Original Article

Pharmacokinetic characteristics of vincristine sulfate liposomes in patients with advanced solid tumors

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Aim: To evaluate the single- and multiple-dose pharmacokinetics of vincristine sulfate liposomes (VSLI) in patients with advanced solid tumors.
Methods: In single-dose pharmacokinetic study, 16 patients were administered VSLI (1.5, 2.0, or 2.3 mg·m^{-2}) through intravenous infusion. Another 6 patients receiving vincristine sulfate (VCR, 2.0 mg) were taken as the control. In multiple-dose pharmacokinetic study, 12 patients were administered VSLI (1.5 or 1.8 mg·m^{-2}) through intravenous infusion weekly for 4 consecutive weeks. The plasma concentration of VSLI was determined using the liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.
Results: After intravenous infusion of the single dose of VSLI, the plasma concentrations were characterized by bi-exponential decline curves. No statistically significant differences were observed between the main pharmacokinetic parameters in the 3 dose groups. Compared with the patients receiving VCR, the patients treated with VSLI displayed an increase in the area under the plasma concentration vs time curve (AUC), and a decrease in plasma clearance rates. On the 4th cycle in the multiple-dose study, the plasma concentration of VCR in all subjects prior to the weekly administration was below the lower limit of quantification (LLOQ). The calculated pharmacokinetic parameters from the subjects in the multiple- and single-dose (1.5 mg·m^{-2}) groups had no significant differences. Although the administration of liposomal VCR may significantly elevate the plasma concentration of VCR, VSLI-associated adverse events were similar to those associated with conventional VCR.
Conclusion: VSLI exhibits a lower clearance and a higher AUC compared with conventional VCR. No accumulation was observed in patients exposed to VSLI for 4 consecutive weeks. VSLI was generally tolerated in the subjects. The phase II dose of VSLI may be recommended as 4 doses of 1.5 mg·m^{-2} for treatment of patients with advanced solid tumors.

Keywords: advanced solid tumors; vincristine sulfate; liposome, pharmacokinetics, liquid chromatography-mass spectrometry

Introduction

Vinca alkaloids vincristine (VCR) is a widely used chemotherapeutic agent since the 1960s[1] and its cytotoxic activity is based on its capability to alter the tubulin polymerization equilibrium and arrest cell growth during metaphase[2, 3]. VCR has a broad antitumor activity and is an important component of combination chemotherapy regimens for the treatment of childhood and adult acute lymphocytic leukemia, Hodgkin's and non-Hodgkin's lymphoma, rhabdomyosarcoma, neuroblastoma and Wilms' tumor[4, 5]. However, the rapid elimination of VCR from the blood after IV administration due to a short plasma half-life as well as dose-limiting peripheral neu-

rotoxicity limits its anticancer activity[6].

VCR is a cell cycle-specific anticancer agent, so its therapeutic efficacy may be enhanced by prolonging the retention time of free VCR in the blood, resulting in the exposure of more tumor cells to the drug during the sensitive stage of their cell cycles[7]. Previous studies have demonstrated that the liposomal encapsulation of VCR increased its antitumor efficacy without increasing toxicity[8–11]. Marqibo® (Vincristine sulfate liposomes injection, Hana Biosciences, Inc) has been extensively studied for its capability to prolong the pharmacokinetics and subsequent exposure of VCR to cancer cells[5, 6, 12], thus increasing its antitumor activity[5, 13, 14]. However, Marqibo® was developed as a 3-vial kit containing 100 mg/mL of injectable sphingomyelin/cholesterol liposomes, 14.2 mg/mL of injectable sodium phosphate and Oncovin (injectable vincristine sulfate). Moreover, the preparation of vincristine sulfate

liposomes (VSLI) requires an encapsulation procedure.

The liposomal formulation of VCR (VSLI) was rediscovered in the People's Republic of China the past ten years and a wide range of preclinical studies have been completed. VSLI (Luyesike Pharmaceutical Co, Ltd) was supplied as a two-vial kit containing 1 mg of VSLI freeze-dried powder and 5.68 mg/mL of injectable sodium phosphate. Thus, this formulation could be more convenient for pharmacists because an encapsulation procedure would not be necessary at the time of administration.

Pharmacokinetic (PK) studies of VSLI in Chinese subjects have not been reported to date. Therefore, this study was performed to characterize the PK profiles of VSLI in Chinese subjects with advanced solid tumors and to observe the toxicities after IV administration of this new agent. The PK profile of VSLI was also compared with conventional, unencapsulated VCR.

Materials and methods

Patient selection

This study received approval from the Ethics Committee of the Tianjin Medical University Cancer Institute and Hospital. All patients were informed about the study and were requested to sign informed consent forms prior to participating. Eligible patients had histologically confirmed malignant tumors that were either refractory to conventional forms of cancer therapy or for which no effective conventional therapy existed. It was required that patients had not undergone surgery, chemotherapy, biotherapy, endocrine therapy, or radiotherapy for at least 4 weeks prior to the start of the study. Other inclusion criteria were as follows: age of between 18 and 75 years; life expectancy of at least 12 weeks; Eastern Cooperative Oncology Group performance status of 0 to 2; adequate bone marrow function, as defined by a leukocyte count exceeding 4.0×10^9/L, an absolute granulocyte count of more than 1.5×10^9/L, a platelet count exceeding 100.0 g/L and a hemoglobin count beyond 9.0 g/L; adequate hepatic function, as defined by alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin concentration of less than 1.5 times normal; adequate renal function, as defined by a serum creatinine concentration of less than 1.5 times normal; and no evidence of preexisting neurologic dysfunction. The exclusion criteria were sensitivity to VSLI or vinca alkaloids, neurological disease, severe complications that may have a negative effect on compliance, and pregnancy or lactation.

Dosage and administration

The VSLI for injection was obtained from Luyesike Pharmaceutical Co, Ltd (Jiangsu, China). VSLI is manufactured as freeze-dried powder. Each vial contains 1 mg of VSLI powder. To prepare VSLI, 10 mL of disodium hydrogen phosphate solution was added to the VSLI powder and mixed. The solution was heated in a 50 °C water bath and mixed for 5 min in order for the internal and external aqueous phases of the liposomes to reach acid-base balance and to ensure that 90% of the VCR was encapsulated. The control drug, injectable VCR,

was obtained from Shenzhen Main Luck Pharmaceuticals Inc. (Guangdong, China). A total of 16 patients in the single-dose PK study received 1.5, 2.0, or 2.3 mg·m^{-2} VSLI as a 60 min IV infusion. Six patients in the control group received 2.0 mg of VCR. Patients in the multiple-dose PK study received 1.5 or 1.8 mg·m^{-2} of VSLI weekly (one cycle) for 4 consecutive weeks.

PK sample collection

Single-dose PK 3 mL blood samples were taken at the following time points: before treatment, after infusion for 30 min, at the end of the 60 min infusion, at 5, 15, 30, and 45 min, and subsequently 1, 2, 4, 8, 12, 24, 36, and 48 h after the end of infusion. On the second and third weeks of the multiple-dose PK study, 3 mL blood samples were collected before and after the infusion. On the 4th cycle, blood samples were collected before treatment, after infusion for 30 min, at the end of the 60 min infusion, at 5, 20, and 40 min, and subsequently 1, 2, 4, 8, 12, and 24 h after the end of infusion. Blood samples were collected in heparinized tubes. Plasma was prepared by centrifugation (10 min at 600×g) and was subsequently stored at -80 °C until analysis.

Reagents and instruments

VCR was obtained from Luyesike Pharmaceutical Co, Ltd (Jiangsu, China). Vinblastine sulfate (VBL, internal standard) was purchased from Yifang Science and Technology Co, Ltd (Tianjin, China). High-performance liquid chromatography (HPLC) grade acetonitrile, methanol and isopropanol were purchased from Honeywell Burdick & Jackson (Muskegon, MI). Analytical grade n-hexane and dichloromethane were purchased from Fuchen Chemical Reagent Factory (Tianjin, China).

The chromatographic system consisted of an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA, USA) coupled to an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Concord, Ontario, Canada) and an Agilent Eclipse XDB C$_{18}$ column (50 mm×4.6 mm, 5 μm, Agilent Technologies, Palo Alto, CA, USA). Data were processed using Analyst 1.4.1 software (Applied Biosystems/MDS SCIEX, Concord, Ontario, Canada).

Bioanalytical methods

The solvents used for gradient elution were A) methanol and B) water; each was adjusted to pH 3 by the addition of 10 mmol/L ammonium acetate and 2.9 mL/L formic acid. The conditions for the gradient elution were as follows: 0 to 0.5 min, 55% to 95% solvent A; 0.5 to 2.0 min, isocratic 95% solvent A; 2.0 to 2.1 min, 55% to 95% solvent A; and 2.1 to 5.5 min, isocratic 55% solvent A. The flow rate was 0.45 mL/min, and the column temperature was set to 40 °C. During the analysis, 10 μL of sample was injected using the autosampler, and the sample was then carried into the column. A mass spectrometer with a TurboIonSpray (ESI) source was operated in positive ion mode. The source temperature was maintained at 500 °C, and the spray voltage was set to 5500 V. The nebulizer (Gas 1), heater (Gas 2), curtain, and collision activated

dissociation (CAD) gases were set to 50, 55, 15, and 8 psi, respectively. The declustering potential values were 119.2 and 105 V, and the collision energy values were 50.5 and 59.7 V for VCR and VBL, respectively. Quantification was performed using multiple reaction monitoring of the transitions m/z 825.8→807.5 for VCR and m/z 811.7→224.0 for VBL (Figure 1).
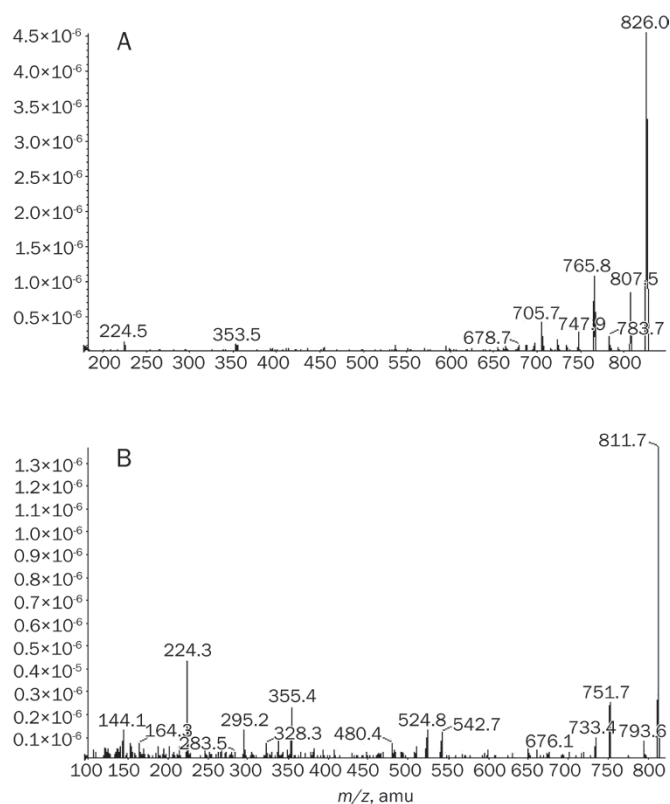

Figure 1. Production of mass spectra of (A) VCR and (B) VBL.

Serial calibration standards at concentrations of 0.5, 2, 10, 40, 100, 400, and 800 ng/mL were prepared by adding 100 µL of the appropriate working solutions to 100 µL of the blank plasma. The calibration curves were established by determining the peak area ratio [VCR/internal standard (Y) versus VCR concentration (X)]. QC samples were prepared in the same way to obtain concentrations of 1 (low), 80 (medium) and 600 (high) ng/mL. All frozen plasma samples were thawed at room temperature. For sample extraction, 100 µL plasma sample was added to a 10 mL centrifuge tube, along with 100 µL solvent A-solvent B (7:3, *v/v*, pH=3), 100 µL 150 ng/mL VBL solution, 100 µL acetonitrile and 200 µL water. The mixture was vortexed for 2 min, and 3 mL *n*-hexane-dichloromethane-isopropanol (2:1:0.1, *v/v/v*) was then added. After vortexing for 7 min, the mixture was centrifuged at 3600 r/min for 10 min. The upper organic phase was placed into another centrifuge tube and evaporated to dryness under a nitrogen stream. The residue was reconstituted in 100 µL of the solvent A-solvent B (7:3, *v/v*) solution, and 10 µL of that solution was injected into the LC-MS/MS system.

PK data analysis and statistical analysis

The plasma concentration-time data were analyzed using non compartmental methods. The PK analysis system DAS 2.1 (Anhui, China) was used to assess the PK parameters. The peak plasma concentration (C_{max}) and the time to peak plasma concentration (T_{max}) were obtained via experimental observations. The elimination half-life ($t_{1/2}$) was calculated as 0.693/Zeta (Zeta is the slope of terminal phase). The area under plasma concentration *vs* time curve (AUC) from zero to infinity ($AUC_{0-\infty}$) was equivalent to the sum of the areas from time zero to the time of the last measured concentration and was calculated using the linear trapezoidal method (until C_{max}), the log-trapezoidal method (until the last measurable concentration), and the extrapolated area. The extrapolated area was determined by dividing the final measured concentration by the slope of the terminal log-linear phase. Trough values on cycle 2 and cycle 3 were averaged for each dose level. All statistical tests were two-tailed, and a *P* value of 0.05 was considered significant. Differences in the mean values of the physical examinations and in PK parameters among the 3 groups were compared with analysis of variance or the Kruskal-Wallis test using SPSS (Statistical Package for the Social Sciences) software, version 16.0. The *t*-test or Wilcoxon's test was used to investigate the differences between the two groups.

Results

Representative chromatogram and validation of the analytical method

The retention times of VCR and the internal standard were 2.7 and 2.8 min, respectively. VCR and the internal standard in plasma were completely separated without significant interferences. The calibration curve was linear over the concentration range of 0.5 ng/mL to 800 ng/mL. The equation for the calibration curve was $Y=0.00157X+0.00273$ ($r=0.9982$, $n=5$). The LLOQ was 0.5 ng/mL. The intra-day precision for the low, medium and high concentration QC samples was 9.1%, 4.1%, and 4.8%; the inter-day precision was 3.2%, 5.2%, and 6.6%; and the accuracy was 99.5%, 102.8%, and 100.4%. The relative standard deviation (RSD) was less than 10.0%. The extraction recoveries for the three gradient concentration of VCR were 77.5%, 76.7%, and 78.7% and the extraction recovery for the internal standard was 86.8%. The matrix suppression for the QC samples and the internal standard was -3.0%, 31.1%, 44.6%, and 26.5%. The concentration of VCR was stable in the working solution at room temperature for 10 h or at -4 °C for one month and was stable in human plasma for 3 freeze-thaw cycles, at room temperature for 12 h, or at -80 °C for 4 months prior to extraction. The differences between stored and freshly prepared solutions were within 15%.

Patient characteristics

A total of 34 eligible and consenting patients with advanced carcinoma were recruited for this study. There were 22

patients enrolled in the single-dose and 12 patients enrolled in the multiple-dose PK studies. No statistically significant differences were found in age, height, weight, body surface area, or body mass index (BMI) among the patient groups. The characteristics of the patients are shown in Table 1. All of the subjects had received prior chemotherapy and 13 of the subjects had received prior radiation therapy.

Single-dose PK study

The total VCR plasma concentration versus time profiles of the patients who received 1.5 to 2.3 mg·m^{-2} of VSLI or 2 mg of VCR were shown in Figure 2. The plasma concentration profiles for all patients were characterized by a biexponential decline after infusion. The VCR concentrations fell below or were marginally above LLOQ within 48 h after infusion.

PK parameters were determined for the 22 subjects. The primary PK parameters are summarized in Table 2. The mean peak concentration (C_{max}) for the patients in the 3 VSLI groups was 141.3, 127.0, and 218.7 ng/mL. The mean AUC from time zero to infinity (AUC_{0-inf}) was 229.3, 242.9, and 316.1 ng·h·mL^{-1} for the 3 groups. The mean values of C_{max}, AUC_{0-inf}, $t_{1/2}$ and clearance (CL_z) were not different among the 3 doses ($P>0.05$); however, significant differences in the mean volume of distribution (V_z) values were observed ($P<0.05$). No correlation was found between the observed PK profile (AUC) and patient characteristics (age, height, weight, body mass index, or body surface area). When compared with patients who received the conventional 2-mg dose of VCR, patients who received 1.5 to 2.3 mg·m^{-2} VSLI had an increased AUC_{0-t} and AUC_{0-inf}, as well as decreased CL ($P<0.05$).

Multiple-dose PK study

A total of 12 patients in the multiple-dose study received 1.5 or 1.8 mg·m^{-2} VSLI weekly for 4 consecutive weeks. The mean

Table 1. Summary of patient characteristics. Data are expressed as mean (Range).

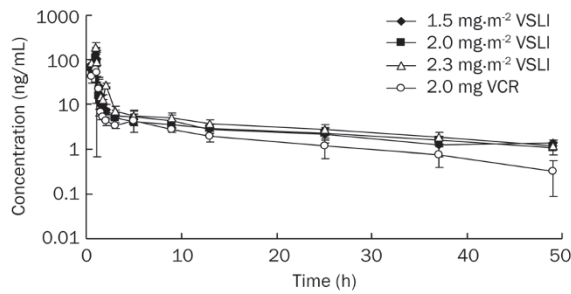|	Single-dose pharmacokinetics				Multiple-dose pharmacokinetics	
	1.5 mg·m^{-2} VSLI (*n*=5)	2.0 mg·m^{-2} VSLI (*n*=6)	2.3 mg·m^{-2} VSLI (*n*=5)	2 mg VCR (*n*=6)	1.5 mg·m^{-2} VSLI (*n*=6)	1.8 mg·m^{-2} VSLI (*n*=6)
Sex						
Male	1	2	2	2	2	3
Female	4	4	3	4	4	3
Age (years)						
Median, Range	64 (56–73)	53 (37–63)	45 (18–59)	54 (22–65)	41 (19–62)	41 (28–54)
Height (cm)						
Median, Range	168 (153–182)	162 (154–178)	168 (155–175)	164 (160–168)	166 (157–178)	167 (156–175)
Weight (kg)						
Median, Range	72 (60–82)	61 (55–69)	70 (61–85)	64 (50–75)	70 (55–85)	64 (42.5–90)
Body surface area (m^2)						
Median, Range	1.83 (1.62–1.98)	1.65 (1.54–1.86)	1.77 (1.61–2.01)	1.69 (1.54–1.82)	1.77 (1.54–2)	1.69 (1.38–2.01)
BMI (kg·m^{-2})						
Median, Range	25.7 (22.6–27.8)	23.1 (21.8–24.6)	24.7 (21.1–28.1)	23.6 (18.1–27.5)	25.6 (22–32.4)	22.7 (17.5–29.4)
ECOG status at entry						
0–1	5	6	5	6	5	6
2	0	0	0	0	1	0
Tumor types						
Lymphoma	4	4	2	6	4	5
Breast cancer	1	0	1	0	0	0
Lung cancer	0	1	1	0	0	1
Others (Ewing's sarcoma, Ovarian cancer, Renal carcinoma, laryngocarcinoma)	0	1	1	0	2	0
Prior therapy						
Chemotherapy	5	6	5	6	6	6
Radiotherapy	3	3	2	0	1	4

Figure 2. Mean logarithmic concentration versus time plot after administration of 1.5 mg·m^{-2} (♦, *n*=5), 2.0 mg·m^{-2} (■, *n*=6), 2.3 mg·m^{-2} (△, *n*=5) of VSLI and 2 mg of VCR (○, *n*=6).

PK parameters for the 4th cycle are summarized in Table 2. As shown in Figure 3, the mean concentration-time curves of the subjects after multiple doses of VSLI were comparable to the corresponding mean values from subjects after a single dose of VSLI. These findings demonstrate that the administration of 4 doses of VSLI does not markedly alter the clearance of total vincristine from the plasma. Total VCR plasma concentrations from all subjects before the weekly treatments were below the
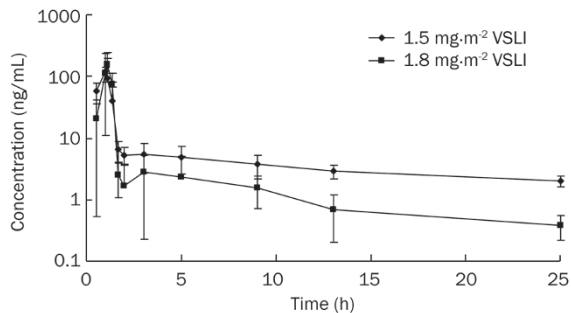

Figure 3. Mean logarithmic concentration versus time plot after multiple doses of VSLI on the 4th cycle (♦, *n*=5; ■, *n*=6).

LLOQ. A comparison of the calculated PK parameters among the subjects in the multiple- and single-dose 1.5 mg·m^{-2} groups did not show significant differences, indicating that that no detectable accumulation was observed in total VCR with repeated doses of VSLI for 4 consecutive weeks.

Safety

The National Cancer Institute's Common Terminology Criteria for Adverse Events (AEs) (version 3.0) were used to grade AEs. Table 3 presents a summary of the toxicities that were associated with VSLI administration. All of the VSLI doses had similar toxicity profiles. The most common toxicities irrespective of grade, causality, or VSLI dose included peripheral neuropathy (75%), neuropathic pain (61%), constipation (46%) and abdominal distention (36%). Nausea, vomiting, anorexia and hypocalcemia were also common. Non-hematologic toxicity was more common than hematologic toxicity. Grade 3/4 toxicities were neuropathic pain, insomnia, alterations in numbers of neutrophils or other leukocytes, hypermagnesemia and hyponatremia. Other reported AEs were mild.

Discussion

A 3-vial kit of Marqibo® consists of empty liposomes, VCR and buffer solution, and requires an encapsulation procedure. However, the VSLI in this study was developed as freeze-dried powder containing encapsulated VCR. Such a freeze-dried formulation of VCR liposomes could be more convenient for pharmacists. In addition, this formulation could increase the stability of VCR and prevent the settlement and aggregation of empty liposomes during storage. The encapsulation efficiency of VSLI exceeds 85%, and its diameter is stable in the range of 100 to 200 nm. Previous stability data showed that the diameter and VCR content of VSLI remained stable within 7 h when administered intravenously. The animal VSLI PK study showed that the V_z and CL of total VCR in VSLI-treated rats was significantly reduced as compared with the V_z and CL in rats that had been treated with the free drug, resulting

Table 2. Main PK parameters of total VCR after IV administration of VSLI or VCR. Values are expressed as mean (SD). [a]P>0.05 *vs* 2.0 mg VCR group. [b]P<0.05 *vs* 2.0 mg VCR group. [d]P>0.05 *vs* 1.5 mg·m^{-2} single-dose VSLI group. [e]P<0.05 *vs* 1.5 mg·m^{-2} single-dose VSLI group. [g]P>0.05 *vs* 2.0 mg·m^{-2} single-dose VSLI group.

Parameters	Single-dose pharmacokinetic study				Multiple-dose pharmacokinetic study	
	1.5 mg·m^{-2} VSLI (*n*=5)	2.0 mg·m^{-2} VSLI (*n*=6)	2.3 mg·m^{-2} VSLI (*n*=5)	2.0 mg VCR (*n*=6)	1.5 mg·m^{-2} VSLI (*n*=6)	1.8 mg·m^{-2} VSLI (*n*=6)
C_{max}/ng·mL^{-1}	141.3 (40.8)[b]	127.0 (80.4)[ad]	218.7 (127.4)[adg]	83.4 (39.5)	134.8 (138.6)[d]	85.2 (28.3)[d]
AUC$_{0-t}$/ng·h·mL^{-1}	205.1 (57.4)[b]	201.2 (51.4)[bd]	281.8 (95.0)[bdg]	121.3 (15.6)	169.8 (88.3)[d]	130.3 (15.7)[d]
AUC$_{0-inf}$/ng·h·mL^{-1}	229.3 (66.3)[b]	242.9 (60.8)[bd]	316.1 (106.5)[bdg]	140.3 (25.0)	216.7 (77.6)[d]	155.5 (25.2)[d]
MRT$_{0-t}$/h	8.9 (1.7)[a]	11.8 (1.6)[be]	10.6 (2.7)[adg]	8.4 (2.8)	5.9 (1.2)[e]	5.8 (0.6)[d]
MRT$_{0-inf}$/h	15.1 (3.9)[a]	24.9 (9.4)[ad]	17.8 (4.5)[adg]	15.9 (8.2)	17.6 (8.2)[d]	12.3 (4.7)[d]
$t_{1/2z}$/h	17.5 (6.7)[a]	24.9 (8.3)[ad]	19.2 (2.5)[adg]	16.0 (7.9)	17.4 (6.2)[d]	13.0 (6.0)[d]
T_{max}/h	1.05 (0.04)[a]	0.85(0.27)[ad]	0.8 (0.27)[adg]	0.89 (0.31)	0.93 (0.21)[d]	0.83 (0.26)[d]
CL$_z$/L·h^{-1}	7.0 (1.9)[b]	8.7 (2.3)[bd]	8.0 (2.7)[bdg]	14.6 (2.7)	7.6 (2.4)[d]	11.8 (1.9)[e]
V_z/L	166.3 (42.2)[b]	309.0 (119.8)[ae]	217.5 (70.8)[adg]	321.9 (123.4)	202.1 (112.2)[d]	212.7(82.0)[d]

Table 3. Summary of VSLI-associated AEs.

| Toxicity (CTC) | No with grade 1–4 toxicity VSLI dose level (mg·m⁻²) | | | | |
| | Single-dose group | | | Multiple-dose group | |
	1.5 (*n*=5)	2.0 (*n*=6)	2.3 (*n*=5)	1.5 (*n*=6)	1.8 (*n*=6)
Neurology					
Peripheral neuropathy	2	5	3	5	6
Neuropathic pain	–	2	5	4	6
Gastrointestinal					
Constipation	2	3	3	1	4
Nausea	2	2	2	1	2
Emesis	2	1	2	1	1
Diarrhea	1	–	–	1	1
Abdominal pain	1	–	1	1	3
Abdominal distention	1	1	1	3	4
Anorexia	–	–	–	4	5
Oral cavity mucositis	–	–	–	1	–
Blood					
Leukocytes	2	2	1	1	3
Anemia	–	1	2	2	3
Lymphopenia	1	–	–	–	1
Neutrophils	–	1	–	2	3
Constitutional					
Fever	–	–	2	3	3
Fatigue	–	1	–	1	3
Insomnia	–	–	1	–	1
Weight Loss	–	–	–	–	1
Skin					
Alopecia	–	–	2	–	–
Cardiovascular					
Supraventricular arrhythmia	–	1	–	1	–
Metabolic					
Hypokalemia	–	–	–	1	–
Hypomagnesemia	1	1	–	–	–
Hypermagnesemia	–	1	–	–	–
Hyponatremia	–	–	–	2	–
Hypocalcemia	–	–	2	4	2
Hypercholesteremia	–	–	–	–	1
Hypertriglyceridemia	–	–	–	1	2
ALT	–	–	–	3	2
AST	–	1	–	1	2
Bilirubin	–	–	–	1	–
Alkaline phosphatase	–	–	–	1	–

in a significantly increased AUC (unpublished data). Previous studies have demonstrated that following the administration of VSLI, levels of free VCR in plasma were below the lower limits of quantitation [12]. Therefore, the VCR levels measured in plasma following the administration of VSLI reflected liposomally encapsulated drug[5].

In this study, the PK of VSLI in patients with advanced solid tumors was evaluated and compared with the corresponding PK data for conventional VCR. The total VCR plasma concentration values for patients treated with VSLI were measured using the LC-MS method. The LLOQ for VCR was 0.5 ng/mL. The method was specific, sensitive and convenient for the assessment of total VCR in biological samples. The plasma concentration of total VCR in all of the patients followed a biexponential decline after a single IV administration of VSLI. Bedikian *et al*[6] reported that the total VCR of some metastatic melanoma patients with adequate liver function followed a biexponential decline, but that of others followed a monoexponential decline. Interpatient variability in the rate of decline resulted in the monoexponential or biexponential profiles. However, differences in elimination at each dose level were not observed in the current study. In addition, the pharmacokinetic parameters in this study were not consistent with previous international studies. One previous study[12] of Marqibo® indicated that the mean±SD AUC_{0-inf} and C_{max} at a dose of 2.0 mg·m⁻² were 15.6±11.9 µg·h·mL⁻¹ and 11.0±0.3 µg/mL, respectively, which were higher than the results obtained in this study. The differences in pharmaceutical formulations of VSLI or in characteristics of the patients (*ie*, race) in the previous study versus the current study may have contributed to these inconsistencies. It is also possible that methodological differences applied in different laboratories may be responsible for these inconsistencies. No significant differences were observed in the main PK parameters among the 3 dose groups (*P*>0.05), indicating that the effect of the dose on single-dose pharmacokinetics of VSLI was insignificant. Moreover, the results of this study indicate that the liposomal encapsulation of VCR significantly increases plasma AUC and decreases plasma clearance rates compared with conventional VCR. Therefore, total plasma VCR exposure following the administration of VSLI appears to be greater than that of conventional VCR because of the difference in elimination. The liposomal encapsulation of VCR protects the drug from the early phase of rapid elimination that is observed with nonliposomal VCR[15]. Previous studies have demonstrated that increasing VCR retention in liposomal systems improves the therapeutic index by increasing the duration of drug exposure to the tumor tissue[8, 10, 16–18]. These PK properties of VSLI may potentially increase VCR accumulation in tumors and obtain greater efficacy over conventional VCR.

The mean AUC, $t_{1/2}$, C_{max}, T_{max}, and V_z from the subjects in the multiple-dose 1.5 mg m⁻² group were similar to those from the subjects in the single-dose 1.5 mg·m⁻² group. No significant differences were observed in the main PK parameters between the two groups, indicating that that no accumulation was observed with repeated administration of VSLI for 4 consecutive weeks. These data indicate that the pharmacokinetics of VSLI had no apparent change after repeated administration, which is consistent with previous data[6].

In this study, PK parameters from patients who tolerated VSLI administration well were calculated. A previous tolerability study reported that 4 subjects who received 1.8 mg·m⁻² of VSLI weekly for 4 consecutive weeks withdrew from the

study because of treatment-associated peripheral neurotoxicity[19]. In the current study, most VSLI-associated AEs varied from mild to moderate. Grade 3/Grade 4 toxicities were neuropathic pain, insomnia, alterations in numbers of neutrophils or other leukocytes, hypermagnesemia and hyponatremia. The most frequently observed AEs included peripheral neuropathy, neuropathic pain and gastrointestinal disorders. Based on the differences in body surface areas, the total dosage of VSLI for 4 cycles ranged from 9.24 to 14.47 mg, which was significantly higher than the routine dosage of VCR. The results of this study show that VSLI-associated AEs were similar to those associated with conventional VCR, although the administration of liposomal VCR may greatly increase the dosage. Correlation analyses were conducted to determine if a relationship could be established between the observed PK profile and toxicities; no correlation was found, possibly because of the small sample size. Out of the 12 patients with 4 consecutive weeks of VSLI treatment, 8 were assessed to have stable disease as measured by an increase in tumor size of less than 25%.

In conclusion, VSLI exhibited a longer circulation half-life and higher AUC compared with conventional VCR, which provides VSLI with an advantage over conventional VCR. After repeated administration of VSLI, the accumulation of total vincristine was not observed in the plasma. Furthermore, the pharmacokinetics of VSLI were not altered significantly after 4 doses of 1.5 or 1.8 mg·m^{-2}. The prolonged plasma retention of VSLI compared with conventional VCR may potentially improve antitumor efficacy. VSLI was generally tolerated in the subjects. Considering previously published data on VSLI tolerability[19, 20], the phase II dose of VSLI may be recommended as 4 doses of 1.5 mg·m^{-2} for the treatment of patients with advanced solid tumors.

Acknowledgements

Author contribution

Zhao YAN designed the research; Zhao YAN, Zhong-ling ZHU, Hua-qing WANG, Ge HU, Zheng-zi QIAN, Wan-hui LIU, and Guang CHENG performed the research; Zhao YAN and Zhong-ling ZHU analyzed the data; Zhao YAN and Zhong-ling ZHU wrote the paper.

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