that at low pH, NH_2 groups on the chitosan backbone can be easily protonated and the nanoparticles swell, promoting drug release.. Cumulative release rate of Paclitaxel in phosphate pH buffers at 36.5 ± 0.5 °C in 112 h.

Fig. 4 shows that the cumulative release rate differs from normal physiological conditions to tumor extracellular conditions. The cumulative release rate of Paclitaxel was as low as 49.60% in normal physiological conditions, and much higher (92.24%) in tumor extracellular conditions. Fig. 4. Cumulative release rate of Paclitaxel at physiological pH and temperature (pH 7.4, 36.8 °C), and in tumor extracellular pH and temperature (pH 6.8, 37.5 °C).

3.3.1. In vitro biological evaluation of nanoparticles

MTT tests for blank nanoparticles showed that an increase in monomer/polymer concentration from 0.025 to 5 mg ml^{-1} was not harmful for survival of human lung cancer cells A549, compared with chitosan and NIPAAm (Fig. 5). A clear positive correlation was found between cell cytotoxicity (MTT) and concentration of NIPAAm monomers (Fig. 5). Moreover, there was an obvious reduction in cell survival as the concentration increased above 1 mg ml^{-1}. However, conspicuous cytotoxicity of nanoparticles and chitosan was not found in the MTT test, and noticeable cytotoxicity was not found after incubation. This result indicated that the biocompatibility of the nanoparticles was satisfactory. Insert Fig. 5 about here Fig. 5. Cytotoxicity of blank nanoparticles, chitosan and NIPAAm against human lung cancer cells A549 as a

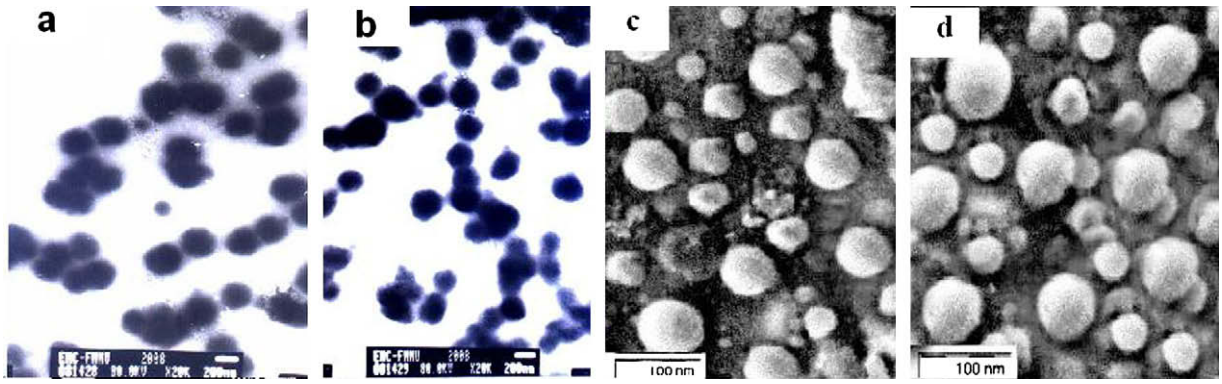

Fig. 2. TEM and SEM micrographs of blank and drug-loaded nanoparticles (a), TEM of blank nanoparticles; (b), TEM of drug-loaded nanoparticles; (c), SEM of blank nanoparticles; (d), SEM of drug-loaded nanoparticles.

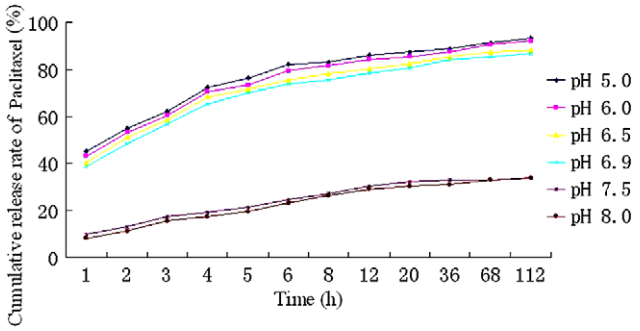

Fig. 3. Cumulative release rate of Paclitaxel in pH phosphate buffers at 36.5 ± 0.5 °C in 112 h.

function of polymer/monomer concentration. Data represent mean ± SD, n = 7.

For detailed observation of the behavior of the nanoparticles with the cells, nanoparticles were labeled with a fluorescence probe and the moving and locating tendency towards cells was monitored by fluorescence microscopy to visualize this interaction. At pH 6.8, blue DPH released from nanoparticles was swallowed by A549 cells because of endocytosis, so the red nucleus became orange. However, at pH 7.2 the amount of DPH released was much smaller than at pH 6.8, so there were still many blue nanoparticles around cells, and the cells were still red. Thus decreasing pH augmented the degree of moving and locating tendency between the nanoparticles and cells (Fig. 6).

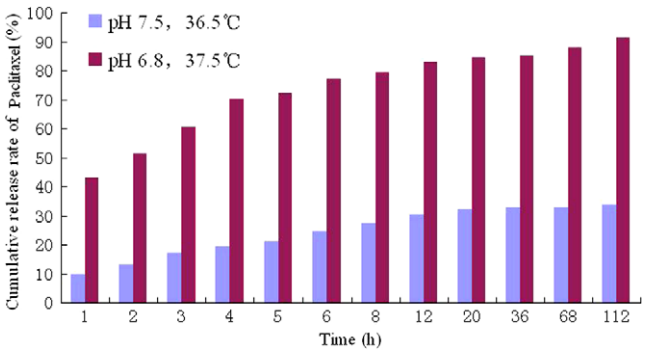

Fig. 4. Cumulative release rate of Paclitaxel at physiological pH and temperature (pH 7.4, 36.8 °C), and in tumor extracellular pH/temperature conditions (pH 6.8, 37.5 °C).

Fig. 6. Luminescence images of A549 cells treated with fluorescent nanoparticles at (a) pH 6.8, and (b) 7.4. The A549 cells were incubated at 37 °C for 5 h.

3.3.2. In vivo evaluation of anti-tumor activity of nanoparticles

Although S-180 cells have been one of the classical tumor models for a very long time, the characteristics including density of transferrin receptors on the surface of S-180 cells have not been reported. For mice treated with 20 mg $kg^{-1} \times 5$ of drug-loaded nanoparticles, the decrease in body weight was within 4% of the initial weight, and tumor regression was significantly observed with complete regression for five out of nine mice. The tumor burden in mice that had been treated with Paclitaxel-loaded nanoparticles was much smaller compared with mice treated with Paclitaxel injection. In addition, the life span of tumor-bearing mice was significantly increased when they were treated with Paclitaxel-loaded nanoparticles: three out of nine mice survived for more than 60 days.

4. Conclusions

Research efforts have been devoted to demonstrating in vitro that the pH-sensitive characteristics of poly NIPAAm/chitosan nanoparticles can be applied to targeting tumor cells. Paclitaxel-loaded nanoparticles show drastically enhanced cytotoxicity compared with that at normal pH. For mice treated with Paclitaxel -loaded nanoparticles, the decrease in body weight is limited, and significant tumor regression is observed, with complete tumor

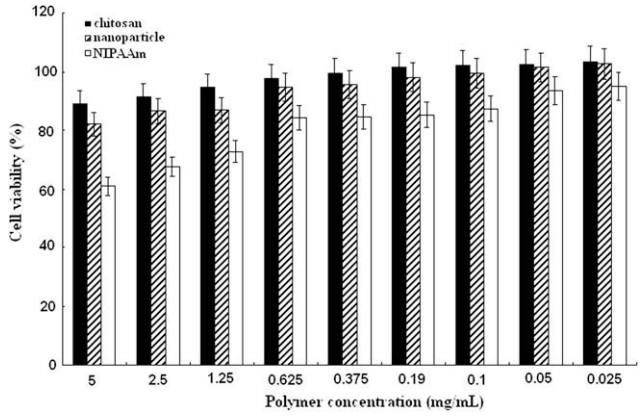

Fig. 5. Cytotoxicity of blank nanoparticles, chitosan and NIPAAm against human lung cancer cells A549 as a function of polymer/monomer concentration. Data represent mean ± SD, *n* = 7.

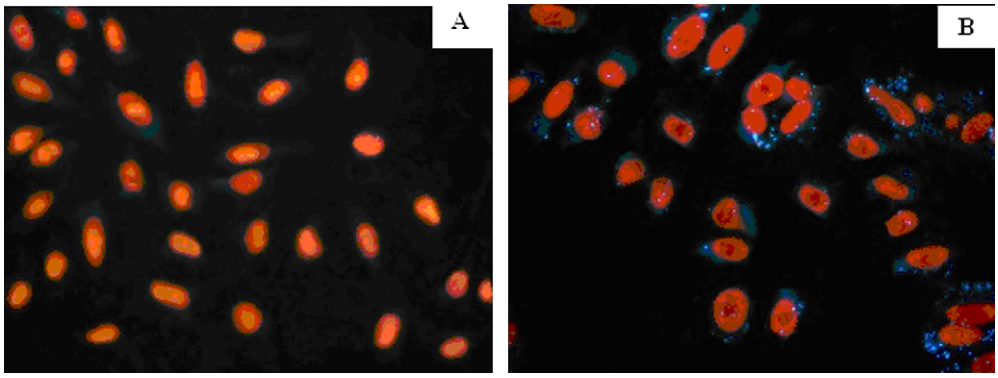

Fig. 6. Luminescence images of A549 cells treated with fluorescent nanoparticles at (a) pH 6.8 and (b) 7.4. The A549 cells were incubated at 37 °C for 5 h.

regression for more than 50% of mice. In addition, the life span of tumor-bearing mice is significantly increased when they are treated with Paclitaxel-loaded nanoparticles. Thus the atoxic poly-NIPAAm/chitosan nanoparticles have potential as a novel anticarcinogen carrier.

Acknowledgments

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