School of Pharmacy, Hebei University, Baoding, China

Preparation and characterization of solid lipid nanoparticles loaded with epirubicin for pulmonary delivery

LianDong Hu, Yanhong Jia, WenDing

Received January 26, 2010, accepted February 8, 2010

Liandong Hu, College of Pharmacy, Hebei University, No.180, WuSi Road, Baoding, 071002, P.R. China
hbupharm@126.com

Pharmazie 65: 585–587 (2010) *doi: 10.1691/ph.2010.0023*

In this study, epirubicin (EPI)-loaded solid lipid nanoparticles (EPI-SLNs) were successfully prepared as an inhalable formulation for treatment of lung cancer. The physicochemical properties and *in vitro* pulmonary deposition of EPI-SLNs was studied. Pharmacokinetics were studied in rats by inhalation administration of EPI-SLNs and EPI-solutions respectively, the concentrations of EPI in blood and lungs were determined. Cytotoxicity was determined in A549 alveolar epithelial cells and found to be not toxic in blank SLNs and higher cytotoxicity of EPI-SLNs was found compared with that of EPI solution. *In vitro* deposition study suggested that SLNs remained stable during nebulization with improved respirable fraction (RF) compared to EPI-solutions. *In vivo* pharmacokinetic study showed that the drug concentration achieved by inhalation of EPI-SLNs was much higher than the drug concentration in plasma. Furthermore the drug concentration in lungs after inhalation of EPI-SLNs was much higher than that after administration of epirubicin solution. These findings suggest that EPI-SLNs could be used as an inhalable delivery system for treatment of lung cancer.

1. Introduction

Epirubicin (EPI) is an anthracycline and a stereoisomer of doxorubicin that has shown activity against various types of tumors (Arcamone et al. 1975; Casazza et al. 1979). The major side effects of EPI are haematological and cardiac toxicity (Coukell et al. 1997; Robert et al. 1993, 1994). The risk of cardiotoxicity increases with blood concentration (Plosker and Faulds 1997). Focus should be made on efforts to kill cancer cells by more specific targeting while sparing normal cells. Simple, safe and stable formulations are also needed.

Drug delivery to the lungs by inhalation has attracted tremendous scientific and biomedical interest in recent years. Diseases that can be targeted with local pulmonary administration include chronic obstructive pulmonary disease, asthma, cystic fibrosis, infectious diseases, tuberculosis and lung cancer (Pison et al. 2006). Delivering drugs via the lungs also provides a non-invasive route of delivery for targeting the systemic circulation, as the lungs provide a large surface area, a thin epithelial barrier, high blood flow, and less enzymatic activity compared to other areas in the body. Pulmonary administration of drugs is a useful way to avoid problems associated with parenteral formulations, such as tissue invasion, and also to improve patient compliance (Sung et al. 2007; Sakagami et al. 2006; Scheuch et al. 2006). First-pass metabolism can be avoided by pulmonary administration, which can be especially useful for drugs, which are extensively degraded following oral delivery (Shoyele and Cawthorne 2006).

SLNs have been reported as an alternative drug delivery system to traditional polymeric nanoparticles (Mehnert and Mäder 2001). SLNs combine the advantages of polymeric nanoparticles, fat emulsions and liposomes (Schwarz and Mehnert 1999) the nanoparticles are in submicron size range and they are composed of physiologically tolerated lipid components, at room temperature the particles are in solid state (Müller and Lucks 1996). Recently, SLNs have been investigated for pulmonary delivery of insulin (Liu et al. 2008; Bi et al. 2009). However, few investigators have addressed pulmonary applications of SLNs as systemic delivery carriers for anti-cancer drugs. The aim of this study was to prepare EPI-SLNs for pulmonary administration to reduce side effects. The physicochemical characteristics, the cytotoxicity against cancer cells and deposition behaviors of SLNs were investigated. The current investigation illustrates that SLNs can be used as a powerful approach for non-invasive pulmonary delivery of therapeutic drugs.

2. Investigations, results and discussion

2.1. Characterization of SLNs

The physicochemical properties of SLNs before and after nebulization are shown in Table 1. The size of the freshly prepared SLNs was 223.7 nm. After nebulization, the particle diameters increased slightly. The EE and z-potential did not show any significant alteration 78.9%/−30.6 mV before and 77.6%/−28.4 mV after nebulization for EPI-SLNs, indicating that their properties have not been significantly affected during nebulization.

2.2. In vitro nebulization

Selected formulations were characterized for *in vitro* deposition by a Twin Stage Impinges (TSI). Blank SLNs, EPI-SLNs and epirubicin solution showed respirable fractions (RF) of 77.03%, 78.46% and 59.51%, respectively. No significant difference in

Table 1: Physicochemical properties of EPI-SLNs before and after nebulization (n = 3)

Formulation	Size (nm)	Zeta potential (mV)	Entrapment efficiency(%)
Before nebulization	223.7 ± 4.6	-30.6 ± 1.8	78.9 ± 1.23
After nebulization	231.1 ± 5.1	-28.4 ± 1.1	77.6 ± 0.85

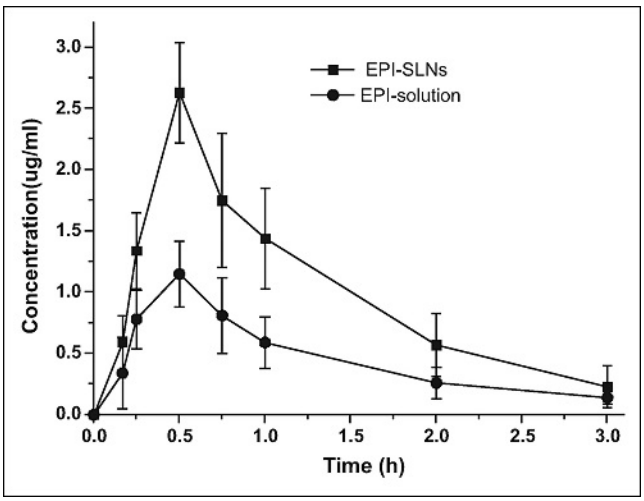

Fig. 1: Plasma concentration–time profiles of EPI after inhalation administration of EPI-SLNs and EPI-solution to rats (n = 6)

RF was found between EPI-SLNs and blank SLNs while RF for EPI-SLNs was significantly higher than that for epirubicin solution ($p < 0.05$). Thus, the use of EPI-SLNs decreases drug loss, and can deliver EPI into the deep lung.

2.3. In vitro cytotoxicity

Cytotoxicity of EPI-SLNs, and epirubicin solution against A549 cells was determined. The result indicated that the blank SLNs had almost no cytotoxicity and A549 cell number remained constant. As a result, A549 cells were more sensitive to EPI-SLNs than to epirubicin solution ($p < 0.05$). The concentration where 50% of the tested cells were killed by EPI-SLNs was 0.5 μg/ml EPI, whereas that of epirubicin solution was about 2 μg/ml. EPI-SLNs after nebulization showed similar cytotoxic properties than freshly prepared EPI-SLNs, which indicated that the process of nebulization did not influence the efficacy of EPI-SLNs. EPI-SLNs showed improved cytotoxicity towards A549 cells. The cytotoxicity results show pulmonary drug delivery is a practicable route for cancer therapy, SLNs might have the potential to be applied as an aerosol to tumor-affected lungs.

2.4. In vivo pharmacokinetic study

The plasma concentration–time curve after inhalation of EPI-SLNs and epirubicin solution in rats is shown in the Fig. 1. The pharmacokinetic parameters are listed in Table 2. The differences were highly significant. The results suggested that EPI-SLNs had excellent lung deposition characteristics compared to epirubicin solution. The drug concentration in the lungs after inhalation was significantly higher than that in plasma both after EPI-SLNs and epirubicin solution, indicating a pulmonary targeting delivery potential of the formulations developed.

EPI-SLNs inhalation resulted in significantly higher C_{max} (2.63 μg/ml) in plasma than that achieved with epirubicin solution (1.15 μg/ml), The plasma AUC values after EPI-SLNs were 2.07-fold higher than that after epirubicin solution. The measured drug concentration in the lung at 1,2,3 h was 20.8, 19.7, 18.5 μg/g for EPI-SLNs and 11.3, 10.5, 9.4 μg/g for epirubicin solution, respectively. It is obvious that the inhalation of SLNs resulted in much higher drug concentrations in the lungs than that of epirubicin solution, indicating improved lung absorption of the drug after inhalation of SLNs. Therefore, it can be concluded that higher drug concentrations in lung and plasma can potentially lead to improved anticancer efficacy after inhalation of EPI-SLNs.

Table 2: Pharmacokinetic parameters of EPI after inhalation administration of EPI-SLNs and EPI-solution to rats (n = 6)

Parameter	EPI-solution	EPI-SLNs
Ke (h^{-1})	0.72 ± 0.12	0.93 ± 0.17
AUC0-∞ (μg·h· mL^{-1})	1.55 ± 0.25	3.23 ± 0.39
AUMC0-∞	2.29 ± 0.38	4.14 ± 0.45
Cmax(μg/ml)	1.15 ± 0.27	2.63 ± 0.41
MRT (h)	1.47 ± 0.32	1.28 ± 0.25
Fr		2.07 ± 0.35

2.5. Conclusions

EPI-SLNs were prepared as carriers for treatment of lung cancer by pulmonary administration. The mean diameters and zeta potential of SLNs were 223.7 ± 4.6 nm and -30.6 ± 1.8 mV, respectively. The respirable fraction (RF) of EPI–SLNs could reach 78.46% and remained stable during nebulization. Cytotoxicity of SLNs against A549 cells was higher than that of epirubicin solution. It was found that drug concentration in the lung was much higher than that in plasma after inhalation. Drug concentration in the lung after inhalation of SLNs was much higher than that after administration of epirubicin solution. These findings suggested that SLNs could be used as a potential carrier for pulmonary delivery of anticancer drugs.

3. Experimental

3.1. Materials

Epirubicin (EPI) hydrochloride was obtained from Zhejiang Haizheng Pharmaceutical Co. Ltd., Compritol 888 ATO was bought from Gattefosse Co. (Shanghai, China). Soy lecithin was provided by TaiWei Pharmaceutical Co. (Shanghai, China). Poloxamer 188 was obtained from Shanghai XieTai Chemical Industry Co. Ltd. (Shanghai, China). All other chemicals were of analytical grade. A549 cell was kindly provided from Cell Resource Center, Peking Union Medical College.

3.2. Preparation of SLNs

EPI-SLNs were prepared with a solvent diffusion and high pressure homogenization (HPH) method. Epirubicin hydrochloride 50 mg was converted into free base form by adding 0.1N NaOH solution and was then dissolved in a mixture of absolute alcohol and DMSO (1:1, v/v). 1.2 g soy lecithin and 1.2 g Compritol 888 ATO were dissolved completely in absolute alcohol in a water bath at 70 °C and then added to the drug solution. 0.25 g Poloxamer 188 was dissolved in 50 ml water and then heated to 70 °C to be used as water phase. The water phase was added dropwise to the organic phase with magnetic stirring for 10 min, after evaporation of organic solvent, the obtained emulsion was passed through a high pressure homogenizer for 5 homogenization cycles. The dispersions were immediately filtered through a 0.45 μm membrane and cooled at room temperature to obtain drug-loaded SLN, stored at 4 °C. Epirubicin solution was prepared by dissolving EPI hydrochloride with distilled water.

3.3. Particle size and zeta potential

The particle size of EPI-SLNs was measured by photon correlation spectroscopy (PCS) using a NICOMP particle sizing system (CW380, Santa

Barbara, California, USA) at a fixed angle of 90 degrees and at a temperature of 25 °C. The particle size analysis data were evaluated using the volume distribution. Zeta potential measurements were operated using the same instrument at an electrical field strength of 10 v/cm and at the same temperature. Prior to measurement, SLN dispersions were diluted 20-fold with distilled water. All the measurements were performed in triplicate.

3.4. Drug encapsulation efficiency and drug loading

A HPLC method was used for the quantitative determination of epirubicin. In brief, the SLNs were dissolved in methanol to preferentially precipitate the lipid. After centrifugation (4000 rpm for 15 min), the drug content in the supernatant was measured.

The system consisted of a HPLC pump and a fluorescence detector with a C_{18} reverse phase column. The mobile phase was composed of acetonitrile, ammonium dihydrogen phosphate ($0.01\ mol \cdot L^{-1}$) and glacial acetic acid (45:55:0.5,v/v/v) at a flow rate of 1 mL/min. The excitation and emission wavelengths were fixed at 480 nm and 560 nm, respectively.

The SLNs were subjected to ultracentrifugation (Hitachi CS120GXL Micro Ultracentrifuge, Japan) at 60000 rpm for 4 h at 4 °C in vacuum. The supernatant containing the free drug was withdrawn for HPLC analysis as described above. The precipitate in the ultracentrifuge tube was desiccated to give an exact weight.

The equations for the drug content and loading efficiency are as follows:

$$\text{Drug content (\% w/w)} = \frac{\text{amount of EPI in the SLNs}}{\text{weight of SLNs}} \times 100 \qquad (1)$$

$$\text{Drug entrapment efficiency (\%)} = \frac{\text{amount of EPI in the SLNs}}{\text{amount of EPI used in formulation}} \times 100 \qquad (2)$$

3.5. In vitro deposition study

The pulmonary deposition of SLNs was investigated *in vitro* using a Twin Stage Impinger (TSI). SLNs were nebulized by a nebulizer (Pari Inhalierboy, Starnberg, Germany). The volume of capturing solvent PBS (pH 7.4) in the upper (stage 1) and lower (stage 2) stages were 5 and 40 ml, respectively (Mendes et al. 2007). EPI–SLNs (6 ml) were placed in a nebulizer with the device mouthpiece directed into the throat of the TSI and each experiment was repeated in triplicate.

The air flow was drawn through the TSI using a vacuum pump and the air flow rate was of 40 L/min. Each TSI stage was rinsed with PBS, the liquid was collected and the volume was adjusted to 1, 10 and 50 ml for stages 0, 1 and 2, respectively. The amount of EPI in each stage was measured by HPLC mentioned above. The respirable fraction (RF), defined as the ratio of the mass of drug recovered from the lower stage of the TSI to the total loaded dose, was expressed as a percentage and calculated for each sample.

3.6. Determination of size and zeta potential of SLNs after nebulization

To examine the nebulization properties of SLNs, they were nebulized and different stages of the TSI were collected in a beaker. The collected droplets were diluted with distilled water and immediately analyzed by PCS and zeta potential.

3.7. In vitro cytotoxicity of SLNs against A549 cells

A549 cells were seeded on 96-well plates and treated with EPI–SLNs. Epirubicin solution with the same drug concentration was also added for comparison. Subsequently the cells were incubated for 24 h, the cells were washed with physiological saline solution and the survival cell concentration was determined by uptake of 0.1% crystal violet, spectrophotometrically at 570 nm. The effect of the blank SLNs was examined as a control. Each sample was studied in replicates of three wells.

3.8. Pharmacokinetic analysis

3.8.1. Pulmonary administration

Male Sprague–Dawley rats (250 ± 20 g) were used in this study. The rats were fasted for 12 h but had free access to water. The rats were put in a chamber and the chamber was connected to the nebulizer. During the experiments, SLNs or epirubicin solution was aerosolized and directed to the chamber for rats inhalation at a single dose of $8\ mg \cdot kg^{-1}$. Each rat was exposed to the aerosols for 20 min. At each time point (10, 15, 30, 45, 60, 120 and 180 min), 0.4 mL blood samples were collected into tubes containing heparin and centrifuged at 3000 g for 10 min, then the plasma was stored at −20 °C until analysis. To study the tissue distribution, rats were randomly assigned to two groups, after inhalation of the same dose of epirubicin solution or EPI–SLNs, animals were dissected at 1, 2, 3 h after administration, the lungs were washed with physiological saline and cut into small parts, stored at −20 °C until analysis. All animal experiments complied with the requirements of the National Act of the People's Republic of China on the use of experimental animals.

3.8.2. Determination of EPI from blood and lungs

Plasma and lung samples were analyzed using the developed HPLC method mentioned above. Frozen plasma samples (0.1 mL) were thawed at room temperature. 10 μl of daunorubicin methanol solution (30 μg/ml) was added to the samples as internal standard and then 2 mL isopropanol and chloroform (1:1, v/v) were added. After vortexing for 5 min, the mixture was centrifuged at 4000 rpm for 10 min. The supernatants were evaporated to dryness under nitrogen and the supernatant was dissolved in 0.1 mL of mobile phase. An aliquot of the clear supernatant (20 μL) was used for HPLC analysis.

In the case of tissue samples, lungs were thawed and homogenized with one volume of methanol followed by two volumes of PBS. The mixtures were mixed with three volumes of acetonitrile and 100 μl of daunorubicin solution. After vortexing for 10 min, the mixture was centrifuged and 20 μL of the clear supernatant was injected for HPLC analysis.

3.8.3. Data analysis

Data were expressed as mean ± S.D. Statistical evaluation of data was performed using one-way analysis of variance (ANOVA). Statistical significance was determined using the Student's t-test.

References

Arcamone F, Penco S, Vigevani A, Redaelli S, Franchi G, Di Marco A, Casazza AM, Dasdia T, Formelli F, Necco A, Soranzo C (1975) Synthesis and antitumor properties of new glycosides of daunomycinone and adriamycinone J Med Chem 18: 703–707.

Bi R, Shao W, Wang Q, Zhang N (2009) Solid lipid nanoparticles as insulin inhalation carriers for enhanced pulmonary delivery. J Biomed Nanotechnol 5: 84–92.

Casazza AM (1979) Experimental evaluation of anthracycline analogs. Cancer Treat Rep. 63: 835–844.

Coukell AJ, Faulds D (1997) Epirubicin. An updated review of its pharmacodynamic and pharmacokinetic properties andtherapeutic efficacy in the management of breast cancer. Drugs 53: 453–482.

Liu J, Gong T, Fu H, Wang C, Wang X, Chen Q, Zhang Q, He Q, Zhang Z (2008) Solid lipid nanoparticles for pulmonary delivery of insulin. Int J Pharm 356: 333–344.

Mehnert W, Mader K (2001) Solid lipid nanoparticles production, characterization and applications, Adv Drug Deliv Rev 47: 165–196.

Mendes, PJ, Pinto, JF, Sousa, JM (2007) A non-dimensional functional relationship for the fine particle fraction produced by dry powder inhalers. Aerosol Sci. 38: 612–624.

Müller RH, Lucks JS (1996) Arzneistoffträger aus festen Lipidteilchen, Feste Lipidnanosphären (SLN). Eur Pat 0605497.

Pison U, Welte T, Giersig M, Groneberg DA (2006) Nanomedicine for respiratory diseases. Eur J Pharmacol 533: 341–350.

Plosker GL, Faulds D (1993) Epirubicin. A review of itspharmacodynamic and pharmacokinetic properties, and ther-apeutic use in cancer therapy. Drugs 45: 788–856.

Robert J (1993) Epirubicin. Clinical pharmacology and dose-effect relationship. Drugs 45 [Suppl 2]: 20–30.

Robert J (1994) Clinical pharmacokinetics of epirubicin. Clin Pharmacokinet 26: 428–438.

Sakagami M (2006) *In vivo*, *in vitro* and *ex vivo* models to assess pulmonary absorption and disposition of inhaled therapeutics for systemic delivery. Adv Drug Deliv Rev 58: 1030–1060.

Scheuch G, Kohlhaeufl MJ, Brand P, Siekmeier R (2006) Clinical perspectives on pulmonary systemic and macromolecular delivery. Adv Drug Deliv Rev 58: 996–1008.

Schwarz C, Mehnert W (1999) Solid lipid nanoparticles (SLN) for controlled drug delivery. II. Drug incorporation and physicochemical characterization. J Microencapsul 16: 205–213.

Shoyele SA, Cawthorne S (2006) Particle engineering techniques for inhaled biopharmaceuticals. Adv Drug Deliv Rev 58: 1009–1029.

Sung JC, Pulliam BL, Edwards DA (2007) Nanoparticles for drug delivery to the lungs. Trends Biotechnol 25: 563–570.