Pharmacokinetics, Mass Balance, and Tissue Distribution of a Novel DNA Alkylating Agent, VNP40101M, in Rats

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ABSTRACT VNP40101M (1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)-2-[(2-methylamino)carbonyl] hydrazine), a novel DNA alkylating agent, is currently under clinical development for the treatment of cancer in Phase I clinical trials. This study investigated the pharmacokinetics, mass balance, and tissue distribution of [¹⁴C]-VNP40101M in rats following a single intravenous dose of 10 mg/kg. After 7 days, the total recovery of radioactivity was 85% for males and 79% for females. Most of the radioactivity was eliminated within 48 hours through urine (70%), with less excreted in feces (6%). Tissue contained relatively high radioactive residues with the highest concentrations in kidneys, liver, lung, and spleen. After 7 days, tissue still contained 9% of the dose. At both 5 minutes and 1 hour post-dose, brain contained relatively high radioactivity (5.9 and 3.3 µg equivalence/g and 50% and 30% of the blood concentration, respectively), suggesting that VNP40101M penetrated the blood-brain barrier. The elimination half-life of VNP40101M was approximately 20 minutes, the peak plasma concentration (C_{max}) averaged 11.3 µg/mL, the volume of distribution (V_{ss}) averaged 0.91 L/kg, and the total body clearance (Cl) averaged 33.5 mL/min/kg. The metabolite profile in urine was complex, indicating VNP40101M was extensively metabolized. There were no apparent sex differences in pharmacokinetic parameters of VNP40101M in the rat.

KEYWORDS: VNP40101M, Mass balance, Pharmacokinetics, Tissue distribution.

METHODS

Materials

[¹⁴C]-VNP40101M (**Figure 1**) with a radiopurity of 99.9% and specific activity of 55 mCi/mmol was purchased from Moravek Biochemicals (Brea, CA). Non-radiolabeled VNP40101M with a chemical purity of 99.1% was manufactured at Vion Pharmaceuticals Inc

(New Haven, CT) and used as a reference standard. A solution formulation of VNP40101M used to prepare the dose solution contained the following components: 100 mg VNP40101M, 7 mL PEG-300, 3 mL ethyl alcohol 200 proof, dehydrated. ¹¹ Blank vehicle was used to dose the control animals. Acetonitrile, formic acid, isopropyl alcohol (IPA), and methanol were obtained from EM Science (Gibbstown, NJ). Absolute alcohol was obtained from Quantum (Morris, IL). Ammonium hydroxide, ammonium phosphate, and citric acid were obtained from J.T. Baker (Phillipsburg, NJ). Heparin (sodium salt) was purchased from Aldrich (Milwaukee, WI). Normal saline (0.9% NaCl) was obtained from Baxter (Deerfield, IL). Water used in this study was produced in the laboratory through a NANOPure® II (Barnstead Co, Dubuque, Iowa) water purification system. Scintillation Cocktails were purchased from R. J. Harvey Instrument Corporation, (Hillsdale, NJ) or Beckman Instruments, Inc (Somerset, NJ). All other solvents and reagents were of high-performance liquid chromatography (HPLC) grade and were purchased from either Aldrich Chemical Co or Fisher Scientific Co (Pittsburgh, PA).

Figure 1. Chemical structure of [¹⁴C]-VNP40101M showing radiolabel position.

Animals

Adult male and female Sprague-Dawley rats (Hilltop Lab Animals, Inc, Scottdale, PA), 6-10 weeks old, body weights ranged from 234-258 g [male] and 227-246 g [female] at dosing) were used in the mass balance and tissue distribution groups. Jugular vein-cannulated rats (Hilltop Lab Animals, Inc, Sprague-Dawley, 6-10 weeks old, body weights ranged from 288-300 g [male], and 263-285 g [female] at dosing) were used in the PK group. All animals were acclimated for at least 5 days before dosing. Care of the animals was in accordance

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with institutional guidelines. Food and tap water were available ad libitum.

Dose Preparation

Dosing solution was prepared as follows: ~0.975 mCi of [^{14}C]-VNP40101M (in acetonitrile) was transferred into a tared vial and the solvent was evaporated. An aliquot of 12 mL of a liquid formulation of VNP40101M (containing 10 mg VNP40101M/mL) and 24 mL 0.9% NaCl (Baxter USP) was added to the vial and stirred for 10 minutes. Absolute ethanol (6 mL) was then added and the solution was stirred and sonicated until a clear solution resulted. The concentration of the dosing solution was assayed by HPLC in duplicate before and after dosing and radiopurity confirmed.

Study Design and Dosing Regimen

Male and female rats, housed individually in stainless steel metabolism cages, were each given a single IV bolus dose of [^{14}C]-VNP40101M at a nominal dose rate of 10 mg/kg body weight (the highest nontoxic dose in rats). Three animals per gender were used in Group 1. Plasma was obtained from these animals at pre-dose and at 2, 5, 15, 30, and 60 minutes and 2, 4, and 8 hours post-dose. Samples were analyzed for VNP40101M by HPLC. Three animals per gender were used in Group 2. Urine was collected at 0-4 hours, 48 hours, and 824 hours, and then daily until 7 days post-dose. Feces samples were collected daily post-dose for a total of 7 days. On day 7, animals were killed for tissue distribution determination. During the study, cages were rinsed daily with water and the cage rinse was collected. At the end of the study, the cages were washed thoroughly with isopropanol/water (1:1). Sample weights at each interval were determined at time of collection. Nine animals per gender were used in Group 3. Three animals per gender were killed at 5 minutes and at 1 hour and 24 hours post-dose for tissue distribution determination. One animal per gender (Group 4) was injected with blank vehicle and animals were killed at 24 hours post-dose. Urine, feces, and tissues were collected; no radioanalysis was done on these samples.

Sample Collection

For chemical specific analysis of plasma and urine by HPLC, samples were acidified prior to analysis to stabilize VNP40101M since VNP40101M undergoes hydrolytic degradation especially under basic conditions.^{10,11} Acidification procedures were evaluated through a series of experiments (results not shown). It was determined that a volume of 1M citric acid equivalent to approximately 5% of the sample volume was needed to stabilize VNP40101M in both blood and urine. Once acidified, VNP40101M is stable during freezer storage and freeze/thaw. For total radioactivity determination (ie, liquid scintillation counting [LSC]), acidification of samples was not carried out.

Plasma: Blood was collected from Group 1 animals via jugular vein cannula. Prior to collecting each PK sample,

a small blood sample (approximately 0.05 mL) was drawn and discarded. After each sample collection, saline (0.5 mL) was injected to flush the catheter, and a heparin lock was then placed. An approximately 0.25-mL blood sample was collected into a syringe (using sodium heparin as anticoagulant), transferred into microcentrifuge tubes, and placed on wet ice immediately. All blood tubes were centrifuged at 10 000 rpm for 10 minutes at 4°C in a Beckman GS-15R centrifuge within 30 minutes after collection. After centrifuging, 100 mmL of plasma was pipetted into another tube, 5 mmL of 1M citric acid was added into each plasma sample and mixed well. The plasma samples were stored in a freezer (-20°C) prior to HPLC analysis.

Urine, Cage Rinse, and Feces (from Group 2 animals): Urine was collected into containers surrounded by dry ice. Cages were rinsed with Nanopure® water on a daily basis for 7 days post-dose. Feces were collected from a stainless steel screen placed at the bottom of the cage and transferred to a specimen cup on a daily basis for 7 days post-dose at room temperature. Upon collection, feces were stored in a freezer until analysis. Sample weights were recorded upon collection. Urine and cage rinse were aliquoted and analyzed for radioactivity immediately.

Tissues: Animals from Groups 2, 3, and 4 were killed at the end of each study. Group 3 animals were killed (3/time point/gender) at 5 minutes and 1 hour and 24 hours post-dose. These time points were selected in an attempt to capture peak tissue concentrations as well as to measure the rapid elimination of VNP40101M. Animals were anesthetized by an overdose of CO₂, and then ~5-7 mL of blood was collected into a heparinized Vacutainer by cardiac puncture. Blood was placed on wet ice upon collection. Tissues from liver, kidneys, spleen, muscle, bone, lungs, heart, brain, and carcass were collected from each animal, rinsed with saline, and blotted dry on tissue paper. Fat (abdominal) and skin (dorsal) were also collected from Group 2 animals. The weight of each tissue was recorded.

Sample Preparation and LSC Analysis

Upon collection, the weight of each urine sample was determined, and an aliquot was acidified immediately with appropriate volume of 1M citric acid equivalent to ~5% of the urine weight to bring the pH to ~4.5. Duplicate aliquots of approximately 0.1 mL urine and 1 mL cage rinse were mixed with 5 mL Ready Value Scintillation Cocktail (Beckman), and counted directly by liquid scintillation counter. The acidified urine was kept frozen and used for HPLC analysis. Feces were mixed with 5-10 volumes of water:acetonitrile (1:1). The weight of the mixture was recorded and then homogenized for 2 minutes using a Tekmar tissuemizer® (Cincinnati, OH). Triplicate aliquots of feces homogenate (~0.6 g or less if the radioactivity level is expected to be high) were combusted using a Harvey Biological Sample Oxidizer (Hillsdale, NJ). Tissues (except bone, muscle, skin, and

fat) were homogenized for 2 minutes with 2 volumes of water using a Tekmar tissuemizer. Duplicate aliquots (~0.3 g) were air dried followed by combustion. Bone, muscle, skin, and fat were minced with scissors, and approximately 0.1 g of bone, muscle, and skin and 0.05 g of fat were combusted in duplicate.

HPLC Analysis

A Agilent Technologies 1100 liquid chromatography system (Wilmington, DE) was used for the analysis of plasma and urine samples. The HPLC system consisted of a quaternary pump, an autosampler, a column heater, and a β -RAM Model 2 flow-through radiometric detector (IN/US Systems, Tampa, FL). Plasma samples were analyzed using a Phenomenex Prodigy column (Torrance, CA) (5 μ m, 250 Å— 2.0 mm) at 40°C. The mobile phases were A) 0.1% acetic acid, and B) acetonitrile. A linear gradient program was used: 0 minute, 90%A/10%B; 30 minutes, 50%A/50%B. The flow rate was 0.4 mL/min. A flow-through radiometric detector equipped with a liquid scintillation cell (1 mL) was used to monitor radioactive components. The scintillation cocktail was set at 1.2 mL/min. The run time was 20 minutes with a 10-minute equilibration delay. For urine samples, a Phenomenex Prodigy column (5 μ m, 250 Å— 4.6 mm) was used, and the flow rate was 1 mL/min. The gradient program was: 0 minute, 90%A/10%B; 30 minutes, 40%A/60%B. The scintillation cocktail flow was 3 mL/min. The run time was 20-30 minutes with a 6 minute equilibration delay.

Each plasma sample (0.05-0.1 mL) was extracted with 0.2 mL of methanol by vortexing thoroughly. The extract was centrifuged at 15 000 rpm for 15 minutes at room temperature. The supernatant was evaporated to dryness under a stream of nitrogen at room temperature. The residue was reconstituted with 0.1 mL of a dilution solvent consisting of acetonitrile and 0.1% acetic acid (1:9). The pre-extraction sample and extract (5 μ L each) were analyzed by LSC for total radioactive residues. Each extract (30 μ L) was analyzed by HPLC with radiometric detection for VNP40101M and metabolite distribution. Each urine sample (0.1 mL) was mixed thoroughly with 0.1 mL of acetonitrile. The extract was centrifuged at 15 000 rpm for 15 minutes. The supernatant was transferred into an HPLC vial and analyzed by HPLC with radiometric detection. The pre-extraction sample and extract (10 μ L each) were also analyzed by LSC for total radioactive residues. HPLC calibration standards were prepared in blank rat plasma and urine with [14 C]-VNP40101M. The calibration range was 0.14-14.2 μ g/mL for plasma, and 2.2-141.5 μ g/mL for urine. Calibration curves were constructed and VNP40101M concentrations in plasma and urine samples were calculated based on peak area.

Pharmacokinetic Analysis

Pharmacokinetic parameters for VNP40101M in plasma were calculated with a 1-compartment model using

WinNonlin software (Pharsight Corporation, Mountain View, CA). An appropriate pharmacokinetic model was chosen based on the lowest weighted squared residuals, lowest Schwartz Criterion (SC), lowest Akaike's Information Criterion (AIC) value, lowest standard errors of the fitted parameters, and dispersion of the residuals. The elimination half-life ($T_{1/2}$) was estimated by linear regression analysis of the terminal phase of the plasma concentration-time profile. The area under the concentration time curve (AUC) was calculated between the first and last sampling times. Peak plasma concentration (C_{max}), total body clearance (Cl), and volume of distribution at steady-state (V_{ss}) were also calculated.

RESULTS

Animal Health and General Observation

At a dose of 10 mg/kg of [14 C]-VNP40101M, animals appeared healthy throughout the study. Male animals gained a reasonable amount of body weight, females did not gain much weight, and some even lost a little weight over the 7-day study period in Group 2.

Excretion Pattern and Mass Balance

Urine, cage rinse, and feces from Group 2 animals were collected and analyzed for total radioactivity. After 7 days, male rats recovered 65.6%, 4.9%, and 5.5% of administered dose in urine, cage rinse, and feces, respectively, while female rats recovered 61.0%, 3.7%, and 5.1% in urine, cage rinse, and feces, respectively. Tissues and carcass contained 8.8% and 9.4% of administered dose in male and female rats, respectively, on day 7. Overall recoveries were 84.9% in male and 79.2% in female rats. Most of the radioactivity (males excreted 68.9% and females excreted 63.4% of the dosed radioactivity) was excreted during the first 48 hours. **Table 1** summarizes the total radioactivity excreted from urine, cage rinse, and feces over a period of 168 hours as well as residual radioactivity in tissues at 168 hours post-dose. The excretion pattern of [14 C]-VNP40101M-derived radioactivity is shown in **Figure 2**. Approximately 15% and 21% of administered radioactivity was not accounted for in male and female rats, respectively. The consistency of the data among all animals and the 14 C-label position of VNP40101M suggests the loss of mass balance might be due to the generation of radioactive CO₂ or other volatile species since a closed system was not used in this study.

Table 1. Summary of Cumulative Total Radioactivity Recovered in Rats 7 Days after IV Administration of [^{14}C]-VNP40101M

Animal ID	Sex	% Dose	% Dose in	% Dose	% Dose	Total (%)
Code		in Urine	Cage Rinse	in Feces	in Tissues	Recovered
G2-001-M	M	70.9	2.5	4.8	9.1	87.4
G2-002-M	M	64.9	4.6	6.0	8.8	84.2
G2-003-M	M	61.0	7.7	5.8	8.6	83.2
Mean	NA	65.6	4.9	5.5	8.8	84.9
SD	NA	5.0	2.6	0.6	0.3	2.1
G2-004-F	F	60.7	6.2	4.3	9.4	80.6
G2-005-F	F	61.0	2.6	6.5	9.7	79.8
G2-006-F	F	61.2	2.2	4.6	9.1	77.2
Mean	NA	61.0	3.7	5.1	9.4	79.2
SD	NA	0.2	2.2	1.2	0.3	1.8

NA indicates Not Applicable; SD, Standard Deviation.

Figure 2. Cumulative recovery of radioactivity in excreta (urine including cage rinse [CR] and feces) of male and female rats following IV administration of [^{14}C]-VNP40101M at 10 mg/kg ($n = 3$ for each sex).

Tissue Distribution

Selected tissues (brain, heart, kidneys, liver, lungs, muscle, blood, spleen, bone, fat, and skin) were collected and analyzed for total radioactivity concentration in Group 3 animals at 5 minutes and at 1 hour and 24 hours post-dose, and in Group 2 animals at 168 hours post-dose. Tissue radioactivity distribution

data are presented in **Tables 2** and **3**. At 5 minutes post-dose, all of the tissue analyzed contained a significant amount of radioactivity, with the highest concentration in kidneys (24.1 mmg equivalence/g in males and 14.5 mmg equivalence/g in females), liver (17.7 mmg equivalence/g in males and 14.9 mmg equivalence/g in females), and lungs (14.0 mmg equivalence/g in males and 14.9 mmg equivalence/g in females). Muscle contained the highest percentage of radioactivity (44.0% in males and 48.9% in females), followed by blood (8.2% in males and 8.7% in females) and liver (7.9% in males and 5.4% in females). At 1-hour post-dose, most tissue concentrations decreased to ~48%-80% of the 5-minute level, except kidneys and blood. At 24-hours post-dose, blood contained the highest concentration of radioactivity (5.9 mmg equivalence/g in males and 7.1 mmg equivalence/g in females). Approximately 11%-12% of administered radioactivity still remained in the tissue at this time point. At 168 hours post-dose in male rats, only blood contained significant radioactivity (3.5 mmg equivalence/g). In female rats, blood radioactivity concentration was 4.5 mmg equivalence/g, whereas heart, lungs, and spleen contained 1.2, 1.5, and 1.4 mmg equivalence/g, respectively. The percent of dosed radioactivity in blood, muscle, bone, skin, and fat was normalized to the total body mass of each tissue.^{12,13}

Therefore, for 168-hour tissue recovery, the total percent of dose should represent a majority of the total tissue residue. It should be noted that brain contained a significant concentration of ^{14}C residue (5.9 mmg equivalence/g at 5 minutes post-dose, 2.8-3.8 mmg equivalence/g at 1 hour, and 0.7-0.9 mmg equivalence/g at 24 hours), indicating that VNP40101M passed the blood-brain barrier.

Table 2. Summary of Total Radioactivity in Tissues of Male Rats at 5 Minutes, 1 Hour, 24 Hours, and 168 Hours Post-dose

	5 Minutes		1 Hour		24 Hours		168 Hours	
	% Dose	$\mu\text{g/g}$	% Dose	$\mu\text{g/g}$	% Dose	$\mu\text{g/g}$	% Dose	$\mu\text{g/g}$
Matrix								
Brain	0.4	5.9	0.2	2.8	0.05	0.7	0.02	0.3
Heart	0.4	11.3	0.2	5.5	0.06	1.7	0.03	0.8
Kidneys	2.1	24.1	1.8	22.4	0.2	1.8	0.05	0.5
Liver	7.9	17.7	4.3	9.6	0.7	1.6	0.3	0.5
Lungs	0.7	14.0	0.4	8.2	0.1	2.3	0.05	0.8
Muscle	43.9	9.8	25.5	5.7	5.5	1.3	4.0	0.8
Blood	8.2	11.9	6.6	9.6	3.9	5.9	2.8	3.5
Spleen	0.3	11.2	0.2	6.5	0.04	2.0	0.02	0.7
Bone	3.0	5.1	2.0	3.3	0.5	0.9	0.2	0.3
Fat	NA	NA	NA	NA	NA	NA	0.2	0.2
Skin	NA	NA	NA	NA	NA	NA	1.1	0.5
Total	67.0	NA	41.2	NA	11.1	NA	8.8	NA

Table 3. Summary of Total Radioactivity in Tissues of Female Rats at 5 Minutes, 1 Hour, 24 Hours, and 168 Hours Post-dose.

	5 Minutes		1 Hour		24 Hours		168 Hours	
	% Dose	$\mu\text{g/g}$	% Dose	$\mu\text{g/g}$	% Dose	$\mu\text{g/g}$	% Dose	$\mu\text{g/g}$
Matrix								
Brain	0.4	5.8	0.3	3.9	0.1	0.9	0.03	0.4
Heart	0.5	12.0	0.2	7.2	0.1	2.0	0.04	1.2
Kidneys	1.2	14.5	1.1	15.7	0.2	2.3	0.1	0.7
Liver	5.4	14.9	3.5	9.1	0.7	2.2	0.2	0.6
Lungs	0.7	14.9	0.6	11.8	0.2	3.3	0.1	1.5
Muscle	48.9	10.9	27.5	6.1	5.7	1.3	4.3	1.0
Blood	8.7	12.7	8.2	11.8	4.7	7.1	3.0	4.5
Spleen	0.3	11.5	0.2	7.6	0.05	2.3	0.04	1.4
Bone	2.3	4.0	1.5	2.6	0.5	1.0	0.2	0.3
Fat	NA	NA	NA	NA	NA	NA	0.2	0.3
Skin	NA	NA	NA	NA	NA	NA	1.3	0.7
Total	68.3	NA	43.1	NA	12.2	NA	9.4	NA

RadioHPLC Analysis of Plasma and Urine

Calibration standards of [^{14}C]-VNP40101M were prepared in blank rat plasma and urine and used to evaluate the analytical method. Based on total radioactivity by liquid scintillation counting, the extraction efficiency averaged $89\% \pm 18\%$ ($n=16$) and $106\% \pm 12\%$ ($n=10$) for plasma and urine standards, respectively. By radioHPLC, the percent recovery of the back-calculated concentration of VNP40101M averaged $101\% \pm 8\%$ in plasma and $100\% \pm 12\%$ in urine. Calibration curves were linear with r^2 values greater than or equal to 0.9875 in both matrices. Under the HPLC conditions, VNP40101M eluted at approximately 13.5 minutes in the plasma method and 16 minutes in the urine method. The acidified plasma and urine samples from the study were extracted and analyzed for quantitation of VNP40101M and metabolite profiles. At least 4 polar (eluted before parent drug with a relative retention time <0.4)

metabolites were observed in plasma samples, and their quantities increased with time. In urine, the metabolites were predominant and the chromatographic profile was complex. At least 6 very polar metabolites were observed. Among the radioactive residues excreted in the urine over the 7-day period (71% in males and 65% in females), more than 85% were metabolites indicating that VNP40101M was extensively metabolized. The low renal clearance of VNP40101M suggests that renal excretion was not a major route of elimination of VNP40101M.

Pharmacokinetics in Plasma

Pertinent pharmacokinetic parameters of VNP40101M in rats are shown in **Table 4**. The elimination of VNP40101M in rats following IV administration was rapid with a mean elimination half-life of approximately 20 minutes. Plasma VNP40101M concentration was the highest at 2 minutes post-dose with a mean peak

Table 4. Pharmacokinetic Parameters of VNP40101M in Rats Determined Using [14 C]-VNP40101M Concentration in Plasma.

Pharmacokinetic Parameters	Male (n=3) Mean $\pm$ SD	Female (n=3) Mean $\pm$ SD
C _{max} (μ g/mL)	12.0 $\pm$ 2.8	10.5 $\pm$ 1.1
AUC (mg/min/L)	241 $\pm$ 21	410 $\pm$ 103
T _{1/2} (minutes)	14.7 $\pm$ 4.8	27.2 $\pm$ 7.1
Total body clearance (CL) (mL/min/kg)	41.7 $\pm$ 4.0	25.3 $\pm$ 5.5
Renal clearance (CL _R)	6.3	3.8
V _{ss} (L/kg)	0.86 $\pm$ 0.22	0.96 $\pm$ 0.11

Figure 3. Plasma concentration time profile of [14 C]-VNP40101M and total radioactive residues (n = 6).

concentration of approximately 11 μ g/mL. By 2 hours, VNP40101M was generally below the method quantitation limit (<0.28 μ g/mL). The plasma concentration time profile is shown in **Figure 3**. The mean total body clearance of VNP40101M (33 mL/min/kg) was high relative to hepatic (55 mL/min/kg)¹⁴ and renal (37 mL/min/kg) blood flow rates of the rat. The mean volume of distribution (0.9 L/kg) of VNP40101M approximated the total body water (0.67 L/kg) in the rat.¹⁴ It should be noted that the total body clearance for males (41.7 mL/min/kg) is quite different from that of females (25.3 mL/min/kg). Due to the limited

number of animals, it is not clear at this time if this difference is real or within experimental variability.

DISCUSSION Following an IV dose of 10 mg/kg in the rat, [14 C]-VNP40101M was eliminated rapidly with a terminal half-life of approximately 20 minutes. Radioactivity was extensively distributed into tissues of rats. Among the tissues, the kidneys, lung, liver, and blood contained the highest radioactivity on a μ g/g of tissue basis. Elimination of radioactivity from the tissues was prolonged with approximately 9% of the dose still remaining after 7 days. A total of approximately 82% of the administered radioactivity was recovered after 7

days. The remaining 18% was presumably due to the formation of volatile metabolites (ie, $^{14}\text{CO}_2$), which were not trapped in this study. Of the excreted radioactivity, approximately 70% was found in urine, and 6% in feces. The excretion was rapid with >60% of the administered radioactivity (80% of total excreted) excreted within 48 hours. Brain was found to contain relatively high radioactive residues (50% and 30% of the blood concentration at 5 minutes and 1 hour, respectively), indicating that VNP40101M penetrated the blood-brain barrier. The curves for total radioactivity and VNP40101M concentration in plasma (**Figure 3**) diverged quickly, suggesting radioactive metabolites were formed at a very early stage. This implies that the radioactivity found in tissues does not represent VNP40101M, even as early as 1 hour.

The metabolism of [^{14}C]-VNP40101M was also investigated in this study although identification of metabolites was not attempted. The rapid elimination of VNP40101M in plasma and the formation of complex polar metabolites were consistent with the expected in vivo metabolic fate of VNP40101M.^{5,9} A known degradation product of VNP40101M, 1,2-Bis(methylsulfonyl)-1-(2-chloroethyl)hydrazine^{9,10} was not detected in either plasma or urine samples. The absence of this compound in plasma and urine at study sampling intervals was consistent with its reactive and transient nature.^{9,10}

In conclusion, the pharmacokinetics of VNP40101M in rats primarily involves metabolism (and/or hydrolytic degradation) of VNP40101M followed by urinary excretion of its polar metabolites. The wide spread of radioactivity in various tissues and the relatively slow tissue clearance of VNP40101M metabolites are consistent with the DNA alkylating properties of VNP40101M. The findings in this study provide the basis for understanding the distribution, metabolism, and elimination characteristics of VNP40101M in cancer patients.

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