Drug Development and Industrial Pharmacy, 34:834–839, 2008

ISSN: 0363-9045 print / 1520-5762 online
DOI: 10.1080/03639040801926733

informa
healthcare

Evaluation of Arsenic Trioxide-Loaded Albumin Nanoparticles as Carriers: Preparation and Antitumor Efficacy

Zhiwen Yang, Muhua Yang, and Jinnian Peng
Department of Pharmaceutical Sciences, Gannan Medical College, Ganzhou, People's Republic of China

Bovine serum albumin (BSA) nanoparticles containing arsenic trioxide (As_2O_3) were prepared by a pH-coacervation method. To investigate the properties of the As_2O_3-loaded BSA nanoparticles, a study on drug-to-polymer ratio was done to determine the drug loading (DL), and a H-600 transmission electron microscope (TEM) was used to examine the particle sizes. The results showed that the DL was 27.8% and the average particle size was about 734 nm. The drug release in vitro test was done, which revealed that the drug release was found to provide a slow release after an initial burst release and the cumulative percentage release reached close to 95%. In vitro cytotoxicity test was carried out using APL NB4 cell lines (acute promyelocytic leukemia), and the anticancer efficacy in vivo against mouse H22 hepatoma cells was evaluated on kungming mice. The results indicated that the anticancer efficacy of the As_2O_3-loaded BSA nanoparticles was very obvious.

Keywords antitumor; bovine serum albumin; nanoparticles; arsenic trioxide

INTRODUCTION

Arsenic trioxide (As_2O_3) is a promising agent that is widely used in the treatment of tumor. Mass research revealed that the mechanism included inhibition of proliferation of tumor cells (Park et al., 2001) and angiogenesis (Rubenstein et al., 2000), induction of incomplete differentiation (Zhu et al., 2002) and apoptosis of tumor cells (Jiang et al., 2001), and so on. Early studies mainly focused on hematologic malignancies (Verstovsek et al., 2006). A recent pharmacological research showed that As_2O_3 can inhibit growth of many kinds of solid tumors (Evens, Tallman, & Gartenhaus, 2004; Liu & Han, 2003). Nevertheless, the usage of As_2O_3 as an antitumor drug in clinic has been limited by the toxicity and poor retention time in the tumor cells (Diaz et al., 2007; Lemarie, Morzadec, Bourdonnay, Fardel, & Vernhet, 2006; Mathews et al., 2006; Siu et al., 2006), that is, As_2O_3 can inhibit tumor cells while showing severe toxicity effect on normal tissue. Fortunately, since the first reports on the preparation of uniformly sized albumin microspheres or nanoparticles, these biodegradable, biocompatible particles have been utilized as drug carrier systems to improve the efficacy and reduce the toxicity of some anticancer agents (Zhou et al., 2005). Drugs were absorbed onto the particle surface, bound by ionic interaction, or were entrapped in the matrix of the nanoparticles (Eatock et al., 1999; Yoo, Oh, Lee, & Park, 1999). The natural polymers such as proteins have been largely employed for the preparation of nanoparticles, and the drugs can bypass the efflux action of drug-resistant cancer cells and are released at slower speed, resulting in their enhanced cellular drug uptake, accumulations, and therapeutic effects (Arnedo, Irache, Merodio, Espuelas, & Millán, 2004; Santhi, Dhanaraj, Koshy, Ponnusankar, & Suresh, 2000). This study indicates the possibility of preparing nanoparticles based on gelatin and human serum albumin (HSA). Encouraging reports have been published about the antitumor efficacy of anticancer drugs loaded onto nanoparticles (Labib et al., 1991; Zhang et al., 2004).

In this article, we made an attempt to design and develop a carrier system as antitumor drugs based on bovine serum albumin (BSA) for the attachment of As_2O_3 and investigate the properties of albumin nanoparticles, including drug loading (DL), average particle size, drug release property, cytotoxicity in vitro, and anticancer efficacy in vivo. The probable hypothesis is that the As_2O_3-loaded BSA nanoparticles were more effective than the free drug on the inhibition of tumor cells, which may allow a reduction in the dose to reduce the systemic toxicity.

MATERIALS AND METHODS

Materials

Aqueous glutaraldehyde solution (25% wt/wt), acetone, ethanol, and As_2O_3 were purchased from Shanghai Chemical Agent Co., Ltd. (Shanghai, China). BSA powder was obtained from Amresco (Solon, OH, USA). RPMI 1640 culture medium

Address correspondence to Zhiwen Yang, Department of Pharmaceutical Sciences, Gannan Medical College, No. 1, Yixueyuan Road, Ganzhou 341000, Jiangxi, China. E-mail: menghua001@sina.com

(P1504) was obtained from Invitrogen Corporation (Carlsbad, CA, USA). Pepsin was purchased from Sigma (Steinheim, Germany).

APL NB4 cell lines (acute promyelocytic leukemia) were obtained from Chinese National Human Genome Center (CHGC) (Shanghai, China). Kunming mice were provided by Medical College of Nanchang University (Nanchang, China). Mouse H22 hepatoma cells were purchased from Institute of Biochemistry and Cell Biology, SIBS, CAS.

Preparation of BSA Nanoparticles Containing As_2o_3

BSA nanoparticles containing As_2O_3 were prepared by a pH-coacervation method with forming albumin nanoparticles and cross-linking with 4% glutaraldehyde. Briefly, 2% aqueous solution of BSA in 100 mL of distilled water, to which 10 mg of As_2O_3 was added, adjusted to pH 9 using 0.5 M sodium hydroxide solution, was transformed into nanoparticles by the continuous addition of dissolved acetone under stirring on a magnetic stirrer till the solution became turbid. After the transformation process, 4% glutaraldehyde in ethanol was added to induce particle cross-linking under continuous stirring at room temperature for 3 h. After the cross-linking process, the nanoparticles formed were filtered by millipore membrane filter with a pore size of 1 μm, then purified by centrifugation at 5,000 *g* at 25°C for 30 min. After removing the supernatant, the suspension was washed thrice with acetone and the nanoparticles obtained were suspended in an acetone–water mixture.

Drug-to-Polymer Ratio Study

To determine the DL of the As_2O_3-loaded BSA nanoparticles, a study on the drug-to-polymer ratio was carried out. Five different batches of nanoparticles containing various concentrations of As_2O_3, such as 10, 20, 30, 40, and 50 mg, were prepared. As_2O_3 was first added to 100 mL of BSA aqueous solution (2%, wt/vol). Afterward, the pH of the solution was adjusted to approximately 9 with 0.5 M sodium hydroxide solution, before the dropwise addition of acetone until the solution became just turbid. The albumin nanoparticles formed were cross-linked by glutaraldehyde then the obtained nanoparticles were purified, and the free drug was removed by centrifugation. Each batch of drug loaded had a constant pH and concentration of BSA with a variation only in the As_2O_3 concentration. The five batches of nanoparticles prepared were named as model A, B, C, D, and E, respectively.

Determination of Particle Size

The particle sizes of albumin nanoparticles were determined by the transmission electron microscope (TEM, Hitachi, Tokyo, Japan). The nanoparticles suspended in the concentrated aqueous solution were spread on a slab, dried under vacuum, and shadowed in a cathodic evaporator with a gold layer 20 nm thick before examination in the TEM.

Estimation of Amount of As_2o_3 Incorporated into BSA Nanospheres

An amount of 10 mg of As_2O_3-loaded nanospheres (As_2O_3-NP) from each batch was suspended in 1 mL of phosphate buffer solution (PBS) and incubated with pepsin at 37°C. After the As_2O_3-NP was completely hydrolyzed, the disrupted and broken nanoparticles were separated by centrifugation at 3,000 rpm and the content of As_2O_3 incorporated into nanospheres was analyzed by an atomic fluorescence analyzer (SK-2003, Beijing Jinsuokun, Beijing, China).

Estimation of Drug by Atomic Fluorescence Analyzer

The amount of As_2O_3 incorporated into the albumin nanoparticles was calculated by the amount of As_2O_3 determined in the supernatants obtained during the purification processes. An amount of 5 mg of As_2O_3-NP from each batch was precisely taken and suspended in 1 mL of PBS. After As_2O_3-NP was completely hydrolyzed by pepsin, the solution was diluted by gradient method stepwise. In all cases, the concentration of As_2O_3 was determined by atomic fluorescence analyzer by extrapolation in a calibration graph, previously estimated with As_2O_3 concentrations in suspension, in the range 0.0, 2.0, 4.0, 8.0, 16.0, and 20.0 μg/L. The DL of As_2O_3-loaded BSA nanoparticles was calculated by the formula

$$\text{DL } (\%) = W_1/W_2 \times 100\%,$$

where W_1 represents the quality of As_2O_3 in the nanoparticles and W_2 the quality of As_2O_3-NS.

Release Studies In Vitro

Eppendorf tubes containing 10 mg of As_2O_3-NS dispersed in a total volume of 50 mL PBS (pH 7.4, 0.15 M) were placed in a shaking bath at 37°C with a constant agitation at 60 rpm. At predetermined time intervals, the samples were centrifuged and the amount of As_2O_3 in the supernatant was determined by an atomic fluorescence analyzer as described above. This analysis was performed thrice for each sample.

In Vitro Cytotoxicity Test

To evaluate the efficacy of drug-loaded nanoparticles against cancer cells, in vitro cytotoxicity test (Bernardini et al., 2006; Sahu & Jena, 2005) was carried out using APL NB4 cell lines. APL NB4 cells (approximately 1×10^5 to 1×10^6 cells/mL) were added to RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and seeded into 24-well microtiter plates, subsequently, with varying amounts of As_2O_3 (2.4, 1.2, 0.6, and 0.3 μmol/L). As_2O_3-loaded nanoparticles equivalent to 2.4, 1.2, 0.6, and 0.3 μmol/L were added along with the control and incubated at 37°C under atmospheric conditions of 5% CO_2 and 95% relative humidity for 5 min with gentle agitation. The number of viable cells remaining in each well was determined by trypan

blue dye extrusion technique. From the in vitro cytotoxicity test, the percentage viability of As_2O_3 nanoparticles was compared with that of the control and free drug at different concentrations.

Study on Antitumor Efficacy In Vivo

The antitumor efficacy of As_2O_3 nanoparticles was compared with that of the free drug and control (Liu et al., 2006) against mouse H22 hepatoma cells in bearing cancer mice. An antitumor study was carried out using 60 kunming strain male mice (weight 18–22 g, age 4–6 weeks), randomly divided into three groups. Groups I animals received the solvent Hanks buffer, administered by i.v. route into the tail vein, and served as control group. Group II animals received free As_2O_3 equivalent to 3 mg/kg; the drug was dispersed in Hanks buffer and injected through the tail vein, and these animals served as free As_2O_3 chemotherapy group. Group III animals were treated with As_2O_3-loaded nanoparticles equivalent to 3 mg/kg in Hanks buffer (injected through the tail vein) and served as As_2O_3-loaded nanoparticles chemotherapy group. The mice were cared for and handled according to the national regulations for experimental animals and were raised conventionally without any limit on water and food.

All the three groups of mice were induced cancer model by implanting mouse H22 hepatoma cells in the abdominal cavity, where the suspension containing 6.5×10^7/mL cells in logarithmic growth phase was conventionally prepared. After inducing cancer, treatment was given to the animals through solvent Hanks buffer, free drug, and nanoparticles-bound drug till 15 days. Treatment was discontinued on the 16th day, and all the mice were killed by cervical dislocation. The tumor tissues from each mouse in each group were removed and entirely transferred to 5 mL of sterile normal saline solution resulting in a suspension. It was measured as the packed cell volume (PCV) (Santhi, Dhanaraj, Joseph, Ponnusankar, & Suresh, 2002).

The PCV can be determined to find out the total number of mouse H22 hepatoma cells present in each animal, which is related to the rate of growth of tumor in each animal. The comparative efficacy of nanoparticles-bound drug and free drug can be evaluated in terms of percentage tumor inhibition. The formula for the calculation of percentage tumor inhibition is given below.

$$\text{Tumor inhibition } \% = 100 - X,$$

where

$$X = \text{test PCV/control PCV} \times \quad 100.$$

RESULTS AND DISCUSSIONS

The Average Particle Size

BSA nanoparticles containing As_2O_3 were readily produced by the pH-coacervation using glutaraldehyde as the cross-linking agent (Lin, Coombes, Davies, Davis, & Illum, 1993). It has been reported that the size and drug yield obtained of albumin nanoparticles can be changed under different pH conditions and the speed of addition of acetone (Lin et al., 1993). The pH-coacervation method for preparation of As_2O_3-loaded BSA nanoparticles enabled us to obtain spherical and discrete nanoparticles (Figure 1) with a size ranging from 472 to 891 nm. The average particle size was found to be 734 ± 1.9 nm for batch C (Table 1), which was selected as the ideal batch based on the DL capacity. The average particle size of the As_2O_3-loaded nanoparticles was larger than that mentioned in the reported studies (sub-100 nm).

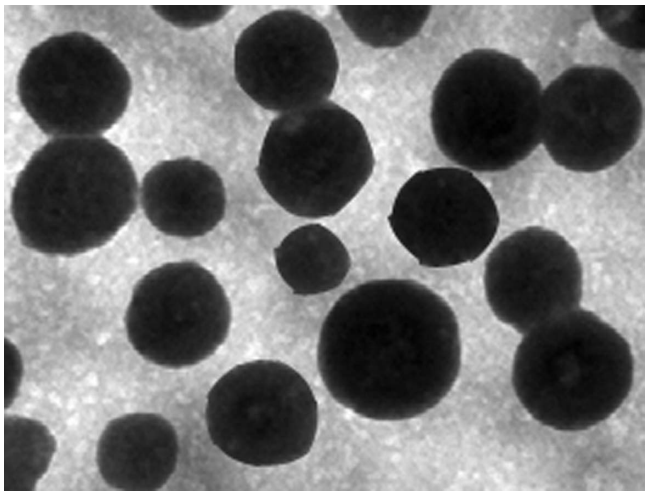

FIGURE 1. Transmission electron microscope (TEM) micrograph of As_2O_3-loaded BSA nanoparticles (×10,000).

The Drug Loading Capacity

The DL capacity of a nanoparticles carrier is mainly altered by the nature of the drug incorporated. The estimation of the drug payload of BSA with respect to As_2O_3 would be an essential parameter to know its limitations and applications as a drug carrier. A preliminary study on the drug-to-polymer ratio was carried out using various concentrations of As_2O_3 (100, 200, 300, 400, and 500 μg/mL), described previously. The DL capacity was determined to be 13.2, 19.6, 27.8, 29.2, and 31.4% by extrapolation in a calibration graph (r^2 = .9986).

$$y = 134.2132x + 2.3869,$$

where x represents the concentration of As_2O_3 and y the absorption determined by atomic fluorescence analyzer.

It was evident that there was a linear increase in the DL capacity with the increase in the concentration of drug. The linearity between the drug concentration and DL was maintained only up to a concentration of 300 μg/mL of drug. Hence, a concentration of 300 μg/mL of drug was considered ideal to achieve the highest loading among the various chosen concentrations of drug. The same batch was selected for the subsequent in vitro and in vivo studies.

TABLE 1
Average Particle Size of As_2O_3-Loaded BSA Nanoparticles

Batch	Average Particle Size (nm)
A	625 + 2.2
B	696 + 1.3
C	734 + 1.9
D	738 + 2.7
E	765 + 3.4

In Vitro Drug Release

The BSA nanoparticles containing As_2O_3, prepared under the experimental conditions, were tested for in vitro release at 37°C. The results showed that the drug was released rapidly within the initial 2 h, and 75% of the drug was released within the first 12 h; afterward, the drug was released in a slow manner over 24 h. The cumulative percentage release was 95% for all the sample batches (Table 2).

The release profiles were related to the presence of As_2O_3 in the nanoparticles. The initial burst release may correspond to the desorption of the As_2O_3 absorbed to the particles surface, and the latter sustained release should be due to the As_2O_3 incorporated into the matrix of the particles, which resulted mainly from the erosion of the particles by the medium. Moreover, in this case, there is an incomplete release (about 5%) that could be attributed to the hydrophobic irreversible interactions of the As_2O_3 particles not affected by a reduction of albumin charge (Arnedo, Espuelas, & Irache, 2002; Irache et al., 2005).

In Vitro Cytotoxicity

Investigations through in vitro cytotoxicity studies on the inhibition of As_2O_3-loaded nanoparticles to the APL NB4 cell lines showed that the drug bound to nanoparticles was more effective than the free drug, in terms of the reduction in percentage cell viability. Two hundred cells were counted and the viable cells were statisticated by trypan blue dye extrusion technique using T test by SPSS software (Figure 2). The percentage cell inhibition of the nanoparticles-bound drug and free drug is shown in Table 3 A test of significance was applied to compare

TABLE 2
In Vitro Release of As_2O_3 from the BSA Nanoparticles (Expressed as Cumulative Release)

Time Interval (h)	Cumulative Release (%)
0.5	20.65 ± 0.64
1	38.44 ± 1.17
2	52.47 ± 1.38
4	60.76 ± 1.15
8	66.87 ± 1.59
12	75.11 ± 3.03
24	95.37 ± 2.44

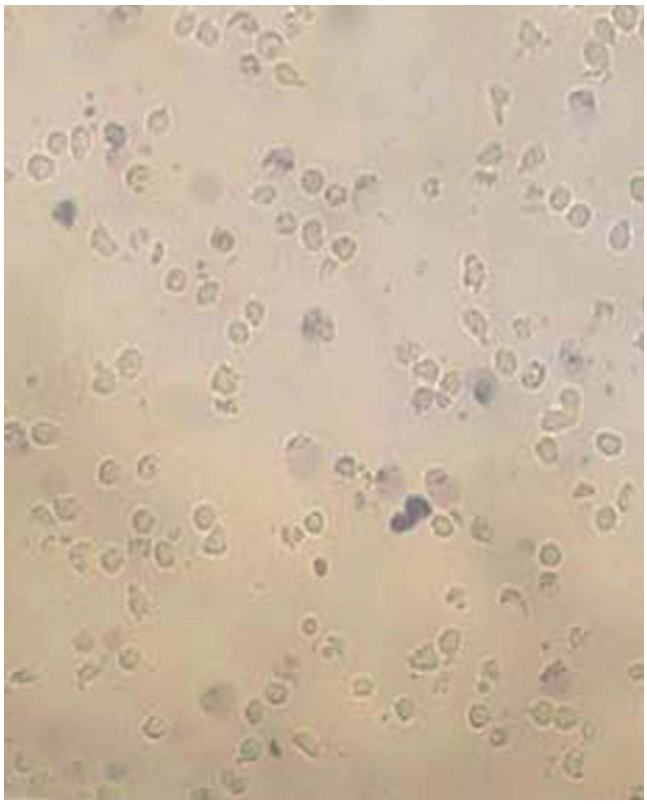

FIGURE 2. Percentage cell viability by using trypan blue dye extrusion technique.

TABLE 3
Percentage Cell Inhibition by Nanoparticles-Bound As_2O_3 and Free As_2O_3

Group	Viable Count ($\times 10^4$)	Viability (%)
Control	9.51	100
As_2O_3 (0.3 µmol/L)	15.8	81.0
As_2O_3 (0.6 µmol/L)	14.7	75.4
As_2O_3 (1.2 µmol/L)	12.1	62.1
As_2O_3 (2.4 µmol/L)	10.3	52.8
As_2O_3 nanoparticles (0.3 µmol/L)	13.9	71.3
As_2O_3 nanoparticles (0.6 µmol/L)	12.3	63.1
As_2O_3 nanoparticles (1.2 µmol/L)	10.5	53.8
As_2O_3 nanoparticles (2.4 µmol/L)	8.6	44.1

Note. Data statistically significant at $p < .05$.

the significance of the difference in percentage cell viability between the nanoparticles-bound drug and the free drug. It was evident that there was a marked difference in the percentage cell viability between the nanoparticles-bound drug and the free drug ($p < .05$).

Antitumor Efficacy

Studies of the antitumor efficacy in vivo were carried out with kunming mice implanted with the mouse H22 hepatoma cells as the cancer model among the three groups with different treatments. The results revealed that the drug bound to nanoparticles was more effective than the free drug by the percentage of tumor inhibition produced, which may allow a reduction in the dose to reduce the systemic toxicity (Table 4) The phenomena attributed to this was achieved through recognition or nonrecognition of the colloidal system by the body's defense system (Moghimi, Porter, Muir, Illum, & Davis, 1999). Particles that are small enough to escape the capillary beds of the lungs are normally sequestered rapidly by the cells of the reticuloendothelial system (RES), particularly, the kupffer cells of the liver (Dreis et al., 2007; Illum et al., 1982). What is more, the uptake by the RES is consequently reduced, thus the particles with a significantly longer circulation half-life are provided (Li et al., 2007; Moghimi et al., 1999). In some cases, the particles can deposit preferentially in a specific organ site such as the bone marrow, spleen, or liver (Moghimi, Hedeman, Muir, Illum, & Davis, 1993; Porter, Moghimi, Illum, & Davis, 1992).

CONCLUSIONS

In conclusion, the BSA nanoparticles containing As_2O_3 can be prepared by pH-coacervation method following cross-linking with glutaraldehyde. Depending on the preparative process and the drug-to-polymer ratio, the average particle size (734 ± 1.9 nm) and the DL capacity (27.8%) can be obtained.

The in vitro release studies indicated that the drug was released in a biphasic way, a burst release in the first 4 h followed by the slower release within the next 20 h. The in vitro cytotoxicity studies showed that the drug-loaded nanoparticles had more effective inhibition to the APL NB4 cell lines than the free drug, in terms of reduction in the percentage cell viability.

The in vivo studies revealed that the drug-loaded nanoparticles have better antitumor efficacy, which may allow a reduction in the dose to reduce the systemic toxicity.

In comparison to As_2O_3 solutions, the nanoparticle formulations showed several benefits. With particle sizes in the range 400–900 nm, a passive tumor targeting was possible due to the so-called enhanced permeation and retention effect (EPR effect) (Maeda, Wu, Sawa, Matsumura, & Hori, 2000). Furthermore, some of these colloidal carriers were able to overcome multidrug resistance in various cancer cell lines. This effect resulted in an increased survival time and in longtime remissions in more than 20% of the treated animals compared with controls (Ambruosi et al., 2005; Barraud et al., 2005; Steiniger et al., 2004).

TABLE 4
Tumor Inhibition Produced by Drug-Loaded Nanoparticles and Free Drug

Group	Total Volume (mL)	PCV (mL)	Tumor Inhibition (%)
Control	24.1 ± 2.7	18.6 ± 2.0	0
As_2O_3	17.1 ± 1.9	13.8 ± 2.4	29.0 ± 3.2
As_2O_3 nanoparticles	11.6 ± 3.0	8.4 ± 1.8	51.7 ± 2.1

REFERENCES

Ambruosi, A., Khalansky, A. S., Yamamoto, H., Gelperina, S. E., Begley, D. J., & Kreuter, J. (2006). Biodistribution of polysorbate 80-coated doxorubicin-loaded[14c]- poly (butyl cyanoacrylate) nanoparticles after intravenous administration to glioblastoma-bearing rats. *J. Drug Target, 14,* 97–105.

Arnedo, A., Espuelas, S., & Irache, J. M. (2002). Albumin nanoparticles as carriers for a phosphodiester oligonucleotide. *Int. J. Pharm., 244,* 59–72.

Arnedo, A., Irache, J. M., Merodio, M., & Espuelas Millán, M. S. (2004). Albumin nanoparticles improved the stability, nuclear accumulation and anticytomegaloviral activity of a phosphodiester oligonucleotide. *J. Control. Release, 94,* 217–227.

Barraud, L., Merle, P., Soma, E., Lefrancois, L., Guerret, S., Chevallier, M., Dubernet, C., Couvreur, P., Trepo, C., & Vitvitski L. (2005). Increase of doxorubicin sensitivity by doxorubicin-loading into nanoparticles for hepatocellular carcinoma cells in vitro and in vivo. *J. Hepatol., 42,* 736–743.

Bernardini, S., Nuccetelli, M., Noguera, N. I., Bellincampi, L., Lunghi, P., Bonati, A., Mann, K., Miller, W. H., Federici, G., & Lo Coco, F. (2006). Role of GSTP1-1 in mediating the effect of As_2O_3 in the acute promyelocytic leukemia cell line NB4. *Ann. Hematol., 85,* 681–687.

Diaz, Z., Laurenzana, A., Mann, K. K., Bismar, T. A., Schipper, H. M., & Miller, W. H. (2007). Trolox enhances the anti-lymphoma effects of arsenic trioxide, while protecting against liver toxicity. *Leukemia, 21,* 2117–2127.

Dreis, S., Rothweiler, F., Michaelis, M., Cinatl, J., Jr., Kreuter, J., & Langer, K. (2007). Preparation, characterization and maintenance of drug efficacy of doxorubicin-loaded human serum albumin (HSA) nanoparticles. *Int. J. Pharm., 341,* 207–214.

Eatock, M., Church, N., Harris, R., Angerson, W., McArdle, C., French, R., & Twelves, C. (1999). Activity of doxorubicin covalently bound to a novel human serum albumin microcapsule. *Invest. New Drugs, 17,* 111–120.

Evens, A. M., Tallman, M. S., & Gartenhaus, R. B. (2004). The potential of arsenic trioxide in the treatment of malignant disease: Past, present, and future. *Leuk. Res., 28,* 891–900.

Illum, L., Davis, R. M., & Wilson, C. G. (1982). Blood clearance and organ deposition of intravenously administered colloidal particles. The effects of particle size, nature and shape. *Int. J. Pharm., 2,* 135–146.

Irache, J. M., Merodio, M., Arnedo, A., Camapanero, M. A., Mirshahi, M., & Espuelas, S. (2005). Albumin nanoparticles for the intravitreal delivery of anticytomegaloviral drugs. *Mini. Rev. Med. Chem, 5,* 293–305.

Jiang, X. H., Wong, B. C., Yuen, S. T., Jiang, S. H., Cho, C. H., Lai, K. C., Lin, M. C., Kung, H. F., & Lam, S. K. (2001). Arsenic trioxide induces apoptosis in human gastric cancer cells through up-regulation of p53 and activation of caspase-3. *Int. J. Cancer, 91,* 173–179.

Labib, A., Lenaerts, V., Chouinard, F., Leroux, J. C., Ouellet, R., & van Lier, J. E. (1991). Biodegradable nanospheres containing phthalocyanines and naphthalocyanines for targeted photodynamic tumor therapy. *Pharm. Res., 8,* 1027–1031.

Lemarie, A., Morzadec, C., Bourdonnay, E., Fardel, O., & Vernhet, L. (2006). Human macrophages constitute targets for immunotoxic inorganic arsenic. *J. Immunol., 177*, 3019–3027.

Li, F. Q., Su, H., Wang, J., Liu, J. Y., Zhu, Q. G., Fei, Y. B., Pan, Y. H., & Hu J. H. (2007). Preparation and characterization of sodium ferulate entrapped bovine serum albumin nanoparticles for liver targeting. *Int. J. Pharm., 11*, 274–282.

Lin, W., Coombes, A. G., Davies, M. C., Davis, S. S., & Illum, L. (1993). Preparation of Sub-100 nm human serum albumin nanospheres using a ph-coacervation method. *J. Drug Targeting., 1*, 237–243.

Liu, P., & Han, Z. C. (2003). Treatment of acute promyelocytic leukemia and other hematologic malignancies with arsenic trioxide: Review of clinical and basic studies. *Int. J. Hematol., 78*, 32–39.

Liu, B., Pan, S., Dong, X., Qiao, H., Jiang, H., Krissansen, G. W., & Sun, X. (2006). Opposing effects of arsenic trioxide on hepatocellular carcinomas in mice. *Cancer Sci., 97*, 675–681.

Maeda, H., Wu, J., Sawa, T., Matsumura, Y., & Hori K. (2000). Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review. *J. Control. Release, 65*, 271–284.

Mathews, V., George, B., Lakshmi, K. M., Viswabandya, A., Bajel, A., Balasubramanian, P., Shaji, R. V., Srivastava, V. M., Srivastava, A., & Chandy, M. (2006). Single-agent arsenic trioxide in the treatment of newly diagnosed acute promyelocytic leukemia: Durable remissions with minimal toxicity. *Blood, 107*, 2627–2632.

Moghimi, S. M., Hedeman, H., Muir, I. S., Illum, L., & Davis, S. S. (1993). An investigation of the filtration capacity and the fate of large filtered sterically-stabilized microspheres in rat spleen. *Biochim. Biophys. Acta, 3*, 233–240.

Moghimi, S. M., Porter, C. J., Muir, I. S., Illum, L., & Davis, S. S. (1999). Non-phagocytic uptake of intravenously injected microspheres in rat spleen: Influence of particle size and hydrophilic coating. *Biochem. Biophys. Res. Commun., 127*, 861–866.

Park, J. W., Choi, Y. J., Jang, M. A., Baek, S. H., Lim, J. H., Passaniti, T., & Kwon, T. K. (2001). Arsenic trioxide induces G2/M growth arrest and apoptosis after caspase-3 activation and bcl-2 phosphorylation in promonocytic U937 cells. *Biochem. Biophys. Res. Commun., 286*, 726–734.

Porter, C. J., Moghimi, S. M., Illum, L., & Davis S. S. (1992). The polyoxyethylene/polyoxy-propylene block co-polymer poloxamer-407 selectively redirects intravenously injected microspheres to sinusoidal endothelial cells of rabbit bone marrow. *FEBS Lett., 305*, 62–66.

Rubenstein, J. L., Kim, J., Ozawa, T., Zhang, M., Westphal, M., Deen, D. F., & Shuman, M. A. (2000). Anti-VEGF antibody treatment of glioblastoma prolongs survival but results in increased vascular cooption. *Neoplasia, 2*, 306–314.

Sahu, G. R., & Jena, R. K. (2005). Significance of intracellular arsenic trioxide for therapeutic response in acute promyelocytic leukemia. *Am. J. Hematol., 78*, 113–116.

Santhi, K., Dhanaraj, S. A., Joseph, V., Ponnusankar, S., & Suresh, B. A. (2002). Study on the preparation and anti-tumor efficacy of bovine serum albumin nanospheres containing 5-fluorouracil. *Drug Dev. Ind. Pharm., 28*, 1171–1179.

Santhi, K., Dhanaraj, S. A., Koshy, M., Ponnusankar, S., & Suresh, B. (2000). Study of bio- distribution of methotrexate-loaded bovine serum albumin nanospheres in mice. *Drug Dev. Ind. Pharm., 26*, 1293–1296.

Siu, C. W., Au, W. Y., Yung, C., Kumana, C. R., Lau, C. P., Kwong, Y. L., & Tse, H. F. (2006). Effects of oral arsenic trioxide therapy on QT intervals in patients with acute promyelocytic leukemia: Implications for long-term cardiac safety. *Blood, 108*, 103–106.

Steiniger, S. C., Kreuter, J., Khalansky, A. S., Skidan, I. N., Bobruskin, A. I., Smirnova, Z. S., Severin, S. E., Uhl, R., Kock, M., Geiger, K. D., & Gelperina S. E. (2004). Chemotherapy of glioblastoma in rats using doxorubicin-loaded nanoparticles. *Int. J. Cancer, 109*, 759–767.

Verstovsek, S., Giles, F., Quintás-Cardama, A., Perez, N., Ravandi-Kashani, F., Beran, M., Freireich, E., & Kantarjian, H. (2006). Arsenic derivatives in hematologic malignancies: A role beyond acute promyelocytic leukemia. *Hematol. Oncol., 24*, 181–188.

Yoo, H. S., Oh, J. E., Lee, K. H., & Park, T. G. (1999). Biodegradable nanoparticles containing doxorubicin–PLGA conjugate for sustained release. *Pharm. Res., 16*, 1114–1118.

Zhang, L., Hou, S., Mao, S., Wei, D., Song, X., & Lu, Y. (2004). Uptake of folate-conjugated albumin nanoparticles to the SKOV3 cells. *Int. J. Pharm., 287*, 155–162.

Zhou, J., Zeng, F. Q., Li, C., Tong, Q. S., Gao, X., Xie, S. S., & Yu, L. Z. (2005). Preparation of arsenic trioxide-loaded albuminutes immuno-nanospheres and its specific killing effect on bladder cancer cell in vitro. *Chin. Med. J (Engl)., 118*, 50–55.

Zhu, Q., Zhang, J. W., Zhu, H. Q., Shen, Y. L., Flexor, M., Jia, P. M., Yu, Y., Cai, X., Waxman, S., Lanotte, M., Chen, S. J., Chen, Z., & Tong, J. H. (2002). Synergic effects of arsenictrioxide and cAMP during acute promyelocytic leukemia cell maturation subtends a novel signaling cross-talk. *Blood, 99*, 1014–1022.