Pharmacokinetics and Biodistribution Study of Paclitaxel Liposome in Sprague-Dawley Rats and Beagle Dogs by Liquid Chromatography-Tandem Mass Spectrometry

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Abstract

Lipusu is the first paclitaxel liposome preparation approved in the world and has been widely used in China for the treatment of ovary, breast and non-small cell lung cancer. In present study we evaluated the pharmacokinetic and tissue distribution characteristics of paclitaxel liposome in Sprague-Dawley rats and Beagle dogs. A rapid and simple liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay was developed for the determination of paclitaxel in plasma and tissues. The plasma concentrations of paclitaxel in both rats and dogs initially declined steeply, followed by slow elimination after intravenous administration of Lipusu at 5 mg/kg and 1 mg/kg, respectively. The pharmacokinetic parameters calculated by a non-compartmental method in rats and dogs were as follows: AUC(0-24): 3566.5 ± 1366.1 and 443.2 ± 165.7 μg·h/L, CL: 1.5 ± 0.5 and 2.1 ± 0.6 L/h/kg, Vd: 20.0 ± 7.8 and 38.4 ± 12.5 L/kg, t1/2: 9.3 ± 2.9 and 14.1 ± 6.9 h, respectively. Biodistribution results in rats showed that except for brain and testis, liposomal paclitaxel was extensively distributed into various tissues, especially highly in liver and spleen.

Introduction

Paclitaxel is an effective antineoplastic agent used extensively in the treatment of a broad spectrum of malignancies including ovarian cancer, breast cancer, and non-small cell lung cancer [1]. Because of its poor solubility in aqueous solution, the surfactant polyoxyethylated castor oil (Cremophor EL) is formulated in the commercial injection (Taxol®). However, this vehicle causes serious hypersensitivity reaction including vasodilation, dyspnea, and hypotension [2,3], and leaches plasticizers di-(2-ethylhexyl) phthalate (DEPH) from polyvinyl chloride (PVC) containers [4]. To overcome the above problems, numerous water-soluble drug delivery systems without Cremophor EL have been investigated, such as albumin-bound formulation [5], microemulsions [6], porous particles [7] and polymeric micellar formulation [8]. Liposome is a promising approach among these preparations. This vehicle is a versatile and advanced nanodelivery formulation formed by phospholipid bilayers which are able to encapsulate a variety of drugs [9]. With the aim to enhance the therapeutic efficiency and reduce the toxicity, a range of liposomal paclitaxel formulations have been developed and evaluated. However, a few of them have reached the clinical trials such as LEP-ETU (NeoPharm) [10] and EndoTAG®-1 (MediGene) [11], and only Lipusu® (Nanjing Luye Sike Pharmaceutical Co., Ltd., China), approved by the State Food and Drug Administration of China, has entered into the market. In view of both preclinical and clinical data, Lipusu exhibits similar antitumor activity with lower toxicity and reduced incidence of serious hypersensitivity reactions compared with the conventional paclitaxel injection [12,13]. Because the encapsulation of paclitaxel into liposome has changed the in vivo behavior of entrapped drug, it is necessary to explore the pharmacokinetics and distribution of Lipusu. The objective of this study was to examine the pharmacokinetic and tissue distribution characteristics of paclitaxel after intravenous administration of paclitaxel liposome (Lipusu) in Sprague-Dawley rats and Beagle dogs by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Materials and Methods

Materials
Paclitaxel (standard substance) was purchased from the National Institutes for Food and Drug...
Control (NIFDC). Docetaxel (the internal standard) was obtained from Shanghai Sunwe Pharmaceutical Co., Ltd. Paclitaxel liposome for injection was purchased from Nanjing Luye Sike Pharmaceutical Co., Ltd. 5% glucose injection solution was obtained from Cisen Pharmaceutical Co., Ltd. HPLC-grade methanol and acetonitrile were purchased from Tedia (Fairfield, Ohio). Formic acid and sodium acetate were of analytical grade.

Animals
Sprague-Dawley rats (200 ± 20 g) of both genders were purchased from Nanjing Medical University Laboratory Animal Centre, and Beagle dogs (9.4 ± 0.3 kg) of both genders were offered by Southeast University Laboratory Animal Centre. Animals were housed under controlled conditions (room temperature: 20 ± 2 °C, relative humidity: 50 ± 20%) and accommodated to the housing environment for 1 week before the experiment. All of the animal studies were approved by the Animal Ethics Committee of China Pharmaceutical University.

Chromatographic conditions
The HPLC system (Shimadzu, Japan) comprised 2 model LC-20AD pumps, a SIL-20AD autosampler, a CBM-20A system controller and a CTO-20A oven. A BDS HYPERSIL C18 column (5 μm, 2.1 × 50 mm, Thermo Scientific) was used for separation. The mobile phase A was water phase (containing 0.1% formic acid and 0.3 mM sodium acetate) and the mobile phase B was methanol. The analytes were eluted with a gradient mode started at 10% mobile phase B for 0.5 min, next increased to 90% B in 2.0 min, then decreased B to the initial percentage during 1.0 min and re-equilibrated at 10% B for 0.5 min. The flow rate was 0.3 mL/min, and the temperature of column was 40 °C.

Mass spectrometric conditions
Mass analysis was conducted by a Thermo Scientific TSQ Quantum MS/MS system equipped with electrospray ionization interface operated in the positive ionization mode. Quantification was accomplished in selective reaction monitoring (SRM) by monitoring the transition of m/z 876.0 → 307.8 for paclitaxel and m/z 830.3→ 549.0 for docetaxel (the internal standard). The spray voltage, the temperature of capillary, sheath gas pressure and auxiliary gas pressure were set at 3500 V, 350 °C, 35 Arb, 25 Arb, respectively.

Pharmacokinetic studies
Paclitaxel liposome dissolved in 5% glucose injection solution was i.v. administrated to Sprague-Dawley rats (5 mg/kg) and Beagle dogs (1 mg/kg). About 100 μL of blood was collected via jugular vein from rats and 1 mL of blood was collected via cephalic vein from dogs into heparinized tubes at 0, 0.083, 0.167, 0.333, 0.5, 1, 2, 4, 8, 12 and 24 h, respectively. Plasma was separated by centrifuging at 4000 rpm for 10 min and stored at −70 °C until analysis. The paclitaxel concentrations were determined by LC-MS/MS. The estimation of pharmacokinetic parameters was performed by a non-compartmental analysis using the WinNonlin computer program (Version 4.0; Pharsight Corporation).

Biodistribution
The Sprague-Dawley rats were divided into 4 groups randomly and then received an i.v. dose (5 mg/kg) of paclitaxel liposome. Each group of rats was sacrificed at 0.25, 1, 4 and 12 h after intravenous injection. Tissues including liver, spleen, lung, kidney, intestine, heart, stomach, ovary, uterus, muscle, testis and brain were collected, rinsed in saline, blotted excess fluid, weighed and stored at −70 °C until analysis.

Plasma and tissue samples preparation
Tissue samples were homogenized in saline (tissue-water ratio of 1:3, w/v). Aliquots of the homogenate (100 μL) or plasma (50 μL) were added to a triple volume of acetonitrile containing 33.3 μg/L docetaxel (the internal standard) to precipitate proteins. The mixture was vortex-mixed for 30 s and centrifuged at 16000 rpm for 10 min. An aliquot of 5 μL of the supernatants was injected into the LC-MS/MS system for the determination.

Results
Method validation
The calibration curve with ten points was established for paclitaxel within the range from 2 to 2000 μg/L and the lowest limit of quantification was 2 μg/L. The precision and accuracy were assessed by the 5 replicates of quality control (QC) samples at low level (5 μg/L), median level (100 μg/L) and high level (1600 μg/L). The RSDs of inter-day and intra-day precision were within 15% of the 3 QC levels, and the accuracy extended from 85% to 115%. The recoveries of paclitaxel from plasma and tissue homogenate were greater than 85%. There was no significant matrix effect observed.

Fig. 1 Concentration-time profiles in plasma of paclitaxel following intravenous administration of paclitaxel liposome (Lipusu) at 5 mg/kg to Sprague-Dawley rats a and 1 mg/kg to Beagle dogs b respectively. Paclitaxel concentrations in plasma were determined by LC-MS/MS assay. Each point represents the mean ± D (n = 8).
Pharmacokinetic studies in rats and dogs
Following i.v. administration of 5 mg/kg and 1 mg/kg of Lipusu in Sprague-Dawley rats and Beagle dogs, respectively, the plasma concentration-time profiles of paclitaxel are showed in Fig. 1. The plasma pharmacokinetic characteristics of paclitaxel in rats and dogs were similar. The AUC\textsubscript{0–24} were 3566.5 ± 1366.1 and 443.2 ± 165.7 μg·h/L, CL were 1.5 ± 0.5 and 2.1 ± 0.6 L/h/kg, t\textsubscript{1/2} were 9.3 ± 2.9 and 14.1 ± 6.9 h, C\textsubscript{max} were 11848.5 ± 4413.3 and 1087.3 ± 733.9 μg/L in rats and dogs, respectively. The pharmacokinetic parameters calculated by a non-compartmental analysis using the WinNonlin software are presented in Table 1.

Tissue distribution of paclitaxel liposome in rats
After intravenous administration of Lipusu at a dosage of 5 mg/kg to Sprague-Dawley rats, the mean concentrations of paclitaxel in selected tissues at 15 min, 1 h, 4 h and 12 h are depicted in Fig. 2. It can be seen that after intravenous injection, liposomal paclitaxel distributed rapidly into various tissues. At 15 min post-dose, the concentrations of paclitaxel displayed maxima in most organs. The maximal concentrations of paclitaxel in spleen and liver were much higher than any other organs, followed by lung, kidney and heart. The concentrations of paclitaxel in other tissues were relatively low. Trace amount of paclitaxel was detected in brain and testis (Fig. 2). To assess the amount of paclitaxel accumulated in each tissue, the AUC\textsubscript{0–12h} values were calculated and summarized in Table 2.

Table 1 Pharmacokinetic parameters of paclitaxel after intravenous administration of Lipusu in rats (5 mg/kg) and dogs (1 mg/kg), respectively (mean ± SD, n = 8).

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Rats</th>
<th>Dogs</th>
</tr>
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<tbody>
<tr>
<td>AUC\textsubscript{0–24} (μg·h/L)</td>
<td>3566.5 ± 1366.1</td>
<td>443.2 ± 165.7</td>
</tr>
<tr>
<td>AUC\textsubscript{0–∞} (μg·h/L)</td>
<td>3655.8 ± 1374.9</td>
<td>540.4 ± 237.3</td>
</tr>
<tr>
<td>t\textsubscript{1/2} (h)</td>
<td>1.4 ± 0.6</td>
<td>4.6 ± 1.2</td>
</tr>
<tr>
<td>CL (L/h/kg)</td>
<td>9.3 ± 2.9</td>
<td>14.1 ± 6.9</td>
</tr>
<tr>
<td>V\textsubscript{d} (L/kg)</td>
<td>1.5 ± 0.5</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td>C\textsubscript{max} (μg/L)</td>
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<td>1087.3 ± 733.9</td>
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</table>

Discussion
Lipusu, the first commercial paclitaxel liposome preparation, is freeze-dried powder and dissolved in 5% glucose prior to injection. Compared with Taxol injection, the toxicity and incidence of hypersensitivity reaction after intravenous administration of Lipusu are much lower, since it is devoid of Cremophor EL and ethanol [12, 13]. It has been reported that anaphylactic reactions are not recorded in patients with Lipusu-based chemotherapy via premedication in a clinical trial [14]. Therefore paclitaxel liposome preparation may take place of the conventional paclitaxel injection. In this work, the pharmacokinetic and biodistribution studies of paclitaxel liposome (Lipusu) have been conducted in Sprague-Dawley and Beagle dogs, which are widely-used animal models in non-clinical pharmacokinetic researches.

To determine the concentrations of the paclitaxel in biosamples, several analytical methods have been developed. Most of them involved multiple steps of sample treatment, such as liquid-liquid extraction [15, 16] or solid-phase extraction [17, 18] followed by evaporation and reconstitution, which are arduous, time-consuming and need a large sample volume. In present study we applied a rapid and simple LC-MS/MS method for the quantitative analysis of paclitaxel in plasma and tissues. After a simple one-step protein precipitation of biosamples with acetonitrile (biosample-acetonitrile ratio of 1:3, v/v), the supernatants was injected into the LC-MS/MS system for the determination. This labor-saving and high-efficiency method is suitable for the quantification of paclitaxel in pharmacokinetic researches.

After an intravenous dose, both of the plasma concentration-time curves of paclitaxel in rats and dogs displayed bi-‐phasic pharmacokinetics (Fig. 1). The plasma concentrations of paclitaxel dropped markedly in the first 1 h post-dose, indicating a rapid distribution phase owing to the effective sequestration of paclitaxel in tissues.
Another way to prevent this phenomenon is by liposomisation. Pharmacokinetic analysis of paclitaxel in rats and dogs showed that its distribution and elimination followed a two-compartment model. The volume of distribution (Vd) at 4 h post-dose was 282.13 ± 29.02 g/l for rats and 202.29 ± 36.82 g/l for dogs. The area under the curve (AUC) at 15 min was calculated as 16.29 ± 1.91 g · h/g for rats and 8.36 ± 0.79 g · h/g for dogs. The elimination half-life (t1/2) was determined as 4.82 ± 0.46 h for rats and 3.86 ± 0.79 h for dogs. The clearance (CL) of paclitaxel in rats was 2.26 ± 0.55 l/h/g, while in dogs it was 1.82 ± 0.46 l/h/g. The interindividual variability in rapid distribution but not elimination was ascribed to the different metabolic pathways of rats and dogs. In conclusion, this study revealed that liposomal paclitaxel was rapidly distributed from plasma to various organs, especially spleen and liver, and eliminated slowly after intravenous administration of Lipusu. Additionally, a rapid, simple and reproducible LC-MS/MS assay was established to analyse the paclitaxel concentrations. This non-clinical research offers the disposition details of liposomal paclitaxel in vivo and may provide help in pharmaceutical researches.

### Acknowledgement

We would like to thank Nanjing YOKO Pharma Co., Ltd for the supply of paclitaxel liposome. The authors are also thankful to Junchi Huang, Shuangxia Ren, Fengjie Tian, Qi Liu, Di Zhao, Jie Xu for their help in this study.

### Author Disclosure Statement

No conflicts of interest exist.

### References