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Pharmacokinetics of 4-[4″-(2″,2″,6″,6″-tetramethyl-1″-piperidinyloxy)amino]-4′-demethylepipodophyllotoxin in mice bearing sarcoma 180

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AIM: To study the pharmacokinetics of 4-[4″-(2″,2″,6″,6″-tetramethyl-1″-piperindyloxy) amino]-4′-demethylepipodophyllotoxin (GP-7) in mice bearing sarcoma 180. **METHOD**: Using HPLC with a uv detector at 285 nm. **RESULTS**: The plasma concentration-time course of GP-7 in mice was best fitted to a 2-compartment open modle after iv 20, 60 mg·kg^{-1}. At both doses the plasma $T_{\frac{1}{2}\beta}$ was around 40 min. The highest concentration was found in liver and lung. The level of GP-7 was higher in tumor than in kidney, spleen, and bone marrow after ip 20 mg·kg^{-1} for 10 d. Urinary excretion of GP-7 as unchanged drug accounted for about 20 % of the administered doses 72 h after injection. **CONCLUSION**: GP-7 disappeared more slowly from the plasma of mice bearing sarcoma 180, distributed extensively over the tissues and was partially excreted from urine. The concentrition of GP-7 in tumor was higher.

KEY WORDS podophyllotoxin; pharmacokinetics; sarcoma 180

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4-[4″-(2″, 2″, 6″, 6″-Tetramethyl-1″-piperidinyloxy) amino]-4′-demethylepipodophyllotoxin (GP-7) is a new spin-labeled podophyllotoxin derivative synthesized by Lanzhou University, China[1]. In our previous

works[2-4], it was found that GP-7 inhibited the growth of transplanted mouse tumors, including sarcoma 180 (S-180), solid carcinoma from ascitic hepatoma, leukemia P388, and Lewis lung carcinoma. *In vitro* it inhibited mouse leukemia L-1210, L-7712, human gastric adenocarcinoma SGC-7901, and human bladder transitional cell carcinoma BU-87 cells. GP-7 was also an immunosuppressive agent[5]. This study was to investigate the pharmacokinetics of GP-7 in mice bearing S-180.

MATERIALS AND METHODS

Mice and tumor Sixty-four kunming mice (either sex, weighing 20 ± *s* 2 g) were bred in the Animal Center of Lanzhou Institute of Biological Products. The mice received a subcutaneous implantation of 2×10^5 viable cells of S-180 (Gansu Institute of New Medicine) to the axillary region. Ten days after sc implantation, the S-180 cells had grown up to a solid tumor about 1.5 g in weight. Then the mice were injected iv with GP-7.

Drugs and instrument GP-7 and podophyllic acid piperinyl hydrazone nitroxide radical (GP-1) used as an internal standard were synthesized by the Department of Chemistry, Lanzhou University. GP-7 was dissolved in Tween-80, Demasorb, and water (1:2:7, vol:vol) for iv and ip to mice. MeOH was of AR grade. Water was redistillated before use. The HPLC instrument consisted of Shimadzu LC-6A HPLC system equipped with a Shimadzu LC-6A pump a Shimadzu SPD-AV multiwavelength uv detector, and a Chromatopac C-R3A data processor.

Plasma, tissues, and urine sampling Mice randomized into 2 groups were injected iv GP-7 20 and 60 $mg\cdot kg^{-1}$. At 1, 5, 15, 30, 60, 120, 240 min after iv, 4 mice at each time were killed. Blood samples were collected into heparinized tubes. Liver, lung, spleen, kidney, and tumor were excised (0.2 g—0.5 g). The heparinized blood samples of 4 mice per time point were mixed. Plasma was immediately obtained by centrifugalization (600 × *g* for 5 min), and tissue homogenate was prepared. Twenty-four hours after sc implantation of S-180 cells, 10 mice were injected ip GP-7 20 $mg\cdot kg^{-1}\cdot d^{-1}$ for 10 d. Twelve hours after the last ip, the mice were killed and tissues homogenates were prepared. Twenty mice randomized into 2 groups were injected iv GP-7 20 or 60 mg $\cdot kg^{-1}$. The urine was collected during 0—12, 12—24, 24—48, and 48—72 h after iv. Specimens were kept at −20 ℃ until analysis.

Drug assay An amount of 30 μL of 100 $mg\cdot L^{-1}$ GP-1 solution was added into a test-tube with 0.5 mL heparinized plasma or tissue homogenate as internal standard. Chloroform (6 mL) was used for extraction. The test-tubes were mechanically shaken for 20 min, centrifuged at 600 × *g* for 5 min, then the organic phase was transferred to a second test-tube. After drying the organic phase under nitrogen stream, the residue was redissolved in 100 μL of mobile phase of MeOH-water (65:35, vol:vol). An amount of 10 μL of this solution was injected into the chromatograph.

The separation was performed on a Zorbax-ODS column (5 μm; 4.6 mm × 150 mm) with the mobile phase. The flow-rate was 1.0 $mL\cdot min^{-1}$, and detection was made at 285 nm. The retention times of GP-7 and GP-1 were 11.3 and 4.9 min, respectively. The regression was liner ($r > 0.99$) within 0.2—50 mg $\cdot L^{-1}$ in plasma and urine, and 0.5—50 $\mu g\cdot g^{-1}$ in tissues. The recoveries were 81.8 ± 6.1 % in plasma, 84.2 ± 5.6 % in urine, and 80.4 ± 7.9 % in tissues. The intra day and day-to-day precision (*CV*) were 3.1 % and 5.4 %, respectively. There was no interference with etoposide, podophyllotoxin, and 4′-demethyl-epipodophyllotoxin. The minimal detectable concentrations of GP-7 were 0.2 $mg\cdot L^{-1}$ in plasma, 0.1 $mg\cdot L^{-1}$ in urine, and 0.5 $mg\cdot L^{-1}$ in tissues.

Pharmacokinetic analysis Pharmacokinetic parameters in plasma were treated using the MCPKP program[6] in an IBM-PC computer.

RESULTS

Pharmacokinetics of GP-7 The concentration-time course of iv GP-7 in mice bearing S-180 was best fitted to a 2-compartment open model (Fig 1).

At both doses GP-7 disappeared biphasically with a rapid distribution half-life of about 1.6 min and an elimination half-life around 40 min (Tab 1).

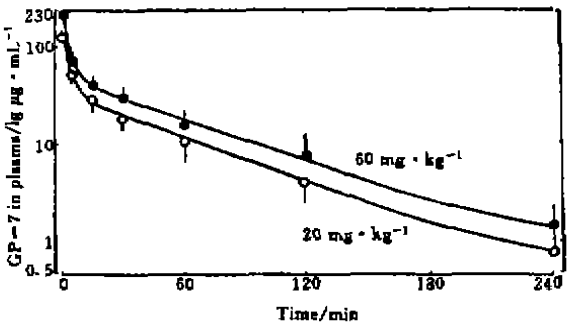

Fig 1. Concentration of GP-7 in plasma of mice bearing sarcoma 180 after iv 20 or 60 mg·kg^{-1}. $n=3$, $\bar{x}\pm s$.

Tab 1. Plasma pharmacokinetic parameters of GP-7 in mice bearing sarcoma 180 after iv 20 or 60 mg·kg^{-1}. $n=3$, $\bar{x}\pm s$.

Parameters	20 mg·kg^{-1}	60 mg·kg^{-1}
α, min^{-1}	0.40±0.08	0.46±0.11
β, min^{-1}	0.018±0.001	0.016±0.002
$T_{\frac{1}{2}\alpha}$, min	1.72±0.24	1.51±0.26
$T_{\frac{1}{2}\beta}$, min	38.9±3.1	44.0±4.6
K_{12}, min^{-1}	0.25±0.05	0.30±0.04
K_{21}, min^{-1}	0.093±0.018	0.084±0.010
K_{10}, min^{-1}	0.077±0.009	0.085±0.006
V_1, L·kg^{-1}	0.113±0.014	0.192±0.021
V, L·kg^{-1}	0.49±0.08	1.04±0.12
Cl, mL·kg^{-1}·min^{-1}	8.8±1.0	16.4±1.3
AUC, mg·L^{-1}·min^{-1}	0.229±0.010	0.365±0.028

Concentration of GP-7 in tissues After iv 60 mg·kg^{-1}, the concentration of GP-7 in liver was the highest. The level of GP-7 in tumor was lowest in the first 15 min, but became higher than those in kidney and spleen 2 h after iv (Tab 2).

Distribution of GP-7 after 20 mg·kg^{-1} ip 10 d The tissue concentraions of GP-7 in the mice: liver>lung>stomach>heart>tumor>spleen>small intestine>kidney>bone marrow>muscle (Tab 3).

Tab 3. Concentration of GP-7 in tissues of mice bearing sarcoma 180 12 h after last injection of ip 20 mg·kg^{-1}·d^{-1} for 10 d. $n=10$, $\bar{x}\pm s$.

Tissue	GP-7 μg·g^{-1}
Tumor	2.1±0.9
Liver	11.3±4.7
Kidney	1.4±0.6
Lung	5.8±2.2
Spleen	1.7±0.7
Heart	2.3±0.8
Stomach	3.1±1.4
Small intestine	1.6±0.8
Muscle	1.2±0.7
Bone marrow	1.4±0.6

Urinary excretion of GP-7 After iv 20, 60 mg·kg^{-1}, about 16.6 %—19.5 % of unchanged GP-7 were recovered from urine for the first 24 h and accounted for 1.8 %—2.5 % of unchanged GP-7 for the next two 24 h. Approximately 18.2 %—22.0 % of the doses

Tab 2. Concentration of GP-7 (μg·g^{-1}) in tissues of mice bearing sarcoma 180 after iv 60 mg·kg^{-1}. $n=3$, $\bar{x}\pm s$.

Time/min	Tumor	Liver	Kidney	Lung	Spleen
1	38.1±6.3	127±23	106±19	98.5±9.6	108±15
5	64±11	184±28	168±21	160±18	146±20
15	95±23	218±44	151±28	165±33	107±24
30	88±17	148±38	114±23	82±18	78±23
60	76±24	106±41	88±19	71±28	51±17
120	36±14	87±32	27±13	47±23	21.3±9.6
240	21.6±9.4	74±37	17±11	31±26	7.5±4.1
360	6.8±4.5	51±32	5.3±2.9	14±11	4.6±2.5

were recovered from urine as unchanged GP-7 72 h after iv. GP-7 was largely eliminated within 24 h in mice bearing S-180 (Tab 4).

Tab 4. Urinary excretion of GP-7 ($mg \cdot kg^{-1}$) iv 20 or 60 $mg \cdot kg^{-1}$ in mice bearing sarcoma 180. $n = 3$, $\bar{x} \pm s$.

Time/h	20 $mg \cdot kg^{-1}$	60 $mg \cdot kg^{-1}$
0—12	2.34±0.42	5.88±1.51
12—24	1.56±0.40	4.09±1.24
24—48	0.34±0.10	0.84±0.22
48—72	0.16±0.10	0.24±0.15

DISCUSSION

The uv absorption spectrum of GP-7 showed that there was a higher absorption peak at 285 nm. So the uv absorption at 285 nm was used as a means of detecting GP-7.

At both doses of 20 and 60 $mg \cdot kg^{-1}$, $T_{\frac{1}{2}\beta}$ of GP-7 in the plasma of mice did not show significant differences, suggesting that GP-7 was eliminated following the course of the first-ordor kinetics. Data (Tab 2, 3) showed that Gp-7 distributed over the five tissues tested, had all higher concentrations. Twelve hours after the last injection of ip 20 $mg \cdot kg^{-1} \cdot d^{-1}$ for 10 d, there were fairly high levels of GP-7 in most tissues of mice. These results suggested that GP-7 has an extensive distribution in body of mice bearing S-180, and it is easy to cumulate in tissues also. In this study, increasing of AUC was not in direct proportion to the doses used, this situation may be related to the fact that GP-7 with higher liposolubility had extensive distribute and more cumulate abilities in mice tissues. The higher levels in liver, lung and stomach suggested that GP-7 may be an effective antitumor agent against carcinomas originating from these tissues.

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4-[4″-(2″,2″,6″,6″-四甲基哌啶氮氧自由基)氨基]-4′-去甲表鬼臼毒素在荷肉瘤180小鼠体内的药物动力学

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目的：研究4-[4″-(2″,2″,6″,6″-四甲基哌啶氮氧自由基)氨基]-4′-去甲表鬼臼毒素在荷肉瘤180小鼠体内的药物动力学. **方法**：用HPLC紫外检测法. **结果**：GP-7在小鼠血浆中的浓度—时间过程符合二室开放模型. 20，60 $mg \cdot kg^{-1}$ iv，$T_{\frac{1}{2}\beta}$约40 min；药物浓度以肝脏和肺中最高；肿瘤组织中药物水平高于脾，肾和骨髓. 72 h 内，经尿以原形排出的GP-7占给药量的20 %左右. **结论**：GP-7在荷肉瘤180在小鼠体内分布较广，消除较慢. 肿瘤组织中浓度较高，部分以原形从尿中排出.

关键词 鬼臼毒素；药物动力学；肉瘤180