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Lung-targeting microspheres of carboplatin

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Abstract

Carboplatin (CPt) was incorporated in the gelatin microspheres by the method of emulsion and the drug content determined spectrophotometrically. The arithmetic mean diameter of the microspheres was 13.20 μm with 98% of the microspheres ranging from 5.0 to 28.6 μm. The average carboplatin content was 23.76% and the yield of the microspheres 85.12%. The microspheres were stable for three months when stored at 37 °C/RH 75%, showing insignificant change in appearance and drug content. The in vitro release profile of the microspheres could be described by a biexponential equation, and the release $t_{1/2}$ was 49.7 min and 92.04% released in 10 h; while for the original drug, CPt, under the same conditions, 92.15% released in the first half an hour. Very high lung-targeting efficiency in vivo was proved by the results of targeting parameters. The S-180 lung neoplasm models were established by i.v. cancer cells in mice and the number of pulmonary nodules examined for evaluation of the treatment effect. The results of therapeutic tests showed that the antitumour effects were increased by injection of the microspheres compared with the injection of CPt solution: half of the dose in the microspheres showed comparable effect to the original drug.

Keywords: Carboplatin; Gelatin; Lung-targeting drug delivery systems; Antitumour drug

1. Introduction

Carboplatin (CPt), an antitumour drug of platinum, has been proven efficient in many types of cancer treatments, including pulmonary small-cell carcinoma, carcinoma of ovary and testis, and epithelium carcinoma of head and neck (Woloschuk et al., 1988), and its main side effects include inhibition of marrow, toxicity to digestive tract and ears, and allergy (Kellend and Mckeage, 1994). Up to now, CPt is available only in the form of injection for clinical use.

Lung cancers are among the most harmful cancers and are increasingly hazardous to human. In China, over 1.3 million patients suffer from lung cancers, increasing especially fast in female, and the malignant lung tumor ranks first in all malignant tumors, both in incidence and death rate. In Beijing, the incidence of lung cancers constitutes 28% of all cancers. Lung cancers rank one of the commonest cancers in Europe and America. In France, the incidence of lung cancers increased 20% for male and 75% for female in the past 20 years; every year 160 thousand Americans die from lung cancers.

Gelatin is a widely used biodegradable coating material for microencapsulation with good biocompatibility, of which the metabolic products are harmless. While various preparative technology can be applied to obtain gelatin microparticles with good drug loading and entrapment rate, their drug release rate is able to be controlled by the reaction time and concentration of the linking agent, usually formaldehyde or glu-

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taraldehyde (Lu et al., 1992; Lu, 1997). When the particle size of the microparticles was controlled in the range of 12–44, 10 or 20 μm, respectively, 95, 70–80 or over 85% of the microparticles can be concentrated in lung after i.v. due to the mechanical trapping effect of the pulmonary capillary blood vessel (Lu, 1998).

In an attempt to decrease the side effects and to increase the therapeutic effect in the treatment of lung cancers, gelatin microspheres for lung targeting were prepared and investigated.

2. Experimental

2.1. Apparatus, reagents and animals

A spectrophotometer, UV-250 (Shimazu, Japan), was used for determination of drug content, and a differential scanning calorimeter, DSC-2C (USA), used for comparison of the characteristic peaks of the microspheres with the controls, and for determination of the activation energy of thermal degradation of the microspheres. An optical microscope with camera (Olympus, Japan) and a scanning electron microscope (1000-B, Amray, USA) were used for physical examination of the microspheres. Atomic absorption spectrophotometer (703 Perkin-Elmer) was used for determination of carboplatin content in animal samples.

Carboplatin (CPt, Qilu Pharmaceutical Factory of Shandong, China, >99%) and gelatin prepared by acidic method (Shanghai Gelatin Factory) were used to prepare the microspheres. All other reagents used were of reagent grade.

Animals: Kunming mice, female, each weighing 20–22 g.

2.2. Preparation of carboplatin gelatin microspheres

2.2.1. Basic protocol

Carboplatin gelatin microspheres, designated CPt-MS, were prepared by emulsion method (Zeng et al., 1996). The procedures were briefly as follows. The gelatin aqueous solution of carboplatin was dispersed in liquid paraffin with the help of an emulsifying agent under stirring. The formed W/O emulsion was treated with formaldehyde to solidify gelatin particles and the obtained microspheres separated, washed and dried. Washing is necessary for washing off the liquid paraffin and excessive formaldehyde, though at the same time the drug on the surface and the drug loading rate will be also reduced.

2.2.2. Optimization of the preparative technology

Single factor test was first conducted to preliminarily optimize the reaction conditions, including the concentration of gelatin in the aqueous solution, the type and amount of the emulsifying agent, the mode and speed of stirring, the temperature and time of emulsifying procedures. The optimized conditions were: 50 ml aqueous solution of gelatin and CPt (weight ratio of CPt/gelatin was 1/1) was added to 50 ml liquid paraffin, then 5 ml Span 85 added under mechanical stirring for 10 min at 200 revolutions/min and $50 \pm 0.5\,^{\circ}C$ to obtain a W/O emulsion, which was then solidified with formaldehyde. The formed microspheres were separated and washed successively with ethyl ether + isopropanol and isopropanol, then air-dried. Secondly, uniform design was applied. Influence of the weight ratio of CPt to gelatin on the drug content was investigated. Also the obtained CPt-MS were examined microscopically for their morphology and uniformity.

2.2.3. Determination of CPt

It was reported that Pt^{4+} ion can be reduced in acidic solution by $SnCl_2$ to give a coloured complex $[PtSn_4Cl_4]^{4+}$ which was used for quantitation of carboplatin (Zhu et al., 1995). In this paper, simple spectrophotometry was used for the determination of CPt in acidic solutions.

The standard regression equation was prepared as follows. 25.0 mg of standard CPt, dried to constant weight, was dissolved in water to make 100 ml stock solution, of which different volumes were used to mix with water to make working solutions, each 5 ml. To each working solution was added 5 ml of 1.0 mol/l $SnCl_2$ in 1.2 mol/l hydrochloric acid. The mixture, after thorough mixing, was thermostatted at $25\,^{\circ}C$ in the dark for 50 min to allow the completion of reaction. After reacting with $SnCl_2$, CPt showed a maximum wavelength at 398 nm. The values of A_{398} of the standard CPt solutions were used to produce a plot of A_{398} versus the amount of CPt, allowing the following standard regression equation in the range of $C =$ 10–50 mg/l to be written:

$$A = 0.01695C - 0.0629,\ r = 0.9999. \quad (1)$$

CPt content in CPt-MS was determined as follows. The CPt-MS were dispersed in about 8 ml water under ultrasonic action for 10 min, then filtered and the microspheres washed. These procedures were repeated for another three times. The filtrates were collected to make 50 ml solution of CPt, of which 5 ml was mixed with 1.0 mol/l $SnCl_2$ in 1.2 mol/l hydrochloric acid, and, after shaking, thermostatted at 25 °C in the dark for 50 min for the reaction to complete. After extracting with ultrasonic treating four times as above, the residual CPt in microspheres was less than 1% of the total CPt.

Absorbance at 398 nm was measured and the concentration calculated according to the standard regression Eq. (1). The blank gelatin MS were treated the same way and used as the blank reference.

When CPt was determined in a pH 7.4 phosphate-buffered solution (PBS), as in the case of release in vitro, the solution was used for direct determination by second-order derivative spectrophotometry. The pH 7.4 PBS was used as the reference. The amplitude D between 224.8 nm (valley point) and 240.8 nm (peak point) in the second-order derivative spectra was used as a measure of the concentration and the standard regression Eq. (3) used for quantitation (see Section 3).

2.3. Determination of recoveries of CPt

To a certain amount of blank MS were added different amounts of standard CPt (for simple spectrophotometry, water was used as the solvent; for the second-order derivative method, pH 7.4 PBS was used) and treated with ultrasonic. The mixture was filtered, and a certain portion of the filtrate mixed with 1.0 mol/l $SnCl_2$ in 1.2 mol/l hydrochloric acid, and, after shaking, thermostatted at 25 °C in the dark for 50 min for the reaction to complete. The above two determination methods were used, respectively, to calculate the recoveries for evaluation of the reliability of the methods.

2.4. Identification of the formation of microspheres and their activation energy of thermal degradation

CPt, blank MS, a physical mixture of CPt and MS (weight ratio 1:3, CPt + MS) and CPt-MS were examined by differential scanning calorimetry (DSC) in the range of 320–600 K under the following conditions: atmosphere—N_2, reference—vacant aluminum crucible, heating rate—20 K/min. The DSC curves were compared to help identify the formation of microspheres.

When the DSC curves of the CPt-MS were conducted at different heating rate (20, 40, 80, and 160 K/min), Kissinger's equation (Kissinger, 1956; Lu et al., 1992) was used to calculate the activation energy of thermal degradation:

$$\ln\left(\frac{\beta}{T_m^2}\right) = \frac{-E}{T_m R} \tag{2}$$

where β is the rate of rise in temperature, T_m is the peak temperature in DSC curves, R is the molar gas constant and E is the activation energy of thermal degradation.

2.5. Release of the drug in vitro

In 50 ml pH 7.4 PBS, a dialysis tube with CPt-MS (containing 25 mg CPt), or 25 mg CPt or a physical mixture of 25 mg CPt and 75 mg MS as the controls, was installed. The device was thermostatted at 37 ± 0.5 °C and shaken at 70 ± 2 revolutions/min. After known immersion times (0.5, 1.0, 2.5, 5.0, 10.0, 24.0 h), 1 ml of the medium was removed and its concentration determined with the second-order derivative method, and 1 ml medium added after each sampling. The content for each sample and the mean cumulative release rate were calculated. The procedures were applied to the three batches of CPt-MS, before and after storage.

2.6. Examination of stability of CPt-MS

2.6.1. Physical stability

CPt-MS were stored for three months at 37 °C/RH 75% in the dark. The appearance of the microspheres was examined microscopically. Since ours was not a product, for research work, we only used the above storage conditions instead of those in ICH guidelines.

2.6.2. Chemical stability: observation after storage

After storage for three months at 37 °C/RH 75%, the drug content and release rate of CPt-MS were determined.

2.7. Body distribution of the drug and lung-targeting characteristics

Sixty mice were randomly divided into 20 groups, each three mice. Groups 1–10 were administrated i.v. through mouse-tail different doses of CPt-MS in physiological salt solution containing 0.25% tragacanth; groups 11–20 were controls, with equivalent doses of CPt in physiological salt solution. After different time periods, the mice were sacrificed. CPt contents in blood, heart, liver, spleen, kidney and lung were determined by atomic absorption spectrophotometry. Three targeting parameters (intake rate r_e, targeting efficacy t_e and peak concentration ratio C_e) (Gupta and Hung, 1989) were calculated for evaluation of lung-targeting characteristics.

2.8. Pharmacodynamic study

A certain amount of S-180 carcinoma cells was injected i.v. to mouse tails (3.6×10^7 cells per 10 g mouse) under sterilized condition. Seven days afterwards, the mice were sacrificed and their lungs taken, fixed with 10% formaldehyde and sliced for pathologic examination. The growth of the carcinoma nodules in the lung indicated that the lung-carcinoma models were successfully developed.

One hundred and twenty-five female mice were divided randomly into five groups, each 25 mice, and treated with S-180 carcinoma cells as above. Seven days afterwards, different doses of CPt-MS were administrated i.v. for consecutive five days to the tails of the groups 1–3. Meanwhile, group 4 was administrated CPt as the positive control and group 5 blank MS as the negative control. On day 14 after inoculation, the mice were sacrificed and dissected, and the lung taken for examination with electron microscope. The number of the carcinoma nodules in the lung of different groups was counted and compared.

2.9. Acute toxicity

One hundred and fifty mice were divided into 15 groups randomly, each 10 mice. Groups 1–5 were administrated i.v. different doses of CPt-MS, and groups 6–10 and groups 11–15 different doses of CPt solution and blank MS, respectively. The number of dead mice was recorded until day 7 after administration when mice of the group of the largest dose all died.

3. Results and discussion

3.1. Preparation of the microspheres

Immersion–absorption method had been preliminarily tested. Blank gelatin microspheres were immersed in the CPt solution, under static or stirring condition, for different time periods. The CPt amount on the surface and inside the microspheres increased with time. Unfortunately, drug content was too low even after immersion for 48 h: for static immersion, total drug content was only 5.33% (of which, 4.21% on the surface and 1.12% inside), and for stirring immersion, total drug content was only 2.58% (of which, 2.56% on the surface and 0.02% inside).

In emulsion procedures, when castor oil was used as the oil phase, the drug content was low (drug content inside the microspheres being lower than 2%), partially because CPt can dissolve in castor oil. So the emulsion method using liquid paraffin as the oil phase was adopted.

When various weight ratios of CPt/gelatin were tested for preparation, CPt-MS with different drug content were obtained, as shown in Table 1. The drug content on the surface of the microspheres was determined in the water which had been mixed and stirred with the microspheres for a certain period of time (30 s) and separated before determination. The total drug content was also determined and the content inside the microspheres was obtained by the difference between the total and the surface content. According to the results, the weight ratio of test no. 3

Table 1
Influence of carboplatin to gelatin ratio on the drug content of CPt-MS

Test no.	CPt:gelatin (g:g)	CPt content (%)			Inside/total ratio (%)
		Surface	Inside	Total	
1	0.5:1	5.24	5.54	10.78	51.39
2	0.75:1	6.57	7.55	14.12	53.47
3	1:1	8.87	14.81	23.68	62.54
4	1.25:1	18.29	12.91	31.20	41.38
5	1.5:1	27.92	14.96	42.88	34.89

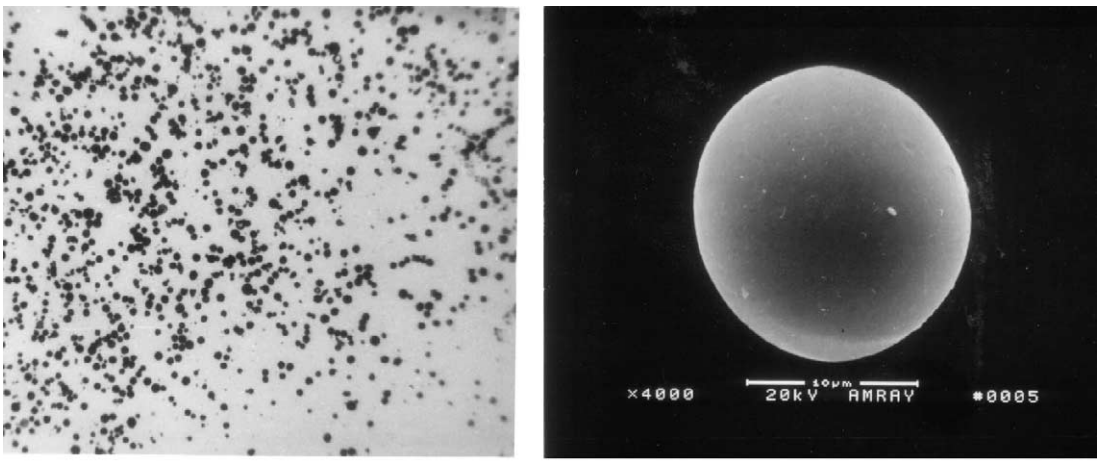

Fig. 1. Carboplatin gelatin microspheres (CPt-MS): (a) optical photomicrograph (100×); (b) scanning electron micrograph (10 000×).

gave good drug content and the best inside/total ratio, hence the weight ratio 1:1 of CPt/gelatin was used for preparation of three batches of the microspheres. Microspheres in whitish color were obtained with drug content of (23.76 ± 0.98%; inside/total ratio 63.22 ± 0.07%) and microsphere yield of (85.12 ± 0.40%; $n = 3$).

3.2. Appearance, particle size and distribution

Under optical and scanning electron microscopes, the CPt-MS looked round and regular, easy to disperse, as shown in Fig. 1. The particle size and its distribution are shown in Table 2. The arithmetic mean diameter was calculated to be 13.32 μm with 91.8% of the total being in the range of 5.0–28.6 μm. The span was 0.91 and the mean volume diameter ($= (\sum nd^3 / \sum n)^{1/3}$) was 12.82 μm.

3.3. Other physical properties

The bulk density of CPt-MS was determined by graduated cylinder method to be (0.36 ± 0.01 g/ml). The angle of repose of CPt-MS was determined by fixed funnel method to be (56.37 ± 1.84°; $n = 3$). CPt-MS were found positively charged by electrophoresis on a cellulose acetate membrane at pH 7.4, and CPt and blank gelatin microspheres were also charged positively under the same condition. The critical relative humidity of CPt-MS was determined to be 61.53% based on the routine procedures (Liu, 1984).

3.4. Determination of CPt

In order to determine the CPt content in CPt-MS, hydrolysis-precipitation method had been prelimi-

Table 2
Particle size distribution of CPt-MS

Diameter range (μm)	Middle value (d) (μm)	Particle number (n)	Frequency (%)	nd (μm)	nd^3 (μm^3)
3.0–4.9	4.0	26	5.2	104.0	1664.0
5.0–14.3	9.7	327	65.4	3171.9	298444.1
14.4–21.5	18.0	104	20.8	1872.0	606528.0
21.6–28.6	25.1	28	5.6	702.8	442771.0
28.7–42.9	50.1	15	3.0	751.5	1886272.5
≥43	0	0	0	0	0

narily tested to eliminate the interference of gelatin. Gelatin was completely degraded by hydrolysis in hydrochloric acid, and under the reaction of sodium deoxycholate and trichloro-acetic acid, the degraded gelatin was precipitated and separated before spectrophotometric determination of CPt. These procedures were unsuccessful because: (a) hydrolysis was time-consuming and CPt was unstable in acidic solution, which resulted in the fluctuation of the recoveries; (b) the solution obtained after hydrolysis was brown and interfered with the determination.

In this paper, simple spectrophotometry was used in acidic solutions for determination of CPt, A_{398} being measured after reacting with $SnCl_2$ in hydrochloric acid.

However, in pH 7.4 PBS, the maximum wavelength of the CPt solution changes with its concentration, so the simple spectrophotometry is not suitable for determination. Fortunately, the second-order derivative spectrophotometry, using amplitude D between 224.8 nm (valley point) and 240.8 nm (peak point), was proved good for accurate quantitation, since the valley and peak wavelengths did not change with concentration (see Fig. 2) and the standard regression equation in the range of $C = 10$–50 mg/l was linear:

$$D = 1.9499C + 0.5960,\ r = 0.9999 \quad (3)$$

3.5. Determination of recoveries of CPt

The recoveries by the two determination methods are shown in Table 3. The results showed that the two methods are both reliable for determination under different conditions: simple spectrophotometry was used in acidic solutions and second-order derivative spectrophotometry in pH 7.4 solutions.

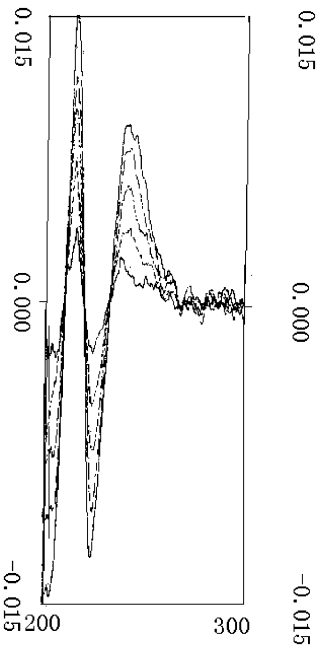

Fig. 2. Second-order derivative spectra of CPt-MS.

3.6. Identification of the formation of microspheres and their activation energy of thermal degradation

Curves of DSC are shown in Fig. 3, from which one can conclude that CPt-MS was not a physical mixture, but real microspheres were formed. The characteristic exothermic peaks at 535.75 K of CPt and 386.87 K of blank MS, respectively, both almost disappeared in the CPt-MS curve, in which a new characteristic peak at 372.34 K appeared. The DSC curve of the physical mixture also differed from that of CPt-MS.

Table 3
The results of recoveries for determination of CPt ($n = 3$)

	CPt added (μg/ml)	CPt found (μg/ml) mean ± S.D. (%)	Recoveries (%)	Mean ± S.D. (%)
A	10	9.70 ± 0.12	97.01 ± 1.22	
	20	19.80 ± 0.15	99.00 ± 0.25	98.40 ± 0.61
	40	39.68 ± 0.14	99.20 ± 0.35	
B	10	9.91 ± 0.07	99.07 ± 0.71	
	30	29.91 ± 0.19	99.69 ± 0.63	99.72 ± 0.89
	50	50.20 ± 0.66	100.4 ± 1.3	

A: simple spectrophotometry; B: second-order derivative spectrohpotometry.

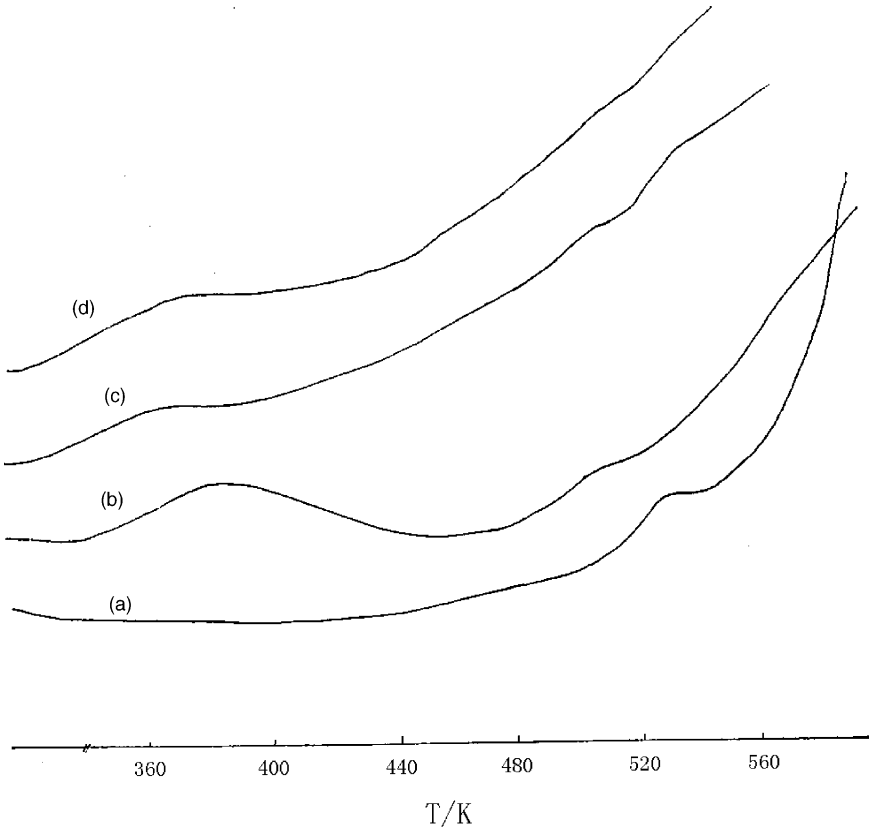

Fig. 3. Curves of differential scanning calorimetry: (a) CPt; (b) MS; (c) CPt + MS; (d) CPt-MS.

Curves of DSC of different heating rate are shown in Fig. 4. According to Eq. (2), the following regression equation was written for the four peaks:

$$\ln\left(\frac{\beta}{T_m^2}\right) = \frac{-9699}{T_m} + 9.827,\ r = 0.973\ (n = 4)$$

The activation energy 80.64 kJ/mol was calculated from the slope. The comparatively large value for the activation energy of CPt-MS thermal degradation, together with the results of the storage tests indicated that the chemical stability of the microspheres was satisfactory.

3.7. Release in vitro

Table 4 lists the results of release in vitro for CPt, CPt + MS and CPt-MS, respectively. About 40% of the total CPt in CPt-MS released in the first half an hour, which reflected the significant amount of CPt adsorbed on or incorporated near the surface of the microspheres, as shown in Table 1. In clinical practice, this would lead to 'burst effect', which enables the preparation to show fast effect to the patients. However, if the burst effect exceeds a certain percentage,

Table 4
Data of release in vitro: mean cumulative release rate vs. time ($n = 3$)

Release time (h)	Cumulative release rate (%)		
	CPt	CPt + MS	CPt-MS
0.5	92.15 ± 1.56	56.20 ± 1.74	40.12 ± 2.67
1.0	98.15 ± 1.22	70.57 ± 3.14	52.07 ± 2.21
2.5		95.48 ± 1.86	80.14 ± 3.99
5.0		97.34 ± 1.35	91.49 ± 3.76
10.0			92.04 ± 2.30
24.0			95.05 ± 2.38

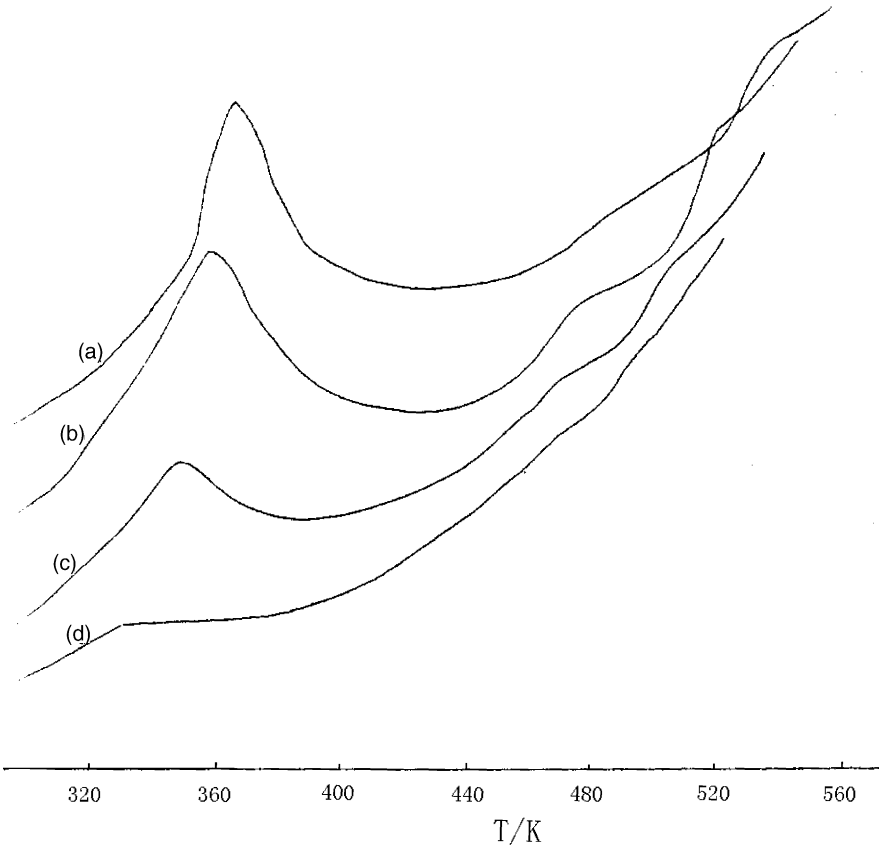

Fig. 4. Differential scanning calorimetric curves of CPt-MS at different rate of rise in temperature: (a) 20 K/min; (b) 40 K/min; (c) 80 K/min; and (d) 160 K/min.

the left amount for sustained release might be insufficient. CPt + MS, which was tested only for comparison, released faster than CPt-MS but, unexpectedly, slower than CPt. The release behavior of CPt + MS can be explained partially by the adsorption of part of the drug by the microspheres, partially by the blank microspheres preventing the drug from diffusion in the dialysis tube. Several equations were used to fit the results (0.5–24.0 h) for the microspheres, as shown in Table 5, which indicated the biexponential equation fits well and the release $t_{1/2}$ is 49.7 min, and 92.04% released in 10 h. While for the original drug, CPt, under the same conditions, 92.15% released in the first half an hour.

Table 5
Regression equations of mean cumulative release rate vs. time for CPt-MS

Equations	CPt-MS	r
Zero-order kinetic eq.	$Q = 0.01749t + 0.6262$	0.6692
First-order kinetic eq.	$\ln(1 - Q) = -0.09225t - 1.1497$	0.8054
Higuchi eq.	$Q = 0.1205t^{1/2} + 0.4787$	0.8063
Baker-Lonsdale eq.	$3/2[1 - (1 - Q)^{2/3}] - Q = 0.01147t + 0.1221$	0.7717
Weibull eq.	$\ln\ln[1/(1 - Q)] = 0.4828 \ln t - 0.1825$	0.9531
Biexponential eq.	$1 - Q = 0.7554 \exp(-0.6701t) + 0.07125 \exp(-0.1157t)$	0.9979

Table 6
The relative content (%) of CPt in blood and various organs (mice sacrificed) after different time of i.v. administration of CPt-MS in mice (mean of three determinations)

Time (h)	Blood	Heart	Liver	Spleen	Kidney	Lung
0.017	79.80	0.52	1.80	0.73	5.73	11.43
0.083	73.81	0.49	1.51	0.58	4.33	19.29
0.25	48.96	0.35	1.24	0.90	1.42	47.14
0.75	35.37	0.35	0.87	1.03	1.37	61.00
2	29.09	0.58	4.80	2.15	1.25	62.16
5	16.65	0.08	3.16	0.99	0.82	78.30
12	29.55	0.34	3.35	1.54	1.31	63.91
24	36.18	0.42	4.17	2.06	5.43	51.75
48	47.80	2.47	6.16	3.24	8.03	32.31
72	44.16	0.50	2.51	3.62	13.71	35.49

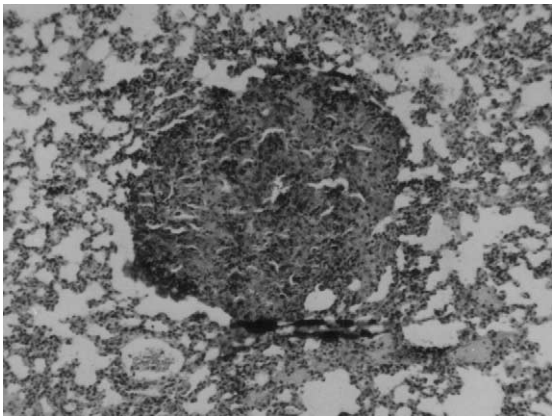

Fig. 5. Growth of S-180 appeared in the lung of mice, 7 days after innoculation (70×).

3.8. Tests for stability

After storage at 37 °C/RH 75% for three months, CPt-MS showed no change in appearance and redispersing ability.

The drug content after storage for three months at 37 °C/RH 75% was ($23.42 \pm 0.19\%$; $n = 3$), which had no significant difference compared with the drug content before storage ($23.76 \pm 0.62\%$; $n = 3$; $P > 0.05$). After storage for three months at 37 °C/RH 75% the biexponential equation for release in vitro became

$$1 - Q = 0.7943\exp(-0.8538t) + 0.06917\exp(-0.02952t), \quad r = 0.9973$$

of which $t_{1/2}$ was 43.0 min (while it was 49.7 min before storage).

3.9. Lung-targeting characteristics

The change in drug content (μg/g or μg/ml) with time was determined in blood, heart, liver, spleen, kidney and lung, and the results (omitted) were calculated to express the percentage amount, as shown in Table 6, the total amount of the contents in blood and all other organs being taken as 100%. Since the volume of the organs of the mice was too small, the blood or organs of three tested mice were mixed and the average content determined. The data of drug content change with time were treated to calculate the targeting parameters, as shown in Table 7. CPt-MS showed the largest value of AUC and r_e for lung; the targeting efficacy (t_e) of lung increased by a factor of 9.4 (compared with spleen) ~90.5 (compared with liver). Compared

Table 7
The lung-targeting parameters of CPt-MS (MS) and CPt (S) after i.v. of mice ($n = 3$)

Sample	AUC[a]		r_e	t_e		$(t_e)_{MS}/(t_e)_S$	C_p[a]		C_e
	MS	S		MS	S		MS	S	
Blood	112.8	115.7	0.97	11.0	0.59	18.4	64.1	111.3	0.58
Heart	19.9	28.8	0.69	61.3	2.4	26.0	5.5	18.9	0.29
Liver	13.4	27.4	0.49	90.5	2.5	36.5	1.9	3.6	0.53
Spleen	129.6	88.4	1.5	9.4	0.77	12.2	11.7	23.1	0.51
Kidney	83.1	54.6	1.5	14.6	1.2	11.8	23.2	62.1	0.37
Lung	1217.0	67.9	18	1	1	1	108.9	26.8	4.1

[a] Unit of AUC: μg h/ml or μg h/g; $r_e = (AUC)_{MS}/(AUC)_S$; $t_e = (AUC)_{lung\ targeted}/(AUC)_{untargeted}$; C_p: peak concentration (μg/ml or μg/g), $C_e = (C_p)_{MS}/(C_p)_S$.

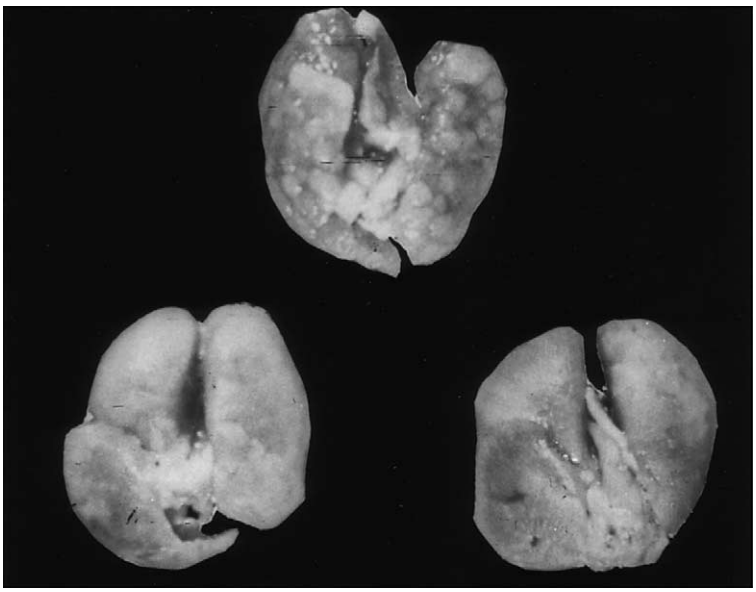

Fig. 6. Antitumor effect of different treatment in mice bearing pulmonary carcinoma. Upper: no treatment; lower left: CPt-MS (8 mg CPt/kg); lower right: CPt (16 mg CPt/kg).

with the original drug CPt, the targeting ratio of lung $((t_e)_{NS}/(t_e)_S)$ increased by a factor of 11.8 (compared with kidney) ~36.5 (compared with liver), and the ratio of peak concentration in lung (C_e) increased by a factor of 4.1 compared with CPt, while all other values of C_e declined. All these strongly indicated very significant lung-targeting characteristics of CPt-MS.

According to the drug content change with time in lung, a two-compartment model was established and the following pharmacokinetic equation for lung obtained

$$C = 60.22343e^{-0.12277t} + 9.27946e^{-0.00300t}$$

For blood, a three-compartment model was established and the following pharmacokinetic equation obtained:

$$C = 112.46645e^{-8.04971t} + 13.22677e^{-0.98124t} + 1.73209e^{-0.01303t}$$

3.10. Pharmacodynamics of CPt-MS

The pulmonary carcinoma nodule (S-180) and the antitumor effect are shown in Figs. 5 and 6 and Table 8. The average number of pulmonary carcinoma nodules after CPt-MS treatment was smaller than that treated with CPt of a doubled dose, though showed unsignificant difference ($P > 0.05$). The results of CPt-MS with a dose of 16 mg CPt/kg were too scattered, so are not cited. One can conclude from these results that

Table 8
Antitumor effect of different treatment for five days in mice bearing pulmonary carcinoma

Drug	Dose (mg CPt/kg per day)	Number of mice	Number of pulmonary nodules per mouse (mean ± S.D.)	P_1[a]	P_2[a]
CPt-MS	8.0	25	18.8 ± 10.8	<0.001	>0.05
CPt	16.0	25	23.3 ± 11.8	<0.001	
MS	33.7[b]	25	64.5 ± 27.0		<0.001

[a] P_1 compared to the value of MS (negative control); P_2 compared to the value of CPt (positive control).
[b] Amount of blank microspheres.

microencapsulation improves the antitumor effect of CPt.

3.11. Acute toxicity

LD_{50} (with 95% confidence limit) of CPt-MS, CPt and blank MS were calculated to be 95.7 ± 11.4 mg/kg, 150.8 ± 15.1 mg/kg and 108.2 ± 12.8 mg/kg, respectively. In the literature (Li et al., 1990), LD_{50} of CPt was reported to be 153.6 ± 13.6 mg/kg, with which our result was in accordance. Blank MS and CPt-MS caused some toxicity because of the mechanical trapping effect of the microspheres in the pulmonary capillary vessel. In short, lung targeting of the microspheres is a double-blade sword: while it has the merit of lung targeting, it causes toxicity or side-effect to the body. However, the reason why CPt-MS showed stronger toxicity than blank MS when CPt is not so toxic still remains unclear.

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