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RESEARCH ARTICLE

Sophoridine-loaded PLGA microspheres for lung targeting: preparation, *in vitro*, and *in vivo* evaluation

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Abstract

Lung-targeting sophoridine-loaded poly(lactide-co-glycolide) (PLGA) microspheres were constructed by a simple oil-in-oil emulsion-solvent evaporation method. The obtained microspheres were systematically studied on their morphology, size distribution, drug loading, encapsulation efficiency, *in vitro* release profile, and biodistribution in rats. The drug-loaded microparticles showed as tiny spheres under SEM and had an average size of 17 μm with 90% of the microspheres ranging from 12 to 24 μm. The drug loading and encapsulation efficiency were 65% and 6.5%, respectively. The *in vitro* drug release behavior of microspheres exhibited an initial burst of 16.6% at 4 h and a sustained-release period of 14 days. Drug concentration in lung tissue of rats was 220.10 μg/g for microspheres and 6.77 μg/g for solution after intraveneous injection for 30 min, respectively. And the microsphere formulation showed a significantly higher drug level in lung tissue than in other major organs and blood samples for 12 days. These results demonstrated that the obtained PLGA microspheres could potentially improve the treatment efficacy of sophoridine against lung cancer.

Keywords

Sophoridine, microspheres, lung targeting, poly(lactide-co-glycolide) (PLGA), biodistribution

History

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Introduction

Lung cancer remains the leading cause of cancer-related deaths among males in most countries (Jemal et al., 2011). Although significant advances have been made in the chemotherapy of lung cancer, side effects, and toxicity for non-tumor tissues after oral or intravenous (i.v.) administration of anticancer drugs became a widely concerned issue (Johnson et al., 2014). Thus, novel therapies functioned with lung-targeting property are needed to make significant improvements in patient adaptability and clinical efficacy.

Microspheres have been extensively studied as carriers for efficient delivery of therapeutic agents to different sites, due to specific particle size and targeting ligands (Guo et al., 2012). And microspheres can also provide a prolonged therapeutic effect by controlled or sustained release of drugs. Among a variety of natural and synthetic polymers applied as matrix for microspheres, the poly(lactide-co-glycolide) (PLGA) has become the most widely used biodegradable and biocompatible material in microparticle production (Pandita et al., 2015). Previous research has reported lung-targeting PLGA-based microspheres carried with a host of therapeutic agents, such as fluorofenidone (Tang et al., 2015), cisplatin (Huo et al., 2005), yuanhuacine (Zhang et al., 2009), etoposide (Feng et al., 2014), and emodin (Chen et al., 2014). In general, the microspheres of 7 μm or more in diameter will be rapidly localized in lung tissue by mechanical filtration through capillary bed of the lung after i.v. injection (Lu et al., 2003; Huo et al., 2005). Therefore, the control of particle size is critical for preparation of lung-targeted microparticles, and higher drug loading and loading efficiency are also preferred for reduction of administration dose and production cost. The oil-in-water (O/W) emulsion method is the most frequently used approach in the production of PLGA microspheres, due to its simplicity and industrialization. But this method generally forms particles with a broad size distribution (Bock et al., 2011), and commonly encounters a large amount of drug loss during the fabrication process especially for drugs with certain aqueous solubility.

Sophoridine (SRI, see Figure 1) is a quinolizidine alkaloid extracted from *Sophora alopecuroides* L. (Leguminosae). SRI shows a range of remarkable pharmacological activities, including various strong anti-cancer effects (Zhang et al., 2006; Huang et al., 2014; Wang et al., 2015). SRI can inhibit DNA topoisomerase I activity, cause cell cycle arrest at the G0/G1 phase, and selectively induce apoptotic cell death in a variety of human cancer cells *in vitro* and *in vivo*. In 2005, Food and Drug Administration of China (CFDA) approved SRI to cure cancer patients, and SRI hydrochloride injection

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has been widely used for treating lung cancer, liver cancer, and gastric cancer in combination with other antitumor drugs (Li et al., 2015). However, SRI possessed a rapid elimination and wide distribution property *in vivo* (Zhang et al., 2006), and certain toxicity at high doses. To address these issues, Dong et al. (2014) developed mesoporous silica nanospheres for controlled release of SRI, yet the *in vivo* performance of these nanospheres was remained unknown.

In the present study, SRI-loaded PLGA microspheres (SRI-MS) were prepared by the emulsion-solvent evaporation method, and the fabrication process was optimized to obtain microspheres with preferable encapsulation efficiency and appropriate size range. The obtained SRI-MS was then characterized in terms of morphology, particle size, and *in vitro* release profile. The lung-targeting performance of SRI-MS administrated intravenously was evaluated and compared with SRI solution.

Materials and methods

Materials

PLGA (lactide:glycolide ratio of 50:50; 20 kDa) was obtained from Shandong Institute of Medical Instruments (Jinan, China). SRI of 98% purity was supplied by Jingzhu Biological Technology Co., Ltd. (Nanjing, China). Poly (vinyl alcohol) (PVA-1788, 44.05 kDa) was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). All other reagents were either of analytical or chromatographic grades. Double distilled water was used throughout the experiment.

Preparation of SRI-MS

The emulsion-solvent extraction method was used for preparation of the SRI-PLGA microspheres. The process parameters for different samples are listed in Table 1.

The commonly O/W and water-in-oil-in-water (W/O/W) emulsion methods were applied to prepare SRI-MS. Typically, 90 mg drug and 270 mg polymer were dissolved in 2 ml dichlomethane by vortexing. The obtained SRI-PLGA solution was then injected into 1% PVA solution (40 ml) and homogenized (Ultra Turrax T18, IKA, Germany) at 8000 rpm for 1 min to form the initial emulsion. The emulsion was subsequently dispersed into 0.5% PVA solution (800 ml) and kept stirring at 40 °C for 3 h to remove the organic solvents. Finally, microspheres were collected by centrifugation at 4000 rpm for 3 min, and vacuum-dried by a FD-1C freezing dryer (Beijing, China). For W/O/W method, SRI was dissolved in 0.2 ml PVA (1%, w/v) and PLGA in 0.18 ml dichlomethane. Then these solutions were mixed and homogenized at 30 000 rpm for 2 min to obtain the internal emulsion phase.

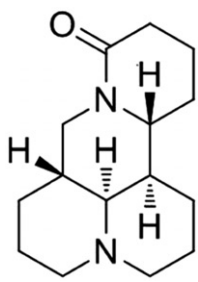

Figure 1. Chemical structure of SRI.

For oil-in-oil (O/O) method, 30 mg drug and 270 mg polymer were dissolved in 3 ml mixture of acetonitrile and dichlomethane (9:1, v/v) by vortexing. The obtained SRI-PLGA solution was then injected into 30 ml liquid paraffin and homogenized at 8000 rpm for 1 min to form the O/O emulsion. The initial emulsion was subsequently dispersed into 200 ml liquid paraffin and kept stirring at 40 °C for 4 h to remove the organic solvents. Finally, microspheres were separated by centrifugation at 4000 rpm for 3 min, rinsed with 50 ml petroleum ether for three times and vacuum-dried for 24 h. The liquid paraffin was pre-saturated with SRI (2.5%, w/v) for the O/Os method.

Before centrifugation and collection, the suspensions of microspheres were dropped on a glass slide for observation of particle shape and size under optical microscopy (Phenix Optical Instrument Group Co., Ltd, China).

Characterization of SRI-MS

Determination of drug loading and encapsulation efficiency

To determine the drug loading (DL) percentage and encapsulation efficiency (EE) of the obtained SRI-MS, 10 mg sample was accurately weighed and dissolved in 0.5 ml dioxane, and then diluted with methanol to 10 ml. The content of SRI was then analyzed at 205 nm by a reversed-phase high pressure liquid chromatography (RP-HPLC) system with a C18 (250 mm × 4.6 mm × 5 μm, DIAMONSIL) column. The mobile phase was a mixture of 80% 20 mM phosphate buffer (pH 6.5) and 20% acetonitrile and set at a flow rate of 1 ml/min. Finally, DL and EE were calculated according to the following equations, respectively:

$$\mathrm{DL}(\%) = \frac{\text{mass of drug in microspheres}}{\text{mass of total microspheres}} \times 100\%$$

$$\mathrm{EE}(\%) = \frac{\mathrm{DL}}{\text{theoretical DL}} \times 100\%$$

Results are expressed as mean ± SD ($n = 3$).

Table 1. Process parameters of emulsion method for SRI-MS preparation.

Code	Method	Solvents for internal oil phase	Solutions for external phase
F1	O/W	Dichlomethane	1% PVA solution
F2	W/O/W	Dichlomethane	1% PVA solution
F3	O/O	Acetonitrile and dichlomethane (9:1, v/v)	Liquid paraffin
F4	O/Os	Acetonitrile and dichlomethane (9:1, v/v)	Liquid paraffin supplemented with 2.5% SRI (w/v)

Surface morphology

The surface morphology of SRI-PLGA microparticles was characterized by a JEOL JSM-7500P (Tokyo, Japan) scanning electron microscopy (SEM). Before observation, samples were affixed on an aluminum stub and sputter-coated with palladium under vacuum (0.1 mmHg) at a current intensity of 20 mA.

Particle size analysis

The mean size and size distribution of microspheres was analyzed by a Microtrac X-100 laser particle sizer (Honeywell, Morris Plains, NJ). Before analysis, 20 mg of sample was added in 10 ml of 0.5% PVA solution (containing 0.1% Tween 80, w/v) and sonicated for 3 min to obtain a well-dispersed suspension. The particle size was expressed as volume weighted mean diameter (D_v) in micrometer. The width of size distribution was calculated according to the equation shown below (Goran & Vladisavljević, 2003).

$$\text{Span} = \frac{d_{90} - d_{10}}{d_{50}} \quad (1)$$

Where d_{10}, d_{50}, and d_{90} are microparticle diameters below which 10, 50, and 90% of the volume of microspheres lie, respectively.

Differential scanning calorimetry (DSC)

Thermograms of SRI, PLGA microspheres, their physical mixture and SRI-MS were obtained using a SETSYS-1750 CS Evolution thermogravimetry analyzer (Seraram, France) under a nitrogen atmosphere. After accurately weighed, dried samples were sealed in aluminum pans and then submitted to a thermal program from 25 °C to 200 °C at 10 °C/min.

X-ray powder diffraction (XRPD)

The crystalline state of SRI, PLGA microspheres, their physical mixture and SRI-PLGA microspheres was characterized by an X-ray powder diffraction equipment (D/MARX2200/PC, Rigaku Co., Tokoyo, Japan) using CuKα radiation at 40 mA and 40 kV. Standard runs were performed with a scanning rate of 8°/min over a 2θ range of 3–60°.

In vitro drug release

Briefly, 10 mg of SRI-PLGA microspheres were suspended in 20 ml pH 7.4 phosphate-buffered saline containing 1% Tween 20 (PBST) and continuously stirred at 37 °C in a THZ-100B thermostatic air bath shaker (Shanghai, China) at 100 rpm. At predetermined time intervals, 5 ml of the supernatant was withdrawn after centrifugation at 4000 rpm for 3 min, and replaced with fresh medium. The drug concentration was measured by above-mentioned RP-HPLC method ($n = 3$).

Tissue distribution of microspheres

Thirty-six SD rats of male, weighing between 200 and 220 g, were obtained from the Laboratory Animal Center of Ningxia Medical University (Yinchuan, China). All animals were maintained in a specific pathogen-free environment at 23 ± 2 °C with free access to water.

Animals were randomly divided into six groups and given a single 5 mg/kg dose of either SRI solution or SRI microspheres by tail-vein injection. At 30 min, 1 d, 4 d, 8 d, 12 d after administration, each animal was euthanized, and heart, spleen, lung, liver, kidney as well as blood samples were collected. Tissues were washed with ice-cold saline, blotted with tissue paper to remove excess fluid, then weighed and stored at −70 °C until assessed for drug content.

The isolated tissue samples were added with a small amount of liquid nitrogen and quickly grinded into powders. Approximately 20 mg tissue powders were accurately weighed and transferred into a test tube. The blood samples were centrifuged at 3000 rpm for 10 min and then 1 ml of supernatants was transferred into a tube. The above tissue and blood samples were then added with 5 ml acetonitrile, vortexed for 3 min, and then centrifuged at 4000 rpm for 5 min. The supernatant was quantitatively transferred into a glass tube and evaporated to dryness at 50 °C. The residue was then dissolved in 1 ml of methanol, and 10 µl aliquot of the solution was injected into the HPLC system for analysis.

Data were expressed as mean ± SD for each group. Statistical analysis was performed using Student's *t*-test. Differences were considered to be statistically significant at $p < 0.05$ or $p < 0.01$.

Results and discussion

Preparation of SRI-PLGA microshpheres

Figure 2 shows the images of SRI-PLGA microspheres prepared by different emulsion-solvent evaporation methods. During the process, emulsion drops were easily formed by homogenization and tiny spheres were obtained after solidification. Both O/W and W/O/W methods formed microspheres with significant size difference. Some particles were broken into pieces for the W/O/W method, probably due to the double emulsion process. Comparatively, the microspheres by O/O methods showed smaller size and narrower distribution, indicating a relatively higher solidification rate of the emulsion droplets. The reason could be mainly assigned to slightly higher miscibility between the inner organic phase (mixture of acetonitrile and dichlomethane) and the outer oil phase (liquid paraffin), in comparison to that between dichlomethane and water for O/W method (Hu et al., 2011). For the basic O/O method, a small amount of spheres were merged to be larger particles with irregular shape. Among all the methods, the O/Os method showed as the most promising approach for preparation of lung-targeting microspheres, from which we obtained relatively uniform particles with their sizes ranging from 10 to 20 µm. It seemed that the addition of SRI in the continuous phase prevented the droplet coalescence during the solidification process, which also indicating the formation of particles with solidified shell in the very beginning of solvent evaporation process (Freitas et al., 2005).

Encapsulation efficiency determination

Various parameters of emulsion method can influence the EE of microspheres during the fabrication process, and the

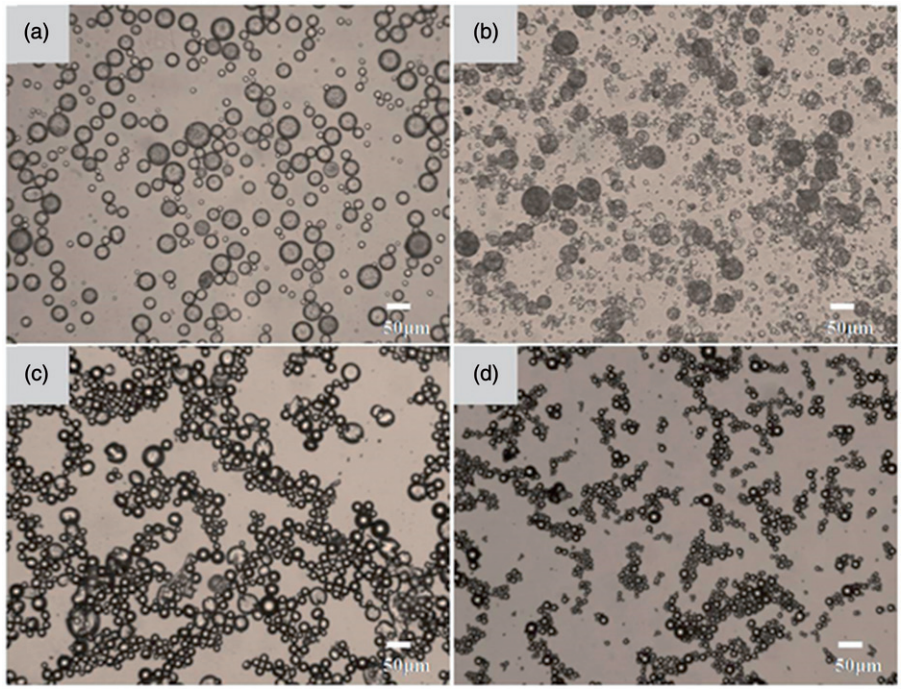

Figure 2. Microscopic images of SRI-loaded micrpospheres prepared by (a) O/W, (b) W/O/W, (c) O/O, and (d) O/Os method, size bar: 50 μm.

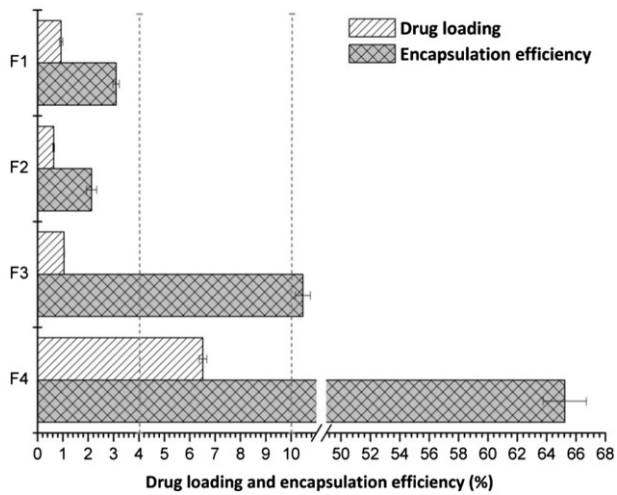

Figure 3. Drug loading and encapsulation efficiency of SRI-PLGA microspheres fabricated by different emulsion methods ($x \pm SD$, $n = 3$).

physiochemical property of drug itself values much among these parameters. It has been proven that the higher solubility of drug in the continuous phase than in the dispersed phase, the more drug loss into the continuous phase during production (Yeo & Park, 2004).

As an active pharmaceutical ingredient with a small molecular weight of 248.36, SRI is extremely soluble in water, freely soluble in dichloromethane or acetonitrile, but sparingly soluble in liquid paraffin (data not shown). Therefore to maximize the EE of SRI-loaded microspheres, it was necessary to enlarge the solubility difference between external and internal phase during the solvent evaporation process of emulsion method.

As presented in Figure 3, the emulsion methods of O/W and W/O/W formed microspheres with an extremely low EE of less than 4%, which mainly due to rapid diffusion of drug molecules into a large volume of external aqueous phase during the preparation process. To improve the EE of water-soluble drugs into PLGA microparticles, Elkharraz et al. (2011) proposed a novel O/O/O-solvent extraction method. A similar result was also observed in our present study. When O/O method was adopted, the EE of obtained microspheres showed a remarkable increase to 10%. The reason for nearly 90% of drug loss may mainly due to the huge gap between the saturation degree of drug in the external oil phase and that in the internal phase. By adding SRI into external oil phase in prior, the EE was further improved up to 65% at a theoretical DL of 10%, despite the remarkable size reduction of microspheres from O/Os method in Figure 1. The similar level of drug saturation between the internal and external phase, and fast solvent immigration during the initial stage of solidification process as well, would retard drug diffusion into the liquid paraffin, which further result in a low drug loss. High DL and EE are preferable for drug-loading microparticles to prolong release term or lower injection dosage (Qi et al., 2013). Therefore, the microspheres prepared by O/Os method were adopted for further investigation.

Characterization of SRI-PLGA microshpheres

The morphology and size distribution of SRI-PLGA microspheres were characterized by SEM and DLS, respectively. As shown in Figure 4, the microspheres with smooth and nonporous surface were obtained by O/Os emulsion method. The particle size was found in the range of 12–24 μm, which was consistent with the SEM images.

One of the key factors that influence lung-targeting performance of the microparticles is the particle size. Previous reports have proved that after i.v. injection, particles of 7–30 μm could be mechanically intercepted by capillary bed and then accumulated in the lungs, thereby achieving

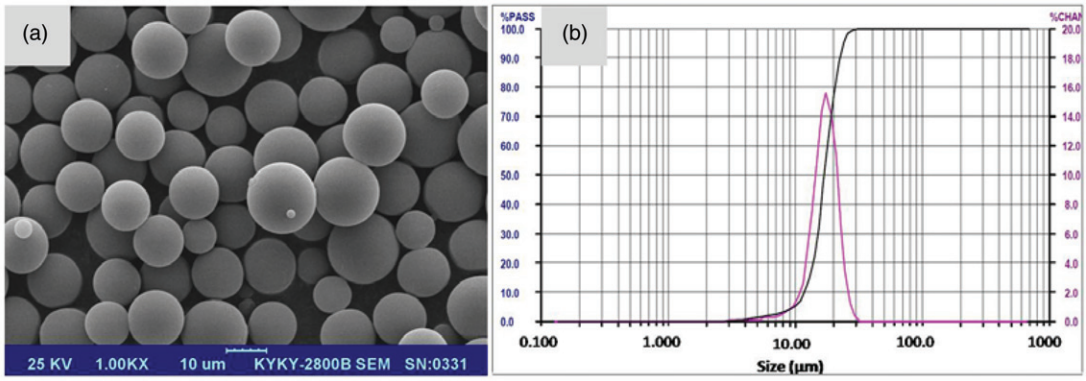

Figure 4. (a) SEM images and (b) size distribution of SRI-PLGA microspheres.

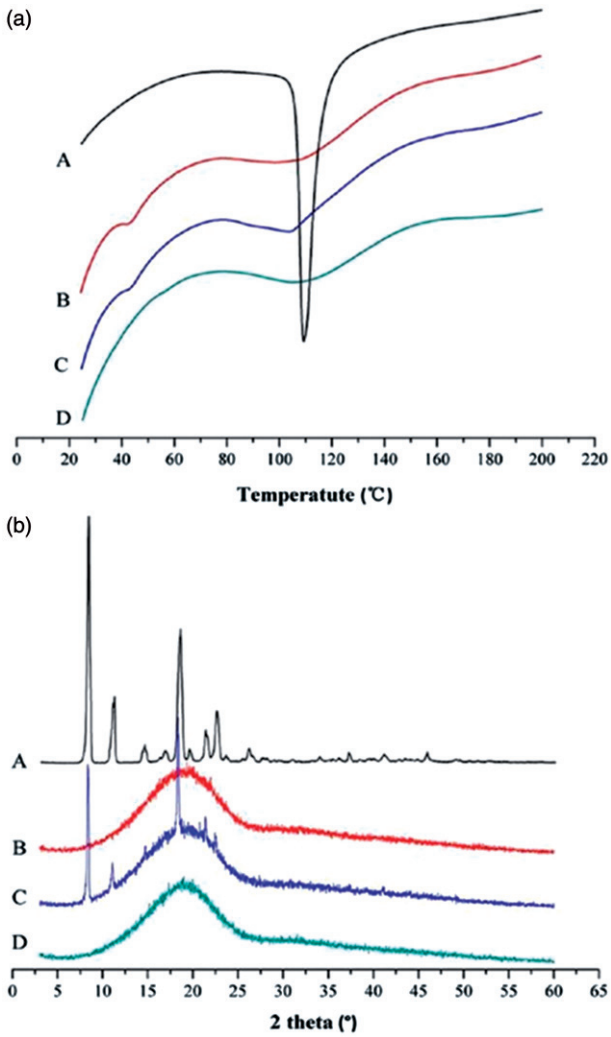

Figure 5. (a) DSC thermograms and (b) X-ray diffractograms of SRI-PLGA microspheres (A: SRI, B: PLGA microspheres, C: physical mixture of GA and PLGA microspheres, D: SRI-PLGA microspheres).

passive lung targeting. In our present study, the obtained microspheres exhibited an average particle size of 17.16 ± 3.94 μm and the span was 0.60, which was favorable for passive-targeting into lung tissue.

Figure 5(a) shows the DSC results of pure SRI and microspheres. PLGA showed a glass transition around 52 °C (Bragagni et al., 2013) for both blank microspheres and physical mixture, but the peak was absent in drug-loaded microspheres. The pure drug exhibited an endothermic peak at 109 °C due to melting, and this peak was broadened and weakened in the physical mixture, probably due to the low content of drug in mixture. For drug-loaded microspheres, the melting point of drug were obscure, indicating that SRI was molecularly dispersed or in an amorphous state in the PLGA matrix (Bohr et al., 2011).

Figure 5(b) shows the X-ray diffractograms of the samples. Presence of sharp peaks in the diffractogram of raw drug indicated the crystalline nature of the compound, and most of these peaks were remained with lower intensities in the mixture of SRI and blank microspheres. However, the drug-loaded microspheres were found to be X-ray amorphous as sharp peaks were absent, which suggested that the crystal structure of SRI was distorted to an amorphous phase (Madan et al., 2013; Saigal et al., 2013).

In vitro drug release

The *in vitro* drug release profiles of SRI from SRI-PLGA microspheres in pH 7.4 PBST are shown in Figure 6. About 14% free SRI was released from the microspheres in the initial 4 h, and the drug release was 34% for the first day. Thereafter SRI was gradually released as time lapsed, suggesting that drug was well entrapped in PLGA matrix. At the end of the experiment, about 94% of SRI was released from PLGA microspheres.

For drug-loaded PLGA microspheres, drug release may occur through polymer erosion, diffusion or a combination of those two. Moreover, physicochemical properties of both polymer and drug can also greatly affect the release pattern of the drug from microspheres (Yeo & Park, 2004). The release data of SRI-MS were then fitted into several models to approach the release kinetics. Ritger–Peppas equation ($Q = 35.659\ t^{0.3971}$, $r^2 = 0.9977$) was found to be the best fitted model for SRI released form the microspheres, revealing drug release from SRI-PLGA microspheres primarily

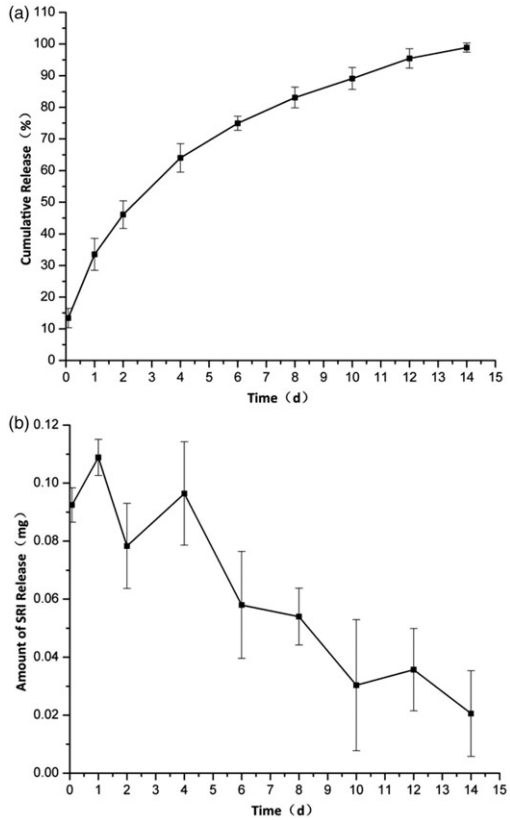

Figure 6. (a) Cumulative and (b) amount *in vitro* release profiles of SRI-PLGA microspheres ($x \pm SD$, $n = 3$).

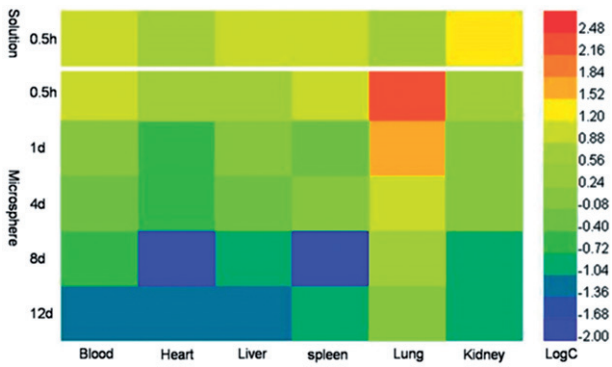

Figure 7. Biodistribution of SRI after intravenous administration of solution and microsphere formulation.

dominated by diffusion. Since SRI was a small molecular compound with good solubility in water, drug molecules were ready for diffusion out of the polymer matrix, once water penetrated into the matrix and dissolved SRI.

Lung targeting performance

In vivo biodistribution behavior of SRI after i.v. administration of SRI-PLGA microspheres to rats was investigated with SRI injection as a control ($n = 6$). The amounts of drug distributed in unit mass of heart, liver, spleen, lung, kidney, and plasma at different time intervals were measured. Figure 7 presents the logarithmic value of mean drug concentration (log C) in unit mass of each organ and blood sample in rats.

After i.v. administration for 30 min, drug concentration of 7.97 μg/ml in blood was observed for SRI solution group. Drug level in the kidney was 26.32 μg/g, which was significantly higher than that in the other tissues. These results suggested that free SRI was quickly cleared from the body, mainly through urinary elimination.

As to SRI microsphere group, tissue distribution was quite different. A high drug level of 220.10 μg/g was observed in the lung after administration for 30 min, which was nearly 33-fold higher than that for the solution group. Meanwhile, drug concentration in the blood was found to be only 12.07 μg/g, and that was even lower for the other tissues. In the following time intervals, the drug level *in vivo* was gradually decreased, but it still remained the highest in lung tissue. SRI content in lung was 39.66 μg/g at 24 h, then dropped to 9.02 μg/g at 4 days and 4.47 at 8 days, but was still more than 1.30 μg/g after injected for 12 days. However, drug concentration was quickly reduced to 1.50 μg/g or less in the other tissues and blood after administrated for 24 h, followed by dropping to the background level after 8 days. The targeting parameters of SRI microspheres showed the largest value of area under the curve (AUC) for the lung. The targeting efficiency (Te) of the lung increased by a factor of 20 compared with kidney, 28 compared with liver, and 22 compared with blood. Therefore, SRI-MS showed a significant lung-targeting characteristics. Drug distribution to the lung tissue was 84.1% of the administered dose. These results indicated that the microspheres could deliver SRI predominantly to lung tissue after i.v. administration, which is preferable for the treatment of lung cancer, and the reduction of side effects as well (Madan et al., 2013; Wang et al., 2014).

Conclusion

In this study, SRI-loaded PLGA microspheres were prepared by O/O emulsion-solvent evaporation method. The obtained microspheres were found to possess suitable physicochemical properties and particle size distribution. The microspheres also showed a combination of lung-targeting and sustained drug release characteristics. Our work has, thus, demonstrated that SRI-loaded sustained-release PLGA microspheres are of potential value in treating lung cancer.

Declaration of interest

The authors declare no conflicts of interest. This work was supported by the National Natural Science Foundation of China (81360644) (81660665) and International Cooperation Project of Ningxia ([2013] No. 21).

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