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Pharmacokinetics of 9-nitro-20(*S*)-camptothecin in rats[1]

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KEY WORDS 9-nitro-20(*S*)-camptothecin; pharmacokinetics; excretion; rats

ABSTRACT

AIM: To study the pharmacokinetics and the excretion of 9-nitro-20(*S*)-camptothecin (9-NC) in rats. **METHODS:** Each rat was given a single dose at random by iv or ig administration. Serial plasma and excreta samples were collected and the pharmacokinetic behavior of 9-NC in rats was characterized by specific liquid chromatographic assays. Individual 9-NC plasma-concentration data were analyzed by both noncompartmental and compartmental analysis. For dose proportionality, AUC- and C_{max}-dose relationships were evaluated by linear regression, and $t_{1/2}$ and CL_{tot} were compared by an analysis of variance model. Also, the excretion of the parent drug was estimated. **RESULTS:** After iv administration of 9-NC at the doses of 1.5, 3, and 6 mg/kg, the $t_{1/2}$ values for 9-NC were estimated to be 0.5, 0.5, and 0.7 h, respectively, and the mean AUC_{0-t} values were 633, 1606, and 3011 h·μg·L^{-1}, respectively. 9-NC was rapidly absorbed, reaching mean C_{max} of 203, 417, and 1150 μg/L at T_{max} of 0.3, 0.2, and 0.3 h at the doses of 3, 6, and 12 mg/kg, respectively. The mean AUC_{0-t} values were 269, 439, and 881 h·μg·L^{-1}, and the mean $t_{1/2}$ values were 1.7, 0.9, and 0.9 h, respectively. The absolute oral bioavailability of 9-NC was calculated to be 14.6 %, which was consistent with the ratio of the total cumulative excretion in the urine and bile by ig to that by iv injection. **CONCLUSION**: The kinetic process of 9-NC in rats *in vivo* was best fitted to a two-compartmental model. For iv administration, the pharmacokinetics are not dose-dependent. The oral bioavailability of 9-NC was low. Renal excretion was the primary elimination route of the parent drug after iv administration, however, after ig administration the unchanged drug was largely excreted in the feces because of the poor absorption.

INTRODUCTION

20(*S*)-Camptothecin (CPT), a natural alkaloid extracted from the leaves and fruits of *Camptotheca acuminata*, is a poorly water-soluble inhibitor of DNA synthesis. It results in single-strand DNA break and finally in cell death, by reversibly stabilizing the cleavable complex between topoisomerase I and DNA. CPT and its analogs are sensitive to a pH-dependent reversible conversion between a lactone form and a carboxylate form[1,2].

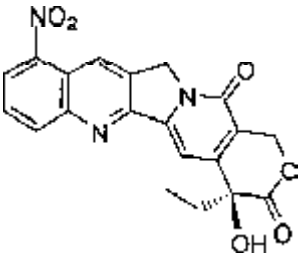

Fig 1. The structure of 9-nitro-20(*S*)-camptothecin.

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During the last years, 9-nitro-20(*S*)-camptothecin (9-NC), an analog of CPT, has been a focus of attention in cancer research and into the stage of early clinical trials[3-5]. 9-NC is water insoluble, and it is partially metabolized *in vivo* into 9-aminocamptothecin[6]. Pharmacological studies have shown that the antitumor activity of 9-NC is superior to the activity of CPT in human tumors xenografted in nude mice[7]. Recently, 9-NC liposome aerosol was developed and applied into the preclinical and clinical studies[8-10]. Knight V *et al*[11] have demonstrated the effectiveness of this formulation in the treatment of the human cancer xenografs in nude mice at doses much smaller than those traditionally used in mice administered by other routes. Pharmacokinetics of 9-nitrocamptothecin in dogs and mice have been reported, and the results of the studies on the conversion of 9-nitrocamptothecin to 9-aminocamptothecin in different species were described[6]. Lately, the modified lactone/carboxylate salt equilibrium *in vivo* by liposomal delivery of 9-NC was studied with rats[12].

In the present investigation, the pharmacokinetic behavior of 9-NC material in rats was characterized by specific HPLC assays, which permitted determination of the sum of the concentrations of the lactone and carboxylate forms. Following iv and ig administration of 9-NC to rats, the oral bioavailability and the dose proportionality were evaluated. Also, the excretion of the parent drug in the urine, feces, and bile was estimated after treatments with 9-NC.

MATERIALS AND METHODS

Chemicals 9-nitro-20(*S*)-camptothecin and 20 (*S*)-camptothecin were provided by Shenlong Biotechnology Co, Ltd (Shanghai, China). The purity of these compounds was 99.6 % and 98.2 %, respectively, which was verified by the supplier using HPLC methods. All other chemicals were purchased from commercial sources and used as received.

Drug disposition Wistar rats (250 g±20 g, Grade II, Certificate No 042) were purchased from the Animal Center of Shenyang Pharmaceutical University (Shenyang, China). Thirty-six rats were used in the experiment of determining plasma concentrations of 9-NC after iv and ig administration. All rats were divided into 6 groups at random, and each rat was given a single 1.5-, 3-, or 6-mg/kg dose of 9-NC by the tail vein injection, or 3-, 6-, or 12-mg/kg doses by ig route. The dose of 3 mg/kg administered to rats were decided according to the dose conversion factors between rats and dogs[6]. Rats were used without fasting before ig administration. The solutions of 9-NC were formulated shortly before administration such that the desired dosages were delivered in a volume of 2 mL. The dimethyl sulfoxide solution of 9-NC was diluted at 1:19 (v/v) with polyethylene glycol 400-sterile water (1:1, v/v) for injection containing 0.01 mol/L phosphoric acid. With an apparent pH of 3.0-3.5, the resulting solution was sufficient acidic to prevent the lactone ring from opening prior to administration.

Blood samples (0.5 mL) were collected into heparinized tubes from each rat by puncture of the retroorbital sinus. This was performed at 0 min (predose), and 10, 20, 30, and 45 min and 1, 1.5, 2, 3, 5, and 8 h after ig administration. With the 2-min iv infusion, the sampling was performed at 0 min (predose), and 10, 20, 30, and 45 min and 1, 1.5, 2, 3, 5, and 7 h after the end of infusion. Blood was immediately processed for plasma by centrifugation at 3000×*g* for 10 min. Plasma samples were frozen and maintained at –20 °C until analysis.

Drug levels in plasma specimens were determined by a validated HPLC method. Concentrations measured were the sum of lactone and hydroxy acid forms. Frozen plasma was thawed in a waterbath and was homogenized by vortex mixing. Next, to 0.2 mL of rat plasma a volume of 100 μL of CPT 400 μg/L in methanol and 100 μL of methanol was added. The phosphoric acid buffer (pH 2.0) of 0.2 mL were added into the sample, which acidified the sample to pH ~3 before the extraction by 3.0 mL of hexane-methylene chloride-isopropanol (20:10:1, v:v:v). Therefore, by effecting the rapid lactonization of drug present in the carboxylate form, the total drug concentrations of the lactone and carboxylate forms were determined in each sample. The sample was vortex-mixed for 30 s and shaken for 10 min. Subsequently, the sample was centrifuged at 3000×*g* for 10 min. The supernatant was collected in a glass tube and evaporated at 40 °C under a gentle stream of air, until a residue was left over. The residue was reconstituted in 100 μL mobile phase, and a volume of 50 μL was injected into the HPLC system. Chromatographic analyses were preformed using a HPLC system consisting of a Shimadzu LC-10AT pump, a SPD-10A UV detector, and a C-R6A integrator (Kyoto, Japan). The analytical column used was packed with Hypersil BDS C_{18} material (150 mm×4.6 mm ID, 5 μm) from Elite (Dalian, China). The column temperature

was maintained at the room temperature. The UV detector was set at 370 nm, which yielded the optimum signal-to-noise ratio for 9-NC. The mobile phase consisted of a mixture of acetonitrile-water-formic acid (35: 65:2, v:v:v), and was delivered at a flow rate of 1.0 mL/min. The mobile phase was degassed before use, and was freshly prepared for each run.

Calibration curves were generated by plotting the 9-NC/CPT peak-height ratios as the ordinate and plasma concentrations as the abscissa. The data were fitted using a $1/x$ weighted least squares linear regression. The linear range of concentrations for 9-NC in plasma were 25–1600 μg/L. The lower limit of quantitation (LLOQ) was 25 μg/L. The correlation coefficient (r) for the typical curve was 0.9988, with a slope of 0.00173, and y-intercept of 0.0162. Data of the analytical method in terms of accuracy (percent deviation) and precision for quality control (QC) samples were shown in Tab 1. The accuracy for 9-NC showed values ranging within ±3 %. The intra- and inter-assay variability assessed by one-way ANOVA varied up to ±10.2 %.

Tab 1. Accuracy and precision of the HPLC method to determine 9-NC in rat plasma.

Concentration/μg·L^{-1}		RSD/%		
Added	Found	Within-day	Between-day	RE/%
25.0	25.0	5.2	8.3	0.1
200.0	193.9	10.2	2.7	–3.0
1600.0	1614.7	7.9	4.4	0.9

Pharmacokinetic analysis Individual 9-NC plasma-concentration data were analyzed by both noncompartmental and compartmental analysis using the TopFit software package (Thomae GmbH, Germany). The area under the curve (AUC_{0-t}) for 9-NC total was calculated by the linear-trapezoidal rule up to the last sampling point with detectable levels (C), with extrapolation to infinity ($AUC_{0-\infty}$) by the equation $AUC_{0-t}+C/k_e$, where k_e represents the terminal disposition rate constant. The latter term was calculated from the slope of data points in the final log-linear part of the drug concentration-time curve by weighted (y^{-1}) least-squares linear regression analysis. The terminal disposition half-life ($t_{1/2}$) value was calculated using the equation: $t_{1/2}=0.693/k_e$. The total body clearance (CL_{tot}) was calculated as dose/$AUC_{0-\infty}$. The apparent volume of distribution (V_d) was calculated as CL_{tot}/k_e. Maximum plasma concentration (C_{max}) and the time to maximum concentration (T_{max}) following ig administration were estimated by visual inspection of the semilogarithmic plot of the concentration-time curve. The absolute oral bioavailability (F) expressed as follows: $F=(AUC_{0-t,ig}/AUC_{0-t,iv})\times 100$ %, and was calculated with AUC mean values by ig and iv, both at the dose of 6 mg/kg. To evaluate dose proportionality, the AUC- and C_{max}-relationships were analyzed by linear regression. Also, $t_{1/2}$, CL_{tot}, and AUC were compared by an analysis of variance (ANOVA) model.

Excretion studies The urinary and fecal excretion of 9-NC was evaluated by treating two groups of five rats with 9-NC 6 mg/kg, iv or ig respectively in the same manner as the disposition study. The animals were housed in stainless steel metabolic cages and given food and water *ad libitum* during the course of the experiment. Pooled urine and feces from each rat was collected at 2- to 12-h intervals for 72-96 h following drug administration, and the feces were dried at the room temperature for 24 h.

Experiments on biliary excretion of 9-NC were performed in another two groups of five rats. The rats were implanted with a PE-10 cannula into the bile duct under anesthesia by ethyl ether, and then allowed to recover for 2 h before drug administration. The drug was administered in the same fashion as in the experiments on urinary and fecal excretion. Bile samples were collected at 2- to 12-h intervals for 24-36 h after drug administration.

The specimens were stored at -20 °C until analysis after measuring the urine (or bile) volume and feces dry weight for each collection period. Blank excreta from untreated rats were similarly obtained during a 12-h period.

The concentration of unchanged compound in urine, bile, and fece specimens was determined by a HPLC method using a gradient elution program. The urine or bile samples (0.1 mL) were diluted with 0.2 mL acetonitrile-water (1:1, v/v), followed the analysis by HPLC. Fecal specimens (0.3 g) were initially homogenized in 1 mL acetonitrile-water (1:1, v/v) containing 10 mg/L of CPT. Subsequently, the suspension was centrifuged at 3000×g for 10 min after ultrasonic vibration for 10 min. The supernatant was collected and filtered by the filter membrane (0.45 μm) before

analysis. The analyses of samples were performed using an HPLC HP series 1100 fitted with a G1314A UV-detector, a G1316A column oven (set at 25 °C), a G1313A autosampler, a vacuum degasser unit, and a G1311 quaternary pump (Hewlett Packard, USA). The mobile phase consisted of a gradient mixed from acetonitrile and water containing 2 % formic acid (pH 2). The column was equilibrated with 10 % acetonitrile at time 0, after injection of the urine and feces sample (50 μL) the acetonitrile content was linearly increased to 30 % at 31 min, then decreased within 2 min to 10 % to equilibrate the column for 3 min before application of the next sample. For the analyses of the bile sample, the percentage of acetonitrile (10 %) was linearly increased to 35 % in 5 min, maintained for 5 min, then decreased within 2 min to 10 % to equilibrate the column. The mobile phase of pH 2 was chosen to prevent the lactone form from hydrolyzing to an open carboxylate form. The linear range of concentrations for 9-NC in bile, urine, and feces was 0.5-25.0 mg/L, 0.2-10.0 mg/L, and 0.33-16.67 μg/g, respectively. Validations of bile 9-NC determination are listed in Tab 2. Determinations of 9-NC in urine and feces were within the same range of variation.

Tab 2. Accuracy and precision of the HPLC method to determine 9-NC in rat bile.

Concentration/mg·L^{-1}		RSD/%		
Added	Found	Within-day	Between-day	RE/%
1.0	0.98	5.9	2.5	–2.4
5.0	5.1	2.5	4.9	0.2
25.0	25.7	1.6	7.1	3.0

RESULTS

Plasma pharmacokinetics The typical chromatograms of 9-nitrocamptothecin in rat plasma are illustrated in Fig 2. The mean plasma concentration-time profiles of 9-NC after iv and ig administration are shown in Fig 3. Plasma concentration versus time data were analyzed by noncompartmental methods, and the pharmacokinetic parameters of three representative doses by iv and ig are shown in Tab 3. Model discrimination was assessed by the analysis of the data, and actually most concentration-time profiles were best fitted to a two-compartment model.

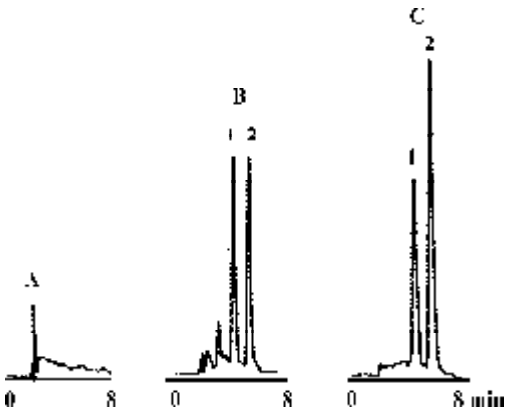

Fig 2. Chromatograms of 9-nitrocamptothecin and camptothecin (internal standard, IS) in rat plasma. A. Blank plasma sample; B. Plasma sample spiked with 9-nitrocamptothecin 200 mg/L and IS; C. Plasma sample 1 h after iv administration of 9-nitrocamptothecin 3 mg/kg to a rat. Peak 1, 2 refer to IS and 9-nitrocamptothecin, respectively.

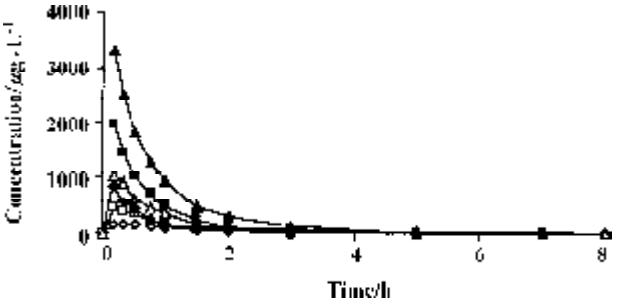

Fig 3. Profiles of mean plasma concentration of 9-NC versus time after ig or iv administration of 9-NC to rats (◇ ig 3 mg/kg; □ ig 6 mg/kg; △ ig 12 mg/kg; ◆ iv 1.5 mg/kg; ■ iv 3 mg/kg; ▲ iv 6 mg/kg.

After iv administration of 9-NC at doses of 1.5, 3, and 6 mg/kg, the $t_{1/2}$ values for 9-NC were estimated to

Tab 3. Pharmacokinetic parameters of 9-NC after iv administration to rats. *n*=6. Mean±SD.

Parameter	Dose/mg·kg^{-1}		
	1.5	3	6
$t_{1/2}$/h	0.5±0.1	0.5±0.1	0.7±0.4
k_e/h^{-1}	1.3±0.2	1.5±0.4	1.2±0.6
AUC_{0-t}/h·μg·L^{-1}	633±134	1606±510	3011±823
$AUC_{0-\infty}$/h·μg·L^{-1}	671±151	1652±525	3225±1146
MRT/h	0.6±0.1	0.6±0.2	0.9±0.5
CL_{tot}/mL·min^{-1}·kg^{-1}	39±9	32±9	34±13
V_d/L·kg^{-1}	1.4±0.3	1.2±0.2	1.6±0.4

be 0.5, 0.5, and 0.7 h, respectively, and the k_e values were 1.3, 1.5, and 1.2 h^{-1}, respectively. The AUC increased with increasing doses for iv administration, and the mean AUC_{0-t} values were 633, 1606, and 3011 $h \cdot \mu g \cdot L^{-1}$, respectively. CL_{tot} was relatively constant from 32 to 39 $mL \cdot min^{-1} \cdot kg^{-1}$.

The analysis of variance of the $t_{1/2}$ and CL_{tot} showed no differences among the three doses of treatments after iv administration ($P>0.05$). These data indicated that the pharmacokinetic parameters did not vary with dose. Additional proof of the linearity of the kinetics of 9-NC after iv administration was found in the regression analysis of the AUC-dose plot, and these plots indicated good linearity ($r>0.85$, $P<0.01$).

9-NC was rapidly absorbed after ig administration, reaching mean C_{max} of 203, 417, and 1150 μg/L at T_{max} of 0.3, 0.2, and 0.3 h for the 3-, 6-, and 12-mg/kg doses, respectively. The mean AUC_{0-t} values were 269, 439, and 881 $h \cdot \mu g \cdot L^{-1}$. The mean $t_{1/2}$ values were estimated to be 1.7, 0.9, and 0.9 h, and k_e were 0.7, 0.8, and 0.9 h^{-1} at the dose of 3, 6, and 12 mg/kg, respectively (Tab 4). For ig administration, the C_{max}-dose relationship was linear ($r=0.65$, $P<0.01$), but the AUC-dose relationship was nonlinear. The absolute oral bioavailability of 9-NC after ig administration at the dose of 6 mg/kg was calculated to be 14.6 %.

Tab 4. Pharmacokinetic parameters of 9-NC after ig administration to rats. $n=6$. Mean±SD.

Parameter	Dose/mg·kg^{-1}		
	3	6	12
$t_{1/2}$/h	1.7±0.9	0.9±0.3	0.9±0.5
k_e/h^{-1}	0.7±0.6	0.8±0.2	0.9±0.3
T_{max}/h	0.33±0.15	0.22±0.08	0.25±0.09
C_{max}/$\mu g \cdot L^{-1}$	203±72	417±370	1150±775
AUC_{0-t}/$h \cdot \mu g \cdot L^{-1}$	269±137	439±508	881±637
$AUC_{0-\infty}$/$h \cdot \mu g \cdot L^{-1}$	404±224	478±518	943±638
MRT/h	2.9±1.6	1.3±0.4	1.2±0.6
CL_{tot}/$mL \cdot min^{-1} \cdot kg^{-1}$	187±145	359±207	356±289
V_d/$L \cdot kg^{-1}$	22±10	28±20	30±38

Drug excretion The typical chromatograms of 9-nitrocamptothecin in rat urine, feces, and bile were shown in Fig 4. The cumulative excretion of unchanged total drug in rats after treatment with 9-NC is illustrated in Fig 5. After iv administration of 9-NC for 84 h, 10.6 % of the dose was found in the urine, which greatly exceeded the amount present in feces (Tab 5). However, after ig administration urinary excretion was a minor pathway, with only 2.1 % of the dose recovered for 96 h after treatment. During this time, 15.8 % of the dose was detected in the feces, primarily in the first 48 h (15.2 %).

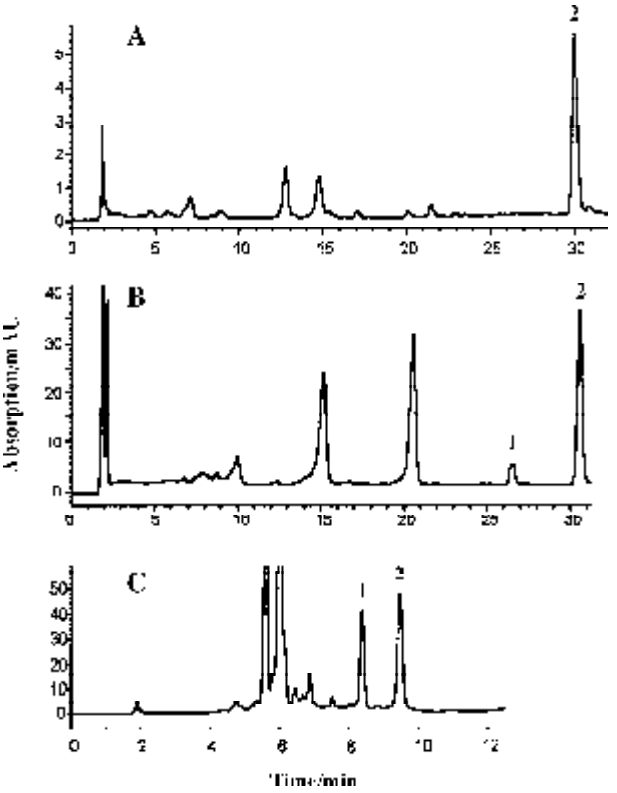

Fig 4. Chromatograms of 9-nitrocamptothecin (3 mg/kg, iv) and camptothecin (internal standard, IS) in rat urine, feces, and bile. A: Urine sample 0-4 h after iv administration; B: Feces sample 24-36 h after iv; C: Bile sample 0-2 h after iv administration. Peak 1, 2 refer to IS and 9-nitrocamptothecin, respectively.

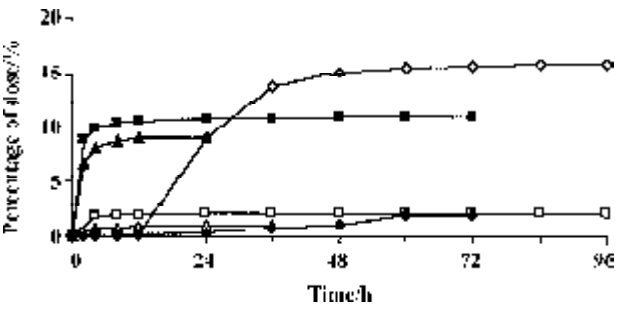

Fig 5. Mean plots of cumulative excretion of 9-NC into urine, feces, and bile after ig or iv administration to rats (□ Urine after ig; ◇ Feces after ig; △ Bile after ig; ■ Urine after iv; ◆ Feces after iv; ▲ Bile after iv).

Tab 5. Excretion of 9-NC into urine, feces, and bile after iv or ig administration to rats. *n*=5. Mean±SD.

Route of administration	Percent of dose excreted/%		
	Urine	Feces	Bile
iv	10.6 ± 1.7	1.9 ± 0.6	9.1 ± 3.4
ig	2.1 ± 0.7	15.8 ± 7.8	1.0 ± 0.5

The biliary excretion of 9-NC was 9.1 % and 1.0 %, respectively, by the iv injection and the ig administration. 9-NC excretion in the bile after iv administration was higher than that observed in the feces. It may suggest the existence of an enterohepatic cycle. The ratio of the total cumulative excretion in the urine and bile by ig to that by iv was 15.7 %, which is consistent with the value of the bioavaliability investigated in the drug disposition studies, suggesting a poor absorption of 9-NC in rats after ig administration.

DISCUSSION

For iv administration, ANOVA showed no difference in the pharmacokinetic parameter of $t_{1/2}$ and CL_{tot} among the doses. This fact, in addition to the linear plots of AUC-dose, indicates that the pharmacokinetics of 9-NC is not dose-dependent. For the dose range studied, 9-NC plasma concentrations are clearly dose-proportional. After ig administration, the linearity between C_{max} and doses was good, however, the AUC-dose relationship was nonlinear. 9-NC is poorly absorbed in rats, which is consistent with the low cumulative excretion of the drug into the bile and the urine after ig administration.

After treatment with 9-NC by iv, the renal excretion of the parent drug was higher than that excreted in the feces, however, the renal climination was a minor pathway after ig administration, which was lower than that after iv administration. It was considered that the drug was badly absorbed so that a part of the drug was directly excreted into the feces.

When comparing the pharmacokinetic parameters of dogs, mice, and rats, it is obvious that their absorptions of 9-NC are different. In dogs after a single oral dose of 1.0 mg/kg 9-NC, the C_{max} was 19.1 μg/L at 0.7 h, and the C_{max} of 9-NC in the mouse after a single oral dose of 4.1 mg/kg of 9-NC was 732 μg/L at time 0.1 h[6]. However, after a single oral dose of 3 mg/kg to rats, the mean C_{max} of 203 μg/L was reached at T_{max} of 0.3 h. Comparing the values of T_{max} and C_{max}, it is estimated that: (a) 9-NC is rapidly absorbed after ig administration; (b) the absorption of 9-NC in mice is higher than that in rats, and the absorption of 9-NC in dogs is least.

In summary, these studies have shown the linear pharmacokinetics of 9-NC after iv administration to rats and the low bioavailability of 9-NC after ig administration. The results of excretion studies showed that renal excretion was the primary elimination route of the parent drug after iv administration. However, after ig administration the unchanged drug was largely excreted in the feces because of the poor absorption of 9-NC in rats. Obviously, the low bioavialability would limit the application of the oral preparation of 9-NC in the clinical therapy. Therefore, it is essential to select an appropriate matrix to improve the absorption of 9-NC.

REFERENCES

1 Underbreg WJM, Goosen RMJ, Smith BR, Beijnen JH. Equilibrium kinetics of the new experimental antitumour compound SK&F 104684-A in aqueous solution. J Pharmacol Biomed Anal 1990; 8: 681-3.

2 Staubus AE, Rutherford M, Snuffer P, Feller D. Kinetics of ring opening of camptothecin analogs and topotecan in plasma and whole blood. Proc Am Assoc Cancer Res 1992; 33:531.

3 Verschraegen CF, Natelson EA, Giovanella BC, Kavanagh JJ, Kudelka AP, Freedman RS, *et al.* A phase I clinical and pharmacological study of oral 9-nitrocamptothecin, a novel water-insoluble topoisomerase I inhibitor. Anticancer Drugs 1998; 9: 36-44.

4 Natelson EA, Giovanella BC, Verschraegen CF, Fehir KM, De Ipolyi PD, Harris N, *et al.* Phase I clinical and pharmacological studies of 20-(*S*)-camptothecin and 20-(*S*)-9-nitrocamptothecin as anticancer agent. Ann NY Acad Sci 1996; 803: 224-30.

5 Verschraegen CF, Gupta E, Loyer E, Kavanagh JJ, Kudelka AP, Freedman RS, *et al.* A phase II clinical and pharmacological study of oral 9-nitrocamptothecin in patients with refractory epithelial ovarian, tubal or peritoneal cancer. Anticancer Drugs 1999; 10: 375-83.

6 Hinz HR, Harris NJ, Natelson EA, Giovanella BC. Pharmacokinetics of the *in vivo* and *in vitro* conversion of 9-nitro-20(*S*)-camptothecin to 9-amino-20(*S*)-camptothecin in humans, dogs, and mice. Cancer Res 1994; 54: 3096-100.

7 Giovanella BC, Hinz HR, Kozielski AJ, Stehlin JS, SilberR, Potmesil M. Complete growth inhibition of human cancer xenografts in nude mice by treatment with 20(*S*)-camptothecin. Cancer Res 1991; 51: 3052-5.

8 Verschraegen CF, Gilbert BE, Huaringa AJ, Newman R, Harris N, Leyva FJ, *et al.* Feasibility, phase I, and pharmacological study of aerosolized liposomal 9-nitro-20(*S*)-camptothecin in patients with advanced malignancies in the lungs. Ann

N Y Acad Sci 2000; 922: 352-4.

9 Koshkina NV, Gilbert BE, Waldrep JC, Seryshev A, Knight V. Distribution of camptothecin after delivery as a liposome aerosol or following intramuscular injection in mice. Cancer Chemother Pharmacol 1999; 44: 187-92.

10 Knight V, Koshkina NV, Waldrep JC, Giovanella BC, Kleinerman E, Gilbert BE. Anticancer activity of 9-nitrocamptothecin liposome aerosol in mice. Trans Am Clin Climatol Assoc 2000; 111: 135-45.

11 Knight V, Koshkina NV, Waldrep JC, Giovanella BC, Gilbert BE. Anticancer effect of 9-nitrocamptothecin liposome aerosol on human cancer xenografts in nude mice. Cancer Chemother Pharmacol 1999; 44: 177-86.

12 Chow DS, Gong L, Wolfe MD, Giovanella BC. Modified lactone/carboxylate salt equilibria *in vivo* by liposomal delivery of 9-nitro-camptothecin. Ann NY Acad Sci 2000; 922: 164-74.