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Pharmacokinetic study of vinorelbine in Chinese patients with non-small-cell lung cancer by high-performance liquid chromatography (HPLC) with fluorescence detection

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To investigate the pharmacokinetics of vinorelbine in Chinese subjects with non-small-cell lung cancer (NSCLC), a high-performance liquid chromatographic method using fluorescence detection was established to determine the concentrations of vinorelbine in human blood and plasma. Samples were collected from 10 Chinese patients with NSCLC after intravenous infusion of 40 mg vinorelbine. Pharmacokinetic parameters were calculated using 3P87 software. The pharmacokinetics of vinorelbine in Chinese patients fitted a two-compartment model. The pharmacokinetic parameters calculated from plasma and blood drug concentrations were: AUC (530.99 ± 88.56) ng·ml⁻¹·h and (904.91 ± 194.97) ng·ml⁻¹·h, C_{max} (861.78 ± 247.25) ng·ml⁻¹ and (1,053.85 ± 295.98) ng·ml⁻¹, and t_{1/2β} (33.70 ± 1.58) h and (40.40 ± 21.30) h, respectively. The pharmacokinetic profiles of vinorelbine in Chinese NSCLC patients were similar to those reported for non-Chinese NSCLC patients.

Key words: Vinorelbine, plasma drug concentration, blood drug concentration, pharmacokinetics, high-performance liquid chromatography.

INTRODUCTION

Also known as vinorelbine and Navelbine®, 5'-nor-anhydrovinblastine is a first-line, semi-synthetic anti-cancer agent. It has similar actions to vindesine and vincristine, but lower toxicity than those agents. It has been used widely in China (Liu et al., 2001) and many

other countries (Provencio et al., 2011) because of its significant activity in the treatment of advanced non-small-cell lung cancer (NSCLC) and metastatic breast cancer. Many studies have been reported; its pharmacokinetics in cancer patients after single administration (Wargin and

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Lucas, 1994; Levêque and Jehl, 1996; Gebbia and Puozzo, 2005; Robieux et al., 1996) as well as combination with other chemotherapy (Airoidi et al., 2003; Tokudome et al., 2008). Pharmacokinetic data were obtained with high-performance liquid chromatographic (HPLC) techniques coupled with fluorescence detection (Gauvin et al., 2000; Robieux et al., 1996), ultraviolet detection (Campone et al., 2001; Puozzo et al., 2007) and electrospray ionization mass spectrometry/mass spectrometry (ESI-MS/MS) (Qian et al., 2011).

Body weight and platelet count were confirmed as influencing blood clearance of vinorelbine. Also, pharmacokinetic interaction occurred between vinorelbine and other drugs probably due to their inhibition of CYP450-3A4 (Rezai et al., 2011). The results by Robieux et al. (1996) showed lower clearance rate of vinorelbine in patients with severe liver failure but not with moderate secondary liver involvement and supported correspondingly vinorelbine dose reduction. Moreover, association does not alter the pharmacokinetic profile of both vinorelbine and other combined drugs (Airoidi et al., 2003). However, only one Chinese pharmacokinetics study of vinorelbine has been reported (Qian et al., 2011), which using a LC/MS/MS determination method. Therefore, more and further study on the pharmacokinetics of vinorelbine in Chinese would be beneficial for its dose individualization and reduce the side effect.

Here we describe a study of the pharmacokinetics of vinorelbine in Chinese NSCLC patients using a modified HPLC method with fluorescence detection. This HPLC method could fulfill the requirements for a sufficiently simple, accurate and precise assay to carry out pharmacokinetic studies of vinorelbine. The pharmacokinetic results would be the reference for the vinorelbine individual administration and lead to more clinical safety and efficiency.

MATERIALS AND METHODS

Reagents

Vinorelbine ditartrate was from Pierre Fabre Corporation (Castres, France). Methanol (BDH, Poole, UK), tetrahydrofuran (Merck, Darmstadt, Germany) and diethyl ether (Merck) were of HPLC grade. Phosphoric acid and potassium dihydrogen phosphate were analytically pure reagents. The water used in all experiments was redistilled.

Equipments

The chromatographic system consisted of a Shimadzu 10AT pump (Shimadzu, Kyoto, Japan), a Shimadzu RF-10AXL fluorescence detector, a Rheodyne 7725i loading valve fitted with a 100 μ l sample loop (Rheodyne, Rohnert Park, CA, USA), and a Shimadzu C-R8A integrator. An 80-2 precipitator, a XW-80A vortex mixer, a pH meter (Orion, Beverly, CA, USA), a JL-120DT ultrasonic cleaner (Shanghai Jili Scientific Instruments, Shanghai, China) and liquid-

liquid extraction equipment were used. The excitation wavelength was 280 nm and the emission wavelength was 360 nm. The HPLC analytical column (4.0 mm $\times$ 150 mm) was packed with 5 μ m diameter Hypersil-C₁₈ particles (Dalian Elite Analytical Instruments, Dalian, China).

Chromatographic conditions

The mobile phase was a solution of methanol-phosphate buffer-tetrahydrofuran (45:52.5:2.5). The buffer was prepared using 50 mM potassium dihydrogen phosphate, and was adjusted to pH 4.0 employing phosphoric acid. Before use, the mobile phase was degassed ultrasonically. The flow rate was 1 ml·min⁻¹. The injection volume was 50 μ l. Chromatography was undertaken at room temperature ($\approx$ 15°C).

Extraction procedure

A 5 ml aliquot of diethyl ether was added to 1 ml of plasma sample (or 0.5 ml whole-blood sample diluted with 0.5 ml of water) in a 10 ml ground-glass stoppered glass centrifuge tube. It was vortex-mixed for 3 min, and then centrifuged at 4,000 rpm for 10 min at room temperature. The supernatant organic phase (4 ml) was transferred to a new glass tube and evaporated to 1 ml at 30°C. Then, 200 μ l of potassium dihydrogen phosphate buffer at pH 4.0 was added to it. After vortex-mixing for 2 min and centrifugation at 4,000 rpm for 10 min at room temperature, 50 μ l of the acidic aqueous phase was injected into the chromatographic system.

Precision and extraction recovery

The chromatogram peak area of vinorelbine was determined for known concentrations of vinorelbine in plasma or blood. Calibration graphs of plasma and blood samples from 1 to 1000 ng·ml⁻¹ (n=3) were prepared in duplicate by spiking plasma or blood with increasing amounts of vinorelbine to determine the concentration of unknown samples.

Intra-assay precision was determined by analyzing (n=5) plasma or blood samples spiked with vinorelbine at 2, 50 and 250 ng·ml⁻¹. Inter-assay precision was tested by analyzing samples of the three concentrations on five days.

The extraction recovery was determined three times at 2, 50 and 250 ng·ml⁻¹. The peak areas obtained after extraction were compared with peaks resulting from standard solutions at the same concentrations.

RESULTS

Chromatograms

Chromatograms of vinorelbine in different samples are shown in Figure 1. The retention time of vinorelbine was 8.5 min. There was no obvious interference in plasma or blood samples.

Linearity

Calibration graphs were obtained by plotting the peak area of vinorelbine against its concentrations in plasma or

Figure 1. Chromatograms of vinorelbine.

A: blank plasma; B: plasma sample 1 h after administration of 40 mg vinorelbine; C: blank blood; D: blood sample 1 h after administration of 40 mg vinorelbine.

blood. Calibration graphs were set up three times for plasma concentrations of 1, 2, 5, 20, 50, 100, 250 and 1,000 ng·ml⁻¹ and for blood concentrations of 2, 4, 10, 40, 100, 500 and 2,000 ng·ml⁻¹. They were described by the equations of $C = 2.76 \times 10^{-4}A + 0.8025$ ($r=0.9994$) for plasma samples and $C = 5.34 \times 10^{-4}A + 0.4384$ ($r=0.9998$) for blood samples, where C is the concentration of vinorelbine spiked in blank plasma or blood, and A is the peak area of vinorelbine.

Limit of quantification (LOQ) and limit of detection (LOD)

The LOQs were 1 ng·ml⁻¹ in plasma and 2 ng·ml⁻¹ in blood, respectively. The LODs were 0.5 and 1 ng·ml⁻¹ (signal-to-noise ratio (S/N) ≥ 3), respectively.

Precision and accuracy

The results for the accuracy of the determination assay, relative recovery, and extraction recovery are presented in Table 1.

Pharmacokinetic study

The analytical procedure described above was used to determine the concentrations of vinorelbine in plasma and blood samples from 10 Chinese patients (7 males; age 56 ± 9 years) with NSCLC. The chemotherapeutic protocol comprised vinorelbine and cisplatin. Vinorelbine (40 mg) was administered as a 10 min continuous intravenous infusion. Serial blood samples were collected before administration and 1, 6, 18, 24, 48 and 72 h after infusion. Three milliliters of blood was collected in a

Figure 2. Concentration–time curve of vinorelbine (40 mg) in 10 Chinese NSCLC patients after rapid intravenous infusion (n=10).

Table 1. Accuracy and recovery of vinorelbine in human plasma and blood (n=5).

Concentration (ng·ml ⁻¹)	RSD Intra-day (%)	RSD Inter-day (%)	Relative recovery (%) ± SD	Extraction recovery (%) ± SD
Plasma				
250	2.59	4.38	103.82 ± 2.69	77.52 ± 3.39
50	6.53	6.72	98.77 ± 6.45	70.92 ± 4.63
2	7.91	10.79	101.69 ± 8.04	74.41 ± 8.03
Blood				
250	3.21	4.57	100.03 ± 3.21	75.37 ± 2.42
50	6.24	7.21	102.82 ± 6.42	71.28 ± 4.45
2	8.54	9.91	97.11 ± 8.29	91.77 ± 7.84

RSD: relative standard deviation

heparinized glass tube. A total of 0.5 ml was taken for a blood sample; the rest was prepared for a 1 ml plasma sample after centrifugation at 4,000 rpm for 5 min at room temperature.

Figure 2 shows the mean plasma and blood concentration *versus* time profiles of vinorelbine in 10 NSCLC patients. Pharmacokinetic parameters (Table 2) were estimated using 3P87 pharmacokinetic software (Chinese Pharmacological Society, Beijing, China). The pharmacokinetic characteristics of vinorelbine in Chinese NSCLC patients afforded a two-compartment model calculated by using plasma or blood concentration data. The elimination half-life of vinorelbine was 33.70 ± 1.58 h

for plasma and 40.40 ± 21.30 h for blood samples. We used values of the area under the curve (AUC) to calculate the ratio $AUC_{\text{blood}}/AUC_{\text{plasma}}$, which was 1.7. It was comparable with the ratio of blood concentration /plasma concentration in these 10 patients, remaining almost constant (≈ 1.7) during the 72 h of the study.

DISCUSSIONS

The pharmacokinetic information of vinorelbine as first-line chemotherapy in Chinese NSCLC patients is too few (Qian et al., 2011). In this paper, we modified a HPLC

Table 2. Pharmacokinetic parameters obtained from plasma and blood concentrations of 10 Chinese NSCLC patients after rapid intravenous infusion of 40 mg vinorelbine (mean $\pm$ S.D., $n = 10$).

Parameter	Unit	Plasma	Blood
<i>A</i>	ng·ml ⁻¹	855.53 $\pm$ 248.04	1041.95 $\pm$ 299.92
α	h ⁻¹	3.82 $\pm$ 0.2	3.25 $\pm$ 0.15
<i>B</i>	ng·ml ⁻¹	6.24 $\pm$ 1.6	11.89 $\pm$ 7.64
β	h ⁻¹	0.02 $\pm$ 0.00	0.02 $\pm$ 0.01
<i>V_d</i>	L	49.15 $\pm$ 12.86	39.86 $\pm$ 9.09
<i>t</i> _{1/2α}	h	0.18 $\pm$ 0.01	0.21 $\pm$ 0.01
<i>t</i> _{1/2β}	h	33.7 $\pm$ 1.58	40.4 $\pm$ 21.3
<i>K</i> ₁₂	h ⁻¹	2.16 $\pm$ 0.53	2.00 $\pm$ 0.45
<i>K</i> ₂₁	h ⁻¹	0.05 $\pm$ 0.01	0.06 $\pm$ 0.04
<i>K</i> ₁₀	h ⁻¹	1.63 $\pm$ 0.38	1.21 $\pm$ 0.41
<i>AUC</i>	ng·ml ⁻¹ ·h	530.99 $\pm$ 88.56	904.91 $\pm$ 194.97
<i>CLs</i>	L·h ⁻¹	77.25 $\pm$ 15.37	46.14 $\pm$ 12.07

A, coefficient of exponential functions of distribution phase; α , absorption rate constant; *B*, coefficient of exponential functions of elimination phase; β , elimination rate constant; *V_d*, volume of distribution; *t*_{1/2 α} , half-life of absorption; *t*_{1/2 β} , half-life of elimination; *K*₁₂, rate constant from central-compartment to peripheral-compartment; *K*₂₁, rate constant from peripheral-compartment to central-compartment; *K*₁₀, elimination rate constant from central-compartment; *AUC*, area under the concentration–time curve; *CLs*, clearance.

method (Gauvin et al., 2000; Robieux et al., 1996) by using a less expensive mobile phase (comprising mainly methanol and phosphate buffer) and sensitive fluorescence detection to determine the vinorelbine concentration in both plasma and blood. Fluorescence detection is certainly more routinely usable detection mode for drugs monitoring. An *in-vitro* study (Urien et al., 1993) reported that 84% of vinorelbine is bound to blood cells, mainly to platelets (78%). Hence, we also determined the blood concentration in patients to compare with the literature (Gauvin et al., 2000). It was reported that vinorelbine concentration in blood may remain constant, but that in plasma would vary depending on differences in the centrifuge conditions (Puozzo et al., 2007). So determination of the concentrations in both blood and plasma meanwhile is more significant.

The present method was relatively easy and feasible to perform and allowed to determine vinorelbine in blood and plasma at the nanogram level. Also, we employed two steps of careful vortex-mixing of overall duration 5 min instead of two steps of speed agitation for 60 min as commonly adopted procedure (Gauvin et al., 2000). Thus the whole procedure of sample extraction could save 55min, which made the determination more rapid. Although we did not use an internal standard to simplify the pretreatment procedure, the results showed relatively sensitivity and accuracy and were successfully applied to pharmacokinetic studies.

The pharmacokinetic profile of vinorelbine is often described as a 3-compartment model characterised by a long terminal half-life (*t*_{1/2}) that varies between 20 and 40

h, a large apparent volume of distribution (*V_d*) of around 70 L/kg and a high plasma clearance (*CL*) between 72.54 and 89.46 L/h when determined by HPLC method (Wargin and Lucas, 1994; Levêque and Jehl, 1996).

The disposition of the alkaloid is not altered by concurrent co-administration of cisplatin (Wargin and Lucas, 1994; Levêque and Jehl, 1996; Delord et al., 2009). However, Gauvin et al. (2000) reported the pharmacokinetic profiles exhibiting a three-compartment model with a mean elimination half-life of 42 h. The calculated pharmacokinetic parameters (Table 2) in our study were consistent with these previous study (Wargin and Lucas, 1994; Levêque and Jehl, 1996), with the mean elimination half-life was 33.70 $\pm$ 1.58 h for plasma and 40.40 $\pm$ 21.30 h for blood, in spite of using a simple HPLC method.

Conclusion

The pharmacokinetic characteristics of vinorelbine in Chinese NSCLC patients were similar to those of non-Chinese patients and non-NSCLC patients described in the literature. Based on our and previous findings, dose modifications of vinorelbine of first use in Chinese patients may need not be under consideration generally. Our study may provide the reference for the vinorelbine individual administration and bring more clinical safety and efficiency to Chinese patients. However, more and further study may need to confirm our results.

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