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Studies on the poly(lactic-co-glycolic) acid microspheres of cisplatin for lung-targeting

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Abstract

Lung-targeting cisplatin-loaded poly(lactic-co-glycolic) acid microspheres (CDDP-PLGA-MS) were prepared by a solvent evaporation method. The uniform design was used to optimize the technology of preparation, the appearance and size distribution were examined by scanning electron microscope, and the aspects such as in vitro release characteristics, stability, drug loading, loading efficiency, pharmacokinetics and tissue distribution in rabbit were studied. The experimental results showed that the microspheres were globular in appearance and dispersed well. The average particle size was 12.8 μm with 98% of the microspheres being in the range of 5–30 μm. The drug loading and loading efficiency were 17.68 and 53.2%, respectively. The in vitro release behavior could be expressed by the following equation: $1-Q = 0.424e^{-0.360t} + 0.474e^{-0.001t}$. After i.v. administration (15 min), the drug concentration of microspheres group in lung in rabbits was 212 μg/g, while that of controlled group was 1.37 μg/g. CDDP-PLGA-MS showed a combination of lung-targeting and sustained drug release in experiments on rabbits.

Keywords: Cisplatin; Poly (lactic-co-glycolic) acid; Microspheres; Lung-targeting

1. Introduction

Cisplatin (*cis*-diamminedichloroplatium, CDDP) is one of the most potent anticancer agents known. However, administration of this drug can lead to a number of side effects, including renal disturbances, nausea, vomiting, and auditory toxicity. A promising way of optimizing its action is to target it to tissue sites via selective arterial catheterization with parenteral, controlled-release system. In the present work we report the encapsulation of CDDP using biodegradable polymer, poly(lactic-co-glycolic) acid (PLGA) as carrier and injected into the vein of rabbits. The results showed that the microspheres were accumulated almost entirely in the lung after i.v. injection and have a good sustained release efficacy.

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2. Materials and methods

2.1. Materials

PLGA (75:25, $M_w = 20{,}000$, provided by Organic Chemistry Research Institute of Chinese Academy) was used as carrier. CDDP (content 99.85%, provided by Kunming Precious Metallic Institute, China) was used as model drug. Polyvinyl alcohol (8 mPa s grades, HOPE Medical Instrument, Inc., China); diethyldithiocarbamate (DDTC, analytical grade, HOPE Medical Instrument, Inc., China). The other regents used were of analytical grade.

2.2. Methods

2.2.1. Microsphere preparation

The preparation was based on the solvent evaporation process (Boisdron-celle et al., 1995). The amount of cisplatin was dispersed in methylene chloride containing PLGA by sonication. The organic phase was then emulsified with agitation in solution containing polyvinyl alcohol. Stirring was continued for 4 h, until the methylene chloride was completely evaporated. The system was protected from light. The microspheres were washed four times with cold water, collected by filtration, and dried in vacuum.

2.2.2. Appearance and size distribution measurement

The surface morphology of the microspheres was observed by scanning electron microscope. Particle size distribution and measurement were carried out using optical microscopy (Lu and Wu, 1999).

2.2.3. Determination of CDDP drug loading and loading efficiency

A weighed quantity of microspheres was dissolved in dimethyl formamide. The CDDP content was assayed by spectrometry at 310 nm using a calibration curve (Spenlehauer et al., 1986). The calibration curve: $A = 0.5769C + 0.0245$, $r = 0.9999$. The method recovery was (102.2 + 2.104)%. The experiments were conducted in triplicate. The average value of CDDP content is the drug loading. The loading efficiency was calculated using the following formula (Jia et al., 1997):

$$\text{Loading efficiency}\,(\%) = \frac{M_{\text{actual}}}{M_{\text{theoretical}}} \times 100$$

where M_{actual} is the actual amount of cisplatin in each composite and $M_{\text{theoretical}}$ is the theoretical amount of cisplatin in each composite calculated from the quantity added in the fabrication process.

2.2.4. In vitro dissolution study of microspheres (Zhang et al., 1994)

A weighted quantity of microspheres was suspended in a saline and the resulting emulsion was put into a dialytic-bag. The dialytic-bag was put in a flask containing 25 ml saline and the flask was shaken in a water bath at 37 °C. Aliquots of the dissolution medium were withdrawn at specific times and the same volume of dissolution medium was added to the flask to maintain a constant volume. The DDTC solutions were added to aliquots withdrawn and the resulting mixtures were warmed in a boiling water bath. The mixtures were extracted with chloroform and separated by centrifugation. The drug concentration in upper solution was determined by spectrometry at 347 nm. The accumulating amount of drug released was calculated using a calibration curve. The calibration curve $A = 0.0236C + 0.0431$, $r = 0.9997$. The method recovery was (99.1 + 2.136)%.

2.2.5. The stability of CDDP-PLGA-MS

The microsphere powders were put into a bottle and stored for 3 months at 3–5 °C, 15–25 °C and 37 °C, respectively. The surface morphology and CDDP content were examined periodically.

2.2.6. In vivo pharmacokinetics of microspheres

Twelve healthy rabbits (six males and six females, weight 2.5 ± 0.5 kg) were randomly divided into two group with six for each group. One group were administered 4 mg/kg CDDP injection via ear marginal vein, while another group were administered 4 mg/kg CDDP-PLGA-MS suspension. All rabbits were kept on starvation for 12 h before injection (drinking freely). Blood samples were taken from ear marginal vein at given time and nitrified with mixed acid (nitric acid:perchloric acid = 4:1). Residue was dissolved with double distilled water and 20 μl of sample was used to determine the plasma drug concentration by graphite furnace atomic absorption spectrometry (Zhang et al., 1992).

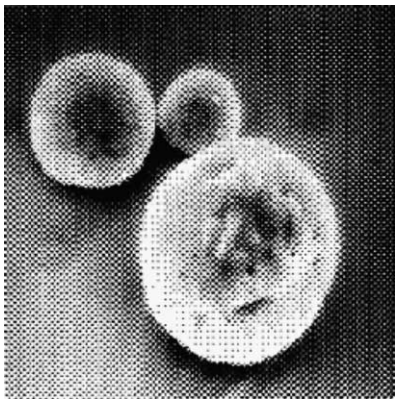

Fig. 1. Scanning electron micrographs of CDDP-PLGA-MS.

2.2.7. Tissue distribution of microspheres

Fourty-eight healthy rabbits (24 males and 24 females, weight 2.5 ± 0.5 kg) were randomly divided into two group with 24 for each group. CDDP injection and suspension were, respectively, injected in the method of pharmacokinetics experiment. Six rabbits in each group were killed at 15 min, 1, 24 and 72 h after administration. Heart, liver, spleen, lung and kidney were taken out immediately and washed with distilled water, and then 1 g of tissue sample was collected, respectively, after surface water was dried. The drug concentration in tissues was determined by graphite furnace atomic absorption spectrometry.

3. Results and discussion

3.1. The characteristics of CDDP microspheres

CDDP-PLGA-MS had different surface and drug loading according to different experimental conditions. In an attempt to identify the optimal conditions for the preparation of microspheres, the influence of the initial drug:polymer ratio, the concentrations of PVA and PLGA were examined. The uniform design was used to optimize experimental conditions. Microspheres prepared by optimal experimental conditions were globular in appearance and dispersed well. Examination using scanning electron photomicrographs showed spherical particles with many pores, which is responsible for solvent evaporation (see Fig. 1). The average drug loading and the average loading efficiency were $(17.68 \pm 0.217)\%$ and $(53.20 \pm 0.621)\%$, respectively.

3.2. Size distribution analysis

The particle size distribution is an important factor since it controls the tissue location of the microspheres after their intra-artery infusion. Previous reports pointed out (Kanke et al., 1980) that the microspheres with the size range of 5–25 μm have a notable lung-targeting efficacy. In this study, the microspheres with the size range of 5–30 μm were prepared and the average particle size was 12.8 μm. The results showed that the microspheres were mainly accumulated in the lung after i.v. injection to the rabbits (Figs. 3 and 4).

3.3. In vitro release characteristics of CDDP microspheres

In vitro release of CDDP from microspheres was performed using oscillation in constant temperature. Fig. 2 shows CDDP release curve from CDDP-PLGA-MS and CDDP injection. The CDDP release curve from CDDP-PLGA-MS suspension presents two phases: the fast release in the first 3 h and sequential slow release. The in vitro CDDP release behavior from CDDP-PLGA-MS could be described by double phase dynamic model and could be expressed by the following equation: $1-Q = 0.424e^{-0.360t} + 0.474e^{-0.001t}$, $t_{1/2(\alpha)} = 1.925$ h, $t_{1/2(\beta)} = 693$ h.

In comparison with CDDP-PLGA-MS composite, the CDDP injection releases the CDDP very fast. In approximately 1 h, 90% of CDDP has been released. The results indicated that the CDDP-PLGA-MS had a well-sustained release efficacy.

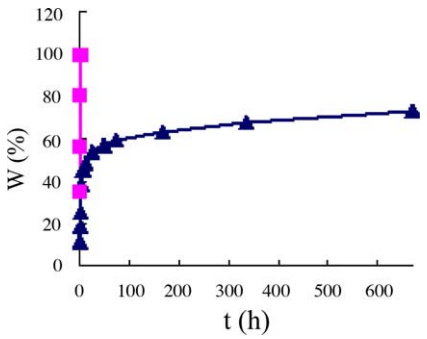

Fig. 2. Cumulative amount of drug release: (▲) microspheres and (■) CDDP injection.

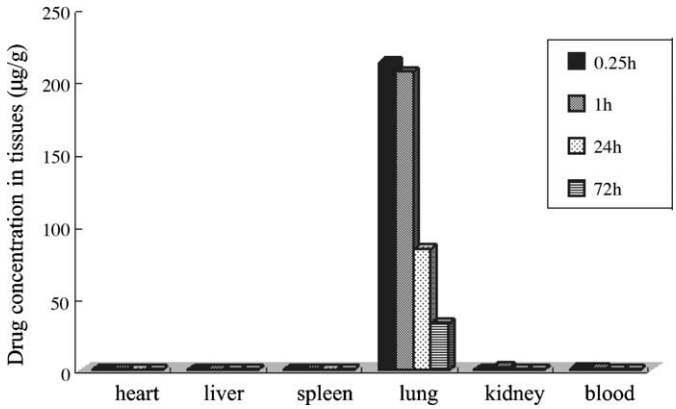

Fig. 3. Distribution in tissue in rabbits after injection of CDDP-PLGA-MS tissues (μg/g) and blood (μg/ml).

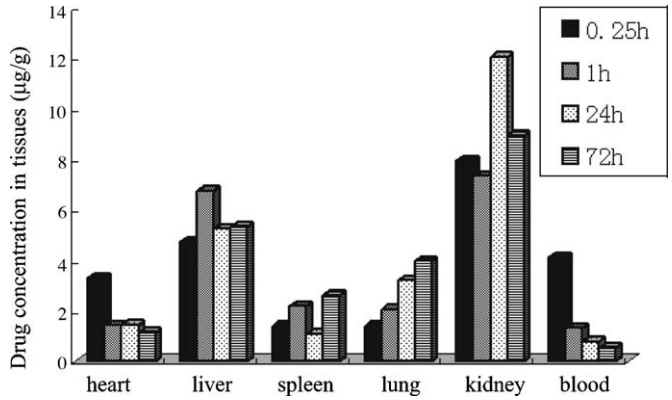

Fig. 4. Distribution in tissue in rabbits after injection of CDDP injection tissues (μg/g) and blood (μg/ml).

3.4. The stability of CDDP microspheres

During stored at 3–5 °C or room temperature (15–25 °C) for 3 months, surface morphology and content of CDDP had no notable changes (see Table 1).

Table 1
Content of CDDP-PLGA-MS in various conditions

Temperature (°C)	Time (month)	Content (S.D.) (%)
3–5	0	17.71 ± 0.095
	1	17.65 ± 0.106
	2	17.74 ± 0.082
	3	17.58 ± 0.106
15–25	0	17.71 ± 0.095
	1	17.63 ± 0.105
	2	17.61 ± 0.036
	3	17.66 ± 0.079

However, at 37 °C and RH 75% the agglutinative phenomenon was observed.

3.5. The in vivo pharmacokinetics of microspheres

The in vivo pharmacokinetics of microspheres was studied with "practical pharmacokinetic program-version 87" and was fitted by one-compartment model, two-compartment model and three-compartment model, respectively. Based on the analysis of the models and parameters, it was concluded that the in vivo pharmacokinetics of microspheres in blood could be described by the model of two-compartment with i.v. injection. The plasma concentration and pharmacokinetical parameters are reported in Tables 2 and 3, respectively. From Table 3, one can see that in comparison with CDDP injection, CDDP-PLGA-MS altered

Table 2
Plasma concentration data

Time (h)	Drug concentration in plasma (μg/ml)								
	0.25	0.5	1	3	6	12	24	48	72
CDDP group	4.12	1.97	1.31	1.17	1.02	0.94	0.77	0.75	0.52
S.D.	2.07	0.64	0.59	0.71	0.39	0.37	0.37	0.24	0.16
CDDP-MS group	0.71	0.68	0.57	0.42	0.33	0.31	0.19	0.19	0.15
S.D.	0.08	0.05	0.06	0.14	0.08	0.06	0.06	0.05	0.07

Table 3
Pharmacokinetics parameters of CDDP-PLGA-MS group and CDDP group in rabbit

Parameter	CDDP-PLGA-MS group	CDDP group
V_c [(mg/kg)/(μg/ml)]	5.344	0.345
$t_{1/2(\alpha)}$ (h)	1.681	0.138
$t_{1/2(\beta)}$ (h)	66.50	63.52
K_{21} (1/h)	0.172	0.501
K_{10} (1/h)	0.025	0.109
K_{12} (1/h)	0.225	4.406
AUC (μg h/ml)	30.01	106.24
CL_s [mg/kg/h/(μg/ml)]	0.133	0.038

the distribution of CDDP in vivo and the half-life after i.v. injection of CDDP-PLGA-MS ($t_{1/2(\alpha)} = 1.681$ h, $t_{1/2(\beta)} = 66.5$ h) were prolonged remarkably than those ($t_{1/2(\alpha)} = 0.138$ h, $t_{1/2(\beta)} = 63.52$ h) after i.v. injection of CDDP injection. The result indicated that the CDDP-PLGA-MS had sustained release efficacy.

3.6. Distribution of cisplatin in tissues

The drug concentration of tissues was determined by graphite furnace atomic absorption spectrometry (FAAS). The results indicated that the microspheres could deliver CDDP mainly to lung after i.v. injection to rabbit and the concentration of CDDP in lung (212 μg/g, 15 min) was significantly higher than those in other tissue and blood. Compared with CDDP injection, the drug concentration of CDDP in lung after i.v. injection of CDDP-PLGA-MS enhanced from 1.37 to 212 μg/g (15 min).

References

Boisdron-celle, M., Menei, P.H., Benoit, J.P., 1995. Preparation and characterization of 5-fluorouracil-loaded microparticles as biodegradable anticancer drug carriers. J. Pharm. Pharmacol. 47, 108–113.

Jia, K.L., Nuo, W., Xue, S.W., 1997. A novel biodegradable system based on gelatin nanoparticles and poly(lactic-co-glycolic acid) microspheres for protein and peptide drug delivery. J. Pharm. Sci. 86, 891–895.

Kanke, M., Simmons, G.H., Weiss, D.L., Brack, A.B., Patrick, P.D., 1980. Clearance of ^{141}Ce-labeled microspheres from blood and distribution in specific organs following intravenous and intra arterial administration in beagle dogs. J. Pharm. Sci. 67, 755–765.

Lu, B., Wu, W., 1999. Optimization of preparation of dexamethasone acetate-loaded poly(D,L-lactide) microspheres by central composite design. Acta Pharm. Sin. 34, 387–391.

Spenlehauer, G., Veillard, M., Benoit, J.P., 1986. Formation and characterization of cisplatin loaded poly(D,L-lactide) microphères for chemoembolization. J. Pharm. Sci. 75, 750–755.

Zhang, M., Hou, S.X., Gong, T., 1994. Derivative assay of bovine serum albumin microspheres loaded cisplatinum for pulmonary targeting. Chin. Pharm. J. 29, 355–357.

Zhang, X.L., Ren, J.P., Zhang, D.P., 1992. Pharmacokinetic study and comparison of carboplatin and cisplatin native to china in rabbits. Chin. J. Hosp. Pharm. 12, 387–389.