Preparation of Aclarubicin PLGA Nanospheres and Related *In Vitro*/*In Vivo* Studies

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Received 31 January 2009; accepted 21 September 2009
DOI 10.1002/app.31464
Published online 27 April 2010 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: The microencapsulation method was used to prepare formulations of the anthracycline drug aclarubicin, and the effects of a variety of different conditions on the preparation process were evaluated. The drug was entrapped in poly(lactic-*co*-glycolic acid) (PLGA) to prepare an aclarubicin nanosphere formulation that could be sustainably released, and had a high drug loading capacity and high entrapment efficiency. The degree of degradation of aclarubicin PLGA nanospheres that were prepared under different conditions was tested *in vitro*. Aclarubicin nanospheres that were intravenously injected into rabbits were found to be slowly and stably released over a period of almost 20 days. HPLC was used to determine the blood concentrations of the products. To provide pharmacodynamic and toxicologic profiles, the aclarubicin PLGA nanospheres were intravenously injected into mice. The results showed that the aclarubicin PLGA nanospheres exerted anti-tumor activity *in vivo*, but did not produce toxic effects that were more serious than those resulting from the standard administration of aclarubicin agents. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 117: 2754–2761, 2010

Key words: biomaterials; degradation; microencapsulation; HPLC

INTRODUCTION

Aclarubicin is a novel second-generation anthracycline anti-tumor antibiotic that was isolated from *Streptomyces galilaeus*. First reported in 1975 by the Japanese scholar Oki,[1] it was developed and marketed in the 1980's by Sanle, in Japan. Its chemical name is 2-Ethyl-1,2,3,4,6,11-hexahydro-2,5,7-trihydroxy-6,11-dioxo-4-[[2,3,6-trideoxy-4-O-[2,6-dideoxy-4-O-[2-trans-tetrahydro-6-methyl-5-oxo-2H-pyran-2-yl]-A-L-lyxo-hexopyranosyl-3-[dimethylamino]-A-L-lyxo-hexopyranosyl]oxy]-,methylester,[1-R-[1alpha,2beta,4beta]]-1-naphthacenecarboxylic acid.[2] Aclarubicin exhibits strong anti-tumor activity against leukemia,[3] ascites carcinoma,[4] lung cancer,[5] and breast cancer,[6] and it also has therapeutic effects on lymphoma[7] and thyroid cancer.[8] Aclarubicin *in vivo* interacts with the heterogeneous topology enzyme (II), and this inhibits the cell cycle progression of cancer cells at the G1 phase. Aclarubicin is able to embed itself in the double-stranded helical structure of DNA and thus interrupt its template function; therefore, its main effect on cancer cells is to interfere with nucleic acid synthesis (and in particular RNA synthesis) and thus inhibit the synthesis of biological macromolecules.[9] The cellular mechanism and biological activity of aclarubicin are different from those of ordinary anthracyclines; specifically, aclarubicin is characterized by a stronger efficacy, a broader spectrum of anti-tumor activity, and lower toxicity.[10] When used for the treatment of leukemia and various other tumors, aclarubicin must either be administered for 10 consecutive days, followed by repeated injections 2–3 weeks later, or it must be given twice a week for a period of 8 weeks.[11,12]

With superior biocompatibility, biodegradability, and a controllable degradation rate, poly(lactic-*co*-glycolic acid) (PLGA) has a wide range of applications in the field of biomedical engineering. In addition to being used as a slow release carrier of drugs, it has also been used to produce scaffolds for a variety of tissue engineering processes.[13,14] PLGA microspheres have been used to deliver a variety of different therapeutic drugs that are currently available.[15] The aim of this study was to use PLGA as the carrier for the production of a novel slow-releasing formulation of injectable aclarubicin nanospheres that could extend the half-life of the drug and improve patient compliance.

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TABLE I
Seven Factors and Three Levels of Each Factor in an Orthogonal Experiment

Factors	Multiple emulsion rate	PLGA conc.	Volume of middle oil phase	Volume of outer water phase	PVA conc. of outer water phase	Stirring rate	Volume of inner water phase
Levels	A1	B1	C1	D1	E1	F1	G1
	A2	B2	C2	D2	E2	F2	G2
	A3	B3	C3	D3	E3	F3	G3

In light of the confidentiality of preparation parameters, detailed data of the factors that influenced the preparation process were not added.

MATERIALS AND METHODS

Materials

Electronic Balance (Sartorious, LA120, Germany); high-speed homogenizer (IKA, T18, Germany); magnetic stirrer (Mei Ying Pu instrument, 90-1B, China); refrigerated ultra-speed centrifuge (Beckman Coulter, L8-60M, USA); S-520 scanning electron microscope (Hitachi, S-5200, Japan); particle size detector (Beckman Coulter, DelsaNano, USA); UV Spectrophotometer (Beckman Coulter, DU640, USA); freeze-dryer (Pu Dong freeze-drying equipment, Glzy, China); high performance liquid chromatography (Agilent, 1200, Germany); suspension vibrator (IKA, UTTD, Germany); constant temperature water bath (Heng Ao Science and Technology Development, HWT-20B, China); constant temperature incubator (Dong Lian Electronic Technology Development, HPG9075, China). Reagents: aclarubicin (Taizhou Jiao Jiang Xing Cheng Pharmaceutical company, China); PLGA (50/50, lakeshore, USA); dichloromethane (Sigma, USA); other chemical reagents are all pure analytical reagents made in China.

Methods

Preparation, entrapment efficiency, and morphology of aclarubicin PLGA nanospheres

The nanospheres were prepared using double emulsion solvent evaporation. A highly concentrated, slightly acidic aqueous solution of aclarubicin was dissolved in ethylene chloride solution that contained dissolved PLGA (prepared in an oil initial emulsion by high-speed mixing). This initial emulsion was then slowly poured into a slightly alkaline water solution of PVA at 0°C. In the self-made double emulsion mixing reactor, the emulsion was mixed to form a water-in-oil-in-water double emulsion, and this was followed by low-speed mixing. During this process, the vacuum pump pumped gas into the reactor, the exhaust fan drew the gas out, and the cured aclarubicin nanospheres were formed after 3 h. The nanosphere powder was obtained by centrifugal recovery of the aclarubicin nanospheres and subsequent freeze-drying.[2]

We first investigated various factors in the preparation process that influenced the rates of entrapment and drug release of the aclarubicin PLGA nanospheres. Manifold factors affected the entrapment rate, including the initial emulsion speed, the initial emulsion time, the pH in the inner water phase, the inner water phase volume, the organic solvent type and volume, the temperature that was used for organic solvent evaporation, the outer water phase volume, the emulsifier type and amount, the drug concentration, PLGA content, the method that was used to remove the organic solvent, and the electrolyte that was added to the outer water phase. The aforementioned factors were considered, and according to the previous single factor test results, seven factors and three levels of each factor were selected for the orthogonal experiment (Table I).

After being dried for 24 h in the desiccator, an appropriate amount of the aclarubicin PLGA nanospheres was precisely weighed, and dissolved in water to form an aclarubicin PLGA nanosphere suspension. This suspension was then centrifuged at 50,000 rpm for 1 h at 4°C. The aclarubicin content in the supernatant was determined using high performance liquid chromatography (HPLC),[16,17] and these values were converted into entrapment efficiency. Aclarubicin PLGA nanospheres (30 mg) were then weighed for particle size detection, and their morphologies were then evaluated under the microscope and electron microscope.

In vitro release of the aclarubicin PLGA nanospheres

An appropriate amount of the aclarubicin PLGA nanospheres was precisely weighed, and dissolved in normal saline. To establish a standard curve of aclarubicin, the concentrations were measured after gradual dilution. Aclarubicin PLGA nanospheres (5 mg) were weighed and added to a specially made test tube. The *in vitro* release test was performed using 30 mL normal saline as the release solution. The test tubes were then placed in a shaker at

90 rpm at 37°C, and then they were removed after 4 h, 8 h, 1 day, 1.5 day, 2 day, 3 day, 4 day, 5 day, 6 day, 8 day, 10 day, 12 day, 14 day, 16 day, 20 day, 24 day, and 28 day. After being left static for 10 min, 10 mL supernatant was removed from the test tubes (without disturbing the nanospheres), and 10 mL *in vitro* release solution was added. The supernatant was centrifuged at 3000 r/min for 3 min, and the resulting supernatant was obtained for samples. The residual nanospheres at the bottom of the tubes were added back to the special test tubes for further release. After filtering through millipore filter membranes, the concentration of aclarubicin in each of the samples (up to the 30-day time period) was measured using high performance liquid chromatography as the amount released during the corresponding time frame. The cumulative percentage of release was calculated according to the measured concentrations at various time points and the marked amounts.

In vivo release of the aclarubicin PLGA nanospheres

Freeze-dried aclarubicin PLGA nanospheres (dispersed with normal saline before use) were intravenously injected into the ear vein of eight groups of healthy Japanese white rabbits (mean weight: 2.0 ± 0.5 kg). Samples of blood were taken from the other (non-injected) ear vein of the rabbits at 4 h, 1 day, 2 day, 4 day, 6 day, 8 day, 12 day, 16 day, 20 day, 24 day, and 28 day time points. To obtain a concentration-time curve and to calculate the cumulative percentage of release, RP-HPLC was used to measure the plasma aclarubicin concentrations in blood samples that were obtained over each 24 h time period.[18]

Toxicology study

A total of 200 Kunming mice (approximate weight 20 g), half male and half female, were randomly assigned to one of two groups: members of the first group were intravenously (into the tail vein) given the long-acting aclarubicin PLGA nanospheres, and mice in the second group received intravenous injection (into the tail vein) of an ordinary aclarubicin formulation. Each group was further divided into four groups according to the dosage that they received; there were high, medium, and low-dose groups, and a blank control group. The highest dose that was administered (20 mg/d) (calculated by aclarubicin) was calculated using the highest intravenous drug concentration and the largest administration volume; the medium dose was 10 mg, and the low dose was 1 mg. To observe potential animal toxicity or death, daily doses were intravenously administered into the tail vein using four injections per day for a period of 20 consecutive days. To evaluate the conditions of the major organs, all of the mice were subjected to autopsy after 20 days.

Pharmacodynamic study

A mouse model of liver cancer was established according to the method reported by Sun et al.,[19] briefly, human liver cancer cell lines were inoculated subcutaneously into nude mice (weight 18 g) using conventional methods for a few passages; 2 weeks later, the tumors were found to be growing well. The subcutaneous tumors were then removed from the tumor-bearing mice under sterile conditions. They were then added to the cell culture medium, and mechanically cut into small (1 mm) pieces for later use. The mice to be operated were anaesthetized with sodium pentobarbital, and coeliotomized under sterile conditions to expose the liver; the small pieces of liver cancer tissue were transplanted into the parenchyma of the right lobe of the liver, and the peritoneum and skin were sutured layer-by-layer after adequate hemostasis and anti-inflammatory procedures. After the operation, the surviving mice were randomly assigned to one of two groups (six mice per group) corresponding to the aclarubicin PLGA nanospheres group and the blank control group. The drug (2 mg/kg) was administered intravenously into the eye tail of the nude mice every 20 days for a total of three times. Normal saline was injected into members of the blank control group. The nude mice were observed for 10 days after discontinuation of the drug, and they were then sacrificed by breaking the neck. Organs that included the heart, liver, spleen, lung, and kidney were removed for histopathological examinations. The liver cancer tissue was separated, weighed, and examined under light microscopy. The tumor inhibition rate was calculated according to the following formula: tumor inhibition rate (%) = (average tumor weight of the control group − average tumor weight of the treatment group)/average tumor weight of the control group × 100%.

RESULTS

Analysis of the entrapment efficiency and appearance of the aclarubicin PLGA nanospheres

Nanospheres that were prepared according to the aforementioned method were washed five times, centrifuged (8000 rpm for 10 min), and then lyophilized. Some nanospheres were weighed and their entrapment efficiency was determined (Table II).

The appearance of the freeze-dried aclarubicin PLGA nanospheres was of a white powder, in which there was no collapsing or shrinking. They had a smooth surface, a uniform particle size, and dense

TABLE II
Entrapment Efficiency Results of Nanospheres Based on Orthogonal Experiments

Exp. no.	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 7	Exp. 8	Exp. 9	Exp. 10	Exp. 11	Exp. 12	Exp. 13	Exp. 14	Exp. 15	Exp. 16	Exp. 17	Exp. 18
Entrapment efficiency %	52.9	57.9	60.5	50.5	56.8	59.4	55.1	51.9	65.2	53.6	54.3	58.6	61.9	46.6	50.4	58.6	63.7	57.7

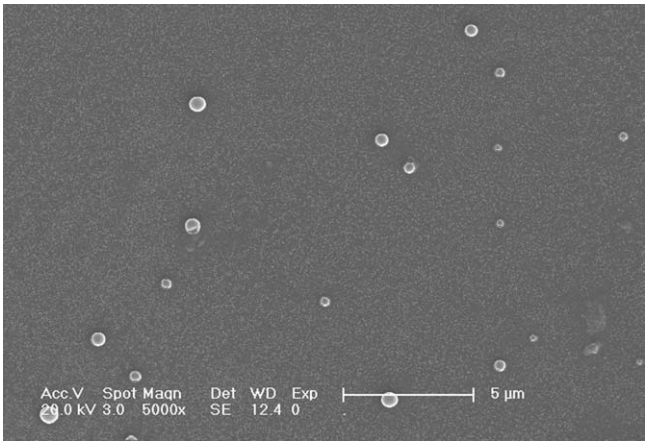

Figure 1 Appearance of the aclarubicin PLGA nanospheres under the electron microscope.

spherical roundness (Fig. 1). The entrapment efficiency was maintained above 50% using double emulsion under different conditions. The average particle size of the aclarubicin PLGA nanospheres was 310 nm (range: 10–500 nm).

In vitro release of the aclarubicin PLGA nanospheres

Within each group, aclarubicin PLGA nanospheres (Exp. 9) with the highest entrapment efficiency (65%) were selected for the *in vitro* and *in vivo* release studies. The preparation method of Exp. 9 was used to prepare 10 batches of the samples, and these were subjected to *in vitro* release experiments under the specific *in vitro* environment. Figure 2 shows the *in vitro* release profiles of each of the 10 groups of aclarubicin PLGA nanospheres over a period of 28 days. Aclarubicin in the nanospheres could be completely released in 30 days. The

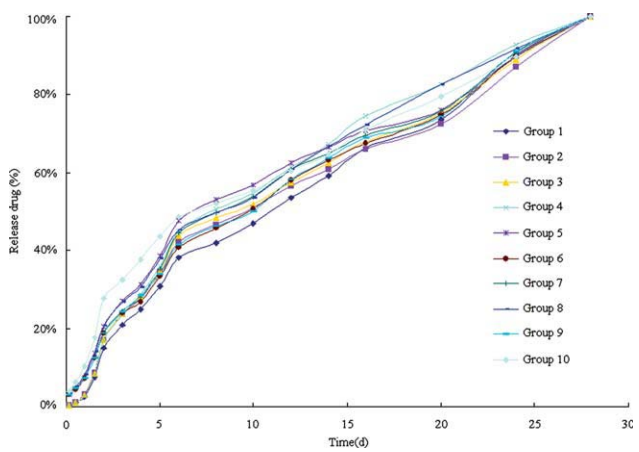

Figure 2 *In vitro* drug release curve of aclarubicin PLGA nanospheres over 28 days (10 groups). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

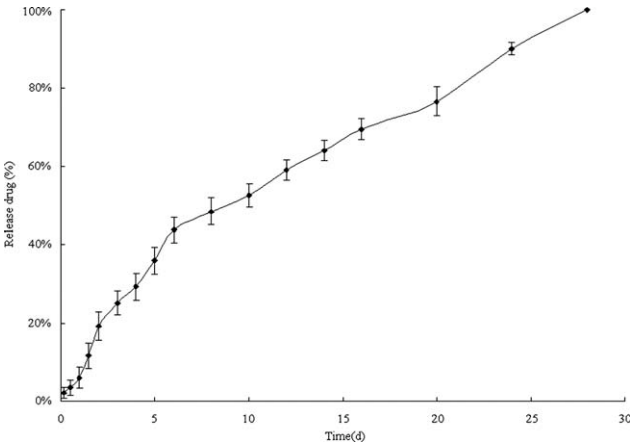

Figure 3 Average *in vitro* drug release curve of aclarubicin PLGA nanospheres over 28 days (10 groups).

average cumulative release rate was about 6% within one day, about 25% within 3 days, about 52% within 10 days, about 77% within 20 days, and about 98% within 28 days (Fig. 3).

In vivo release of the aclarubicin PLGA nanospheres

According to the *in vitro* release results, groups 1–9 were selected for the *in vivo* release study in rabbits. Due to a handling mistake, the *in vivo* release result from Group 9 was invalid. According to the *in vitro* and *in vivo* release curves, the nanospheres had a fast release period at early stages (day 0 to 8); after the drug entered the solution in the nanospheres and dissolved, it could diffuse into the medium across the pores in the nanospheres. The dissolution of the drug from the surface of the nanospheres and diffusion caused the burst release effect at early stages. Thereafter, the nanospheres showed a steady release curve. Figure 4 shows the *in vivo* release profile of aclarubicin PLGA nanospheres over the course of 30 days. The blood concentration in rabbits (detected via HPLC), indicated sustained release over a period of more than 20 days (Fig. 5).

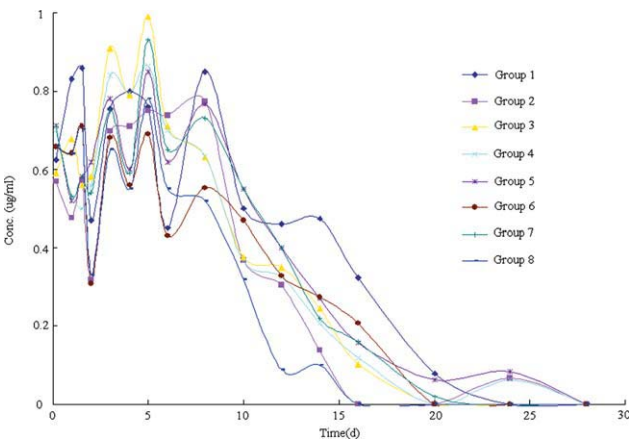

Figure 4 *In vivo* drug release curve of aclarubicin PLGA nanospheres in rabbits over 28 days (8 groups). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

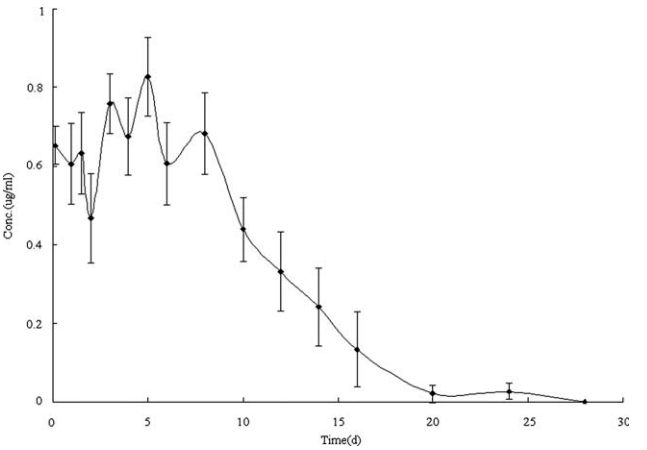

Figure 5 Average *in vivo* drug release curve of aclarubicin PLGA nanospheres in rabbits over 28 days (8 groups).

Toxicology profile of the aclarubicin PLGA nanospheres

Upon intravenous injection of varying doses of the aclarubicin PLGA nanospheres, toxicity reactions were immediately apparent; these included reduced activity, erected hair, cramps, convulsions, shortness of breath, and even death. The severity of these effects was found to decrease with reduced dosage. Intravenous administration of high doses (20 mg) of both aclarubicin PLGA nanospheres and the ordinary aclarubicin formulation resulted in the deaths of the majority of the mice. However, no formulation resulted in deaths when either the medium dose (10 mg/kg) or the low dose (1 mg/kg) was given (Table III).

Because there were no deaths of animals that received the medium dose, the LD50 of the aclarubicin PLGA nanospheres was predicted to be 20 mg/kg; however, the exact LD50 could not be calculated.

Compared to weights before the experiments, the weights of the animals after the experiments were all increased to some extent. However, there was no significant difference in the extent of the weight

TABLE III
Deaths in Each Group in the Toxicity Study

	Deaths in each group ($n = 25$)		
Group	High dose (20 mg/kg)	Medium dose (10 mg/kg)	Low dose (1 mg/kg)
Aclarubicin PLGA nanospheres	8	0	0
Dinary aclarubicin formulations	13	0	0

TABLE IV
Effect of Aclarubicin PLGA Formulations on the Weight of Mice ($\bar{X}$ ± S, n = 25)

Day	Nanosphere formulation (medium dose B) g	Nanosphere formulation (low dose C) g	Blank control (D) g	T-test results B : C	B : D	C : D
1	19.5 ± 2.9	18.2 ± 3.9	18.5 ± 4.1	−	−	−
3	21.8 ± 3.2	20.5 ± 2.6	21.4 ± 3.4	−	−	−
10	25.6 ± 4.5	29.6 ± 4.5	28.6 ± 5.6	+	+	−
20	32.7 ± 6.2	36.5 ± 5.7	35.7 ± 6.3	+	+	−

"+" represents significant difference ($p < 0.05$); "–" represents no difference ($p > 0.05$).

increase between two medium dose groups. However, when compared with the mice in the blank control group, there was a significant difference in weight from day 10 until the end of the observation period. The average weight of animals in the blank control group was 10% heavier than those in the drug groups, and compared to the blank control mice, the mice in the drug groups were less active. There was no significant difference between the weights of the two low dose groups and the blank control group mice for the extent of the observation period; however, the mice in the low dose groups exhibited less activity than those in the blank control group (Tables IV, V, and VI). Animals that received low and medium doses of either the aclarubicin PLGA nanospheres or the common aclarubicin formulation were subjected to autopsy on day 20. No obvious abnormalities were found in their major organs.

Pharmacodynamic profile

Overall, the nude mice in each group had normal activity patterns, food intake and weight gain throughout the dosing period. During the observation period, only two deaths occurred in the control group and a single death occurred in the treatment group; autopsy results indicated that these deaths were attributable to postoperative infections. No abnormalities were found in the rest of the mice. Comparison of the *in vivo* anti-liver cancer activities between the two groups showed that the aclarubicin PLGA nanospheres exerted varying degrees of anti-tumor activity in nude mice that were transplanted with human liver tumors; the tumor inhibition rate was 37% (Table VII). The results of the pathological examination showed that there were large amounts of necrotic tissues among the tumor tissue. Following light microscopic examination, no obvious abnormalities were found in the heart, liver, spleen, lung, kidney, and other organs in any group of the nude mice.

DISCUSSION

Studies on aclarubicin nanosphere formulations conducted in the late 1990s, mainly involved polymer package materials, such as polylactic acid, whereas there are few reports to examine the use of PLGA materials. There is considerable difficulty associated with the preparation of sustainably-releasing aclarubicin formulations that are able to meet the requirements for intravenous injection using traditional preparation parameters. A further reason is that the preparation process of nanospheres is unstable, and this means that small-scale experiments are difficult to amplify.

In this study, there was initial screening of various factors that are involved in the preparation process that affect the entrapment efficiency and release rate of the aclarubicin PLGA nanospheres. There are many factors that influence the entrapment efficiency, including the speed and time of initial emulsion, the pH and volume of the internal aqueous phase, the type, volume, and evaporation temperature of the organic solvent, the volume of the external aqueous phase, the type and volume of

TABLE V
Effect of Ordinary Aclarubicin Formulations on Mice Weight ($\bar{X}$ ± S, n = 25)

Day	Aclarubicin (medium dose F) g	Aclarucbicin (low dose G) g	Blank control (D) g	T-test results F : G	F : D	D : G
1	19.1 ± 3.3	20.2 ± 3.2	18.5 ± 4.1	−	−	−
3	21.1 ± 3.4	21.5 ± 2.6	21.4 ± 3.4	−	−	−
10	26.2 ± 5.8	28.1 ± 5.1	28.6 ± 5.6	−	+	+
20	33.1 ± 7.2	37.1 ± 6.4	35.7 ± 6.3	−	+	+

"+" represents significant difference ($p < 0.05$); "−" represents no difference ($p > 0.05$).

TABLE VI
Effects of Aclarubicin PLGA Nanospheres and Ordinary Aclarubicin Formulation on Mice Weight ($\bar{X} \pm S$, $n = 25$)

Day	Nanoshpere formulation (medium dose B) g	Aclarubicin (medium dose F) g	Nanoshpere formulation (low dose C) g	Aclarubicin (low dose G) g	T-test resutls B : F	T-test resutls C : G
1	19.5 ± 2.9	19.1 ± 3.3	18.2 ± 3.9	20.2 ± 3.2	–	–
3	21.8 ± 3.2	21.1 ± 3.4	20.5 ± 2.6	21.5 ± 2.6	–	–
10	25.6 ± 4.5	26.2 ± 5.8	29.6 ± 4.5	28.1 ± 5.1	–	–
20	32.7 ± 6.2	33.1 ± 7.2	36.5 ± 5.7	37.1 ± 6.4	–	–

"+" represents significant difference ($p < 0.05$); "−" represents no difference ($p > 0.05$).

the emulsifier, the dosage of the drug, the PLGA concentration, the method by which the organic solvent is removed, and the addition of electrolyte to the external aqueous phase. The author selected the entrapment efficiency as the primary influencing factor, and then selected the appropriate technical parameters in combination with *in vitro* release. First, the most important factors to affect the entrapment efficiency were considered using an orthogonal design; however, technical parameters that could be easily improved in later processes were ignored. The resultant aclarubicin nanospheres had an entrapment efficiency of up to 65%, with uniform circular shapes and a high cumulative release rate at fixed intervals.

The particle size of the aclarubicin nanospheres in this study was slightly larger than those previously reported,[2,16] might be due to the different mixing speeds of the double emulsion, or to different preparation methods of the initial emulsion in the double emulsion method. In the author's opinion, although a smaller particle size is safer inside the body (and less likely to cause obstruction of narrow blood vessels), as showed by the *in vitro* release experiments, it is also easily agglutinated, quickly released, and ineffective at maintaining a long-lasting effect. As aclarubicin is soluble in acidic environments but insoluble at pH levels higher than 7, increasing the pH of the external aqueous phase could reduce the solubility of aclarubicin; this could reduce the outward diffusion of the drug to the external aqueous phase and lead to increase in the entrapment efficiency and drug load of the nanospheres. In this study, the pH value of the external aqueous phase was 7.5; this was considered to be optimal. It has been reported that adding Na_2SO_4 to the external aqueous phase could increase its ionic strength; this might assist the adsorption of aclarubicin on the surface of the nanospheres, and thereby enhance the entrapment efficiency and the drug load.[20] However, in this study, the entrapment efficiency did not improve after the addition of 2% Na2SO4. This might be due to the active agent PVA that was used in the experiment and to the appropriate oil to water ratio that offset the effect of ionic strength on the adsorption of aclarubicin to the surface of the nanospheres.

The *in vitro* cumulative release rate of the aclarubicin PLGA nanospheres was lower than the *in vivo* rate. There are several possible reasons for this finding. First, under *in vivo* conditions, in addition to causing drug release, it is possible that degradation of the encapsulation materials could lead to the phagocytosis of macrophages and that this could also cause the early decomposition of many small nanospheres, resulting in the early release of aclarubicin. A second explanation is that the small size of the nanospheres caused the loss of some aclarubicin during the experimental operation of the *in vitro* release process, and this may have led to a reduction in the amount of released aclarubicin. Finally, the fact that aclarubicin is unstable in phosphate buffer and acetate buffer means that these solutions may be more suitable for use as the release buffer for the simulation of *in vitro* release than the normal saline that was used; it is difficult to simulate *in vitro* release with the latter.

In some previous reports, for *in vitro* release experiments, the nanospheres were added to treated dialysis bags with tightened ends,[21] and then put into flasks that were filled with *in vitro* release buffer. These experiments indicated that although

TABLE VII
Comparison of the Anti-Tumor Effects of Aclarubicin PLGA Nanospheres on Mice

Group	Animal amount (initial/end)	Mice weights (initial/end)	Average tumor amount (g)	Tumor inhibition rate (%)	Comparison with control
Treatment	6/5	19.1 ± 1.9/32.2 ± 2.3	1.16 ± 0.23	36.96	$P < 0.01$
Control	6/4	19.8 ± 2.3/31.2 ± 3.1	1.84 ± 0.33	–	

this method is more suitable for the release of micro-particles, it does not confer any advantage for the release of nano-scale particles. Due to the small size of the nanospheres, the limitations on their movement caused by dialysis bags tend to make them stick together, inosculate, and coagulate.

The *in vitro* release curve of nanospheres can be divided into two parts: burst release and sustained release. The speed of release was reduced with the increasing molecular weight of PLGA. Burst release mainly comes from the diffusion of aclarubicin on or near the surface of the nanospheres into the medium, whereas sustained release is mostly the result of either the diffusion of aclarubicin from the center of the nanospheres to the surface, or corrosion.[22] With a relatively high entrapment rate, the majority of aclarubicin was centered in our aclarubicin PLGA nanospheres, and this contributed to the sustained-release phase. Therefore, to raise the initial blood concentration of intravenously injected drug, some ordinary aclarubicin agents could be spread on the surface of the nanospheres.

Due to the hydrophobic surface of the aclarubicin PLGA nanospheres, they are likely to be recognized and phagocytosed as foreign bodies by phagocytes upon intravenous injection; this would result in a reduced retention time of the nanospheres in the blood. Therefore, intravenous injection of PLGA nanospheres is more favorable for the treatment of organs, such as liver and spleen. Thus, the pharmacodynamic properties of the experimental design that was used in this study was suitable for the aclarubicin PLGA nanospheres. The large individual differences that were found in the tumor-inhibition rate of aclarubicin PLGA nanospheres in mice in the pharmacodynamic tests may be due to individual differences in operation. It is also possible that they may be caused by the loss of part of the tumor during tumor transplantation operations. The toxicology profile of the aclarubicin PLGA nanospheres showed that compared with ordinary aclarubicin formulations, there was no increase in the toxicity of aclarubicin formulations that were prepared using the nanosphere technology.

Compared to liquid formulations, the freeze-dried aclarubicin nanospheres prepared in these experiments were more stable. The major drawback of the original aclarubicin formulation is side effects, such as bone marrow suppression, cardiac toxicity, and gastrointestinal discomfort. However, when aclarubicin was entrapped by PLAG, aclarubicin was released slowly into the blood, and this slow release led to increased stability, extended half-life, and reduced toxic side effects. The current formulation was optimized, and the experimental parameters were fixed, and tested repeatedly. The entrapment efficiency and appearance of the resultant nanospheres were basically consistent with the *in vitro* release curve. As the preparation process used in these experiments was highly and demonstrably stable, it may serve as a reference for the future industrial preparation of aclarubicin PLAG nanospheres. Clinically, aclarubicin is often used in combination with other drugs in chemotherapy for the treatment of malignant lymphoma and other tumors. Not only will the application of sustained release entrapment reduce the suffering at the time of aclarubicin injection, it will also reduce the extent of side effects caused by concomitant medications.

CONCLUSION

After considering the effects of a number of different conditions on the preparation process, PLGA was selected as the carrier matrix. To prepare an aclarubicin nanosphere formulation that had high drug loading capacity, aclarubicin was entrapped in PLGA. Both *in vitro* and *in vivo* studies showed that aclarubicin could be sustainably released over a period of almost 20 days. *In vivo* results showed that the aclarubicin PLGA nanospheres had anti-tumor activity *in vivo*, and that they lacked toxic effects that were more serious than those caused by ordinary aclarubicin agents. This novel, slow-releasing formulation of aclarubicin PLGA nanospheres can both extend the half-life of the drug and improve patient compliance.

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