Studies on the Metabolic Fate of NK-104, a New Inhibitor of HMG-CoA Reductase ***(4): Interspecies Variation in Laboratory Animals and Humans***

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Summary: NK-104 is a very potent competitive inhibitor of HMG-CoA reductase. The pharmacokinetic properties of NK-104 were evaluated after intravenous and/or oral administration to rats, rabbits, dogs, monkeys and humans. The plasma concentration of NK-104 showed triexponential elimination after intravenous administration, with a half-life of 4.0–5.3 h in all four animal species. The absorption was relatively rapid after the oral administration. The relationship between the dose and the AUC was linear at a relatively high dose range in the animal species. In humans, the correlation line was located between those of dogs and rats. High bioavailability (about 80%) at a dose of 1 mg/kg was observed in all animal species except monkeys. These results indicated that the pharmacokinetic properties of NK-104 after oral administration in humans resembled those in dogs, suggesting that the BA of NK-104 is large in humans as well as dogs.

NK-104 was excreted mainly into feces *via* biliary route, and the renal handling was negligible in rats, dogs and humans. On the other hand NK-104 was excreted mainly into urine in rabbits, and was excreted poorly into urine and feces in monkeys.

The unchanged drug was excreted mainly in rat bile. Although several β -oxidation products as metabolites of NK-104 were detected, the levels of these metabolites were fairly low. In dogs, the major component in plasma, urine and feces after oral administration was the unchanged NK-104. The β -oxidation products (M-5 and M-8) were also slightly detected only in dogs. In humans, the major components in plasma after repeated oral administration (2 mg/day for 5 days) were NK-104 and its lactone form.

These results suggest that NK-104 may be hardly metabolized in humans than in animal species.

Key words: NK-104, HMG-CoA reductase inhibitor, Rat, Rabbit, Dog, Monkey, Human, Absorption, Excretion, Metabolism

Introduction

In general, lowering low-density lipoprotein (LDL) cholesterol levels or increasing high-density (HDL) cholesterol levels in plasma reduces the risks of coronary heart disease and atherosclerosis. Based on the preclinical findings that NK-104 is a highly potent inhibitor of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis,¹⁾ this agent is promising as a therapeutic drug for hyperlipidemia in humans. The ¹⁴C-labeled NK-104 was absorbed well from the intestinal tract after oral administration to rats and distributed in the liver at a high concentrations in the unchanged form. The drug distributed in the liver was mainly excreted in the bile and reabsorbed by entero-hepatic circulation.²⁾ In addition, the C_{max} of NK-104 reached a steady state within the 4th dosing after repeated administration in rats.³⁾ These pharmacokinetic profiles suggest that NK-104 has selectivity for the liver and long acting HMG-CoA reductase inhibitory effects.

The toxicity and/or the efficacy of a drug in animals or humans are often related to its pharmacokinetics and metabolism. In particular, considerable attention has been paid to interspecies variation in the pharmacokinetics and metabolism. This study examined basic pharmacokinetics of NK-104 in four animal species, rats, rabbits, monkeys, and dogs, and in humans. We also carried out a series of metabolic studies involving bile in rats, feces in dogs, and plasma and urine in dogs and humans.

Materials and Methods

1. Materials

The NK-104 (monocalcium bis [(3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluorophenyl)-3-quinolyl]3,5-dihydroxy-6-heptenoate]) and its metabolites which were determined by NMR and LC-MS spectrometry in the bile of animals after an intravenous infusion⁴⁾ (NK-104 lactone, M-2, M-3, M-4, M-5, M-6, M-7, M-8, M-9, M-10 and M-11) were synthesized by Nissan Chemical Industries, Ltd. (Funabashi, Japan). The structures of

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Fig. 1 Postulated metabolic pathways of NK-104

NK-104 and its metabolites are shown in **Fig. 1**. All other solvents and chemicals were obtained commercially, and were of HPLC grade or extra pure grade.

2. Animals

Wistar strain (specific pathogen-free) male rats, weighing about 200 g, JW strain male rabbits, weighing about 2.5 kg, HRA-male beagle dogs, weighing about 10 kg (Japan Laboratory Animal Inc. Tokyo, Japan) and male cynomolgus monkeys, weighing about 4 kg (Philippines born, Japan SLC. Inc. Shizuoka, Japan) were used. The rats and rabbits were acclimatized for at least 4 days, and all animals were kept in a controlled care room at the temperature of $23 \pm 2^\circ\text{C}$ and a relative humidity of $60 \pm 10\%$. All animals were maintained on laboratory chow diets and were fasted overnight prior to the drug administration, but had free access to water. Three or four animals were assigned to each test group.

3. Pharmacokinetic Study in Animals

1) Intravenous Administration Study

The dosage form of NK-104 was prepared as a solution in physiological saline at a range of 0.1–1 mg/ml for intravenous administration. The rabbits and dogs were intravenously given 0.1 mg/kg, the monkeys and rats were intravenously given 0.3 and 1 mg/kg of NK-104, respectively. The dose levels were quoted from the minimum dose of oral administration and our previous study.²⁾ The intravenous bolus injection was made in the

front leg vein of dogs, the femoral vein of monkeys, the ear vein of rabbits and the tail vein of rats. Blood samples were withdrawn from the jugular vein of rats, from the ear vein of rabbits, from the front leg vein of dogs and from the femoral vein in monkeys at the indicated times. Plasma was separated after centrifugation of blood sample. The plasma samples were stored at -20°C until analyzed.

2) Oral Administration Study

NK-104 was prepared as a suspension in 0.5% sodium carboxymethyl cellulose solution at a range of 0.1–5 mg/ml for oral administration. The rabbits and dogs were given NK-104 in single oral administration of 0.1, 0.3 or 1 mg/kg. The rats and monkeys were given NK-104 at 0.3, 1 or 3 mg/kg. The collection of blood samples was carried out in the same manner as for the intravenous administration.

3) Excretion of NK-104 in Urine and Feces

Rats were housed individually in glass metabolic cages (Metabolica®; Sugiyamagen Iriki, Tokyo, Japan) after intravenous or oral administration of NK-104. The other animals were maintained individually in stainless steel metabolic cages. Urine and feces were collected separately every 24 h up to 72 or 96 h after the administration. Each urinary sample was diluted with water. Each fecal sample was homogenized with water. Aliquots (0.5 ml or 0.5 g) of urine and fecal homogenates were stored at -20°C until analyzed.

4. Biliary Metabolites in Rats

Rats were surgically prepared with common bile duct cannulae under ether anesthesia. After awakening, NK-104 was orally administered at a dose of 1 mg/kg and each animal was housed individually in a Bollman's cage (Natsume Seisakusyo, Tokyo, Japan). The bile was continuously collected for 24 h in the dark to prevent the degradation of biliary metabolites. The biliary samples were stored at -20°C until analysis.

5. Plasma, Urinary and Fecal Metabolites in Dogs

The plasma, urinary and fecal metabolites were investigated in dogs after intravenous administration of NK-104 at a dose of 0.1 mg/kg and oral administration at 1 mg/kg and 10 mg/kg. The administration procedure and the collection of biological samples were carried out as described above. Aliquots of plasma, urinary and fecal samples were stored at -20°C until analysis.

6. Repeated Administration of NK-104 in Humans

Five healthy male volunteers (age, 22–24 years) received repeated oral administrations of NK-104 at a dose of 2 mg as a tablet once a day for five days. In this study, not only the plasma concentration of NK-104 but also the plasma and urinary metabolites of NK-104 were investigated. The protocol was approved by the Niza-Shiki Central Hospital Ethical Committee, and the subjects had given their informed consent. Blood samples were taken *via* an antecubital vein at various times up to 48 h after the final administration for the parent drug

and its lactone assays, and at 1.5, 6 and 24 h on day 1 and day 5 for the metabolites assay. Urine samples were collected every 24 h after daily administration. Aliquots of plasma and urinary samples were stored at -20°C until analysis.

7. Enzymatic Hydrolysis

To confirm the presence of conjugates of NK-104 and its metabolites, the urinary, fecal and biliary samples were treated with enzymatic hydrolysis. The incubations of the urinary, fecal and biliary samples were carried out with β -glucuronidase/arylsulfatase (5000 fishman units and 80 units) at 37°C . The amounts of conjugates were calculated by subtracting the concentration of enzyme-untreated samples.

8. Analytical Methods

1) Preparation of Standard Solution

Quantitative analysis of NK-104 and NK-104 lactone in the biological samples was performed by a column switching-HPLC method described in our previous report.⁵⁾ The other NK-104 metabolites, M-2 (NK-104 dehydrolactone), M-3 (5-keto NK-104), M-4 (3-dehydroxy NK-104), M-5 (pentadienoic acid derivative), M-6 (pentenoic acid derivative), M-7 (hydroxypentenoic acid derivative), M-8 (propenoic acid derivative), M-9 (propanoic acid derivative), M-10 (taurine conjugate of pentadienoic acid derivative) and M-11 (taurine conjugate of pentenoic acid derivative) were measured by the five methods as described in **Table I**. The

Table I Analytical conditions for the determination of NK-104 and its metabolites

Analytes	Method-1 NK-104, lactone, M-4	Method-2 M-10, M-11	Method-3 M-2, M-5, M-6	Method-4 M-3, M-7	Method-5 M-8, M-9
Chromatographic conditions					
Pre-separation					
Pre-column:	Cosmosil ₅ C ₁₈ (150 mm × 4.6 mm)	Cosmosil ₅ C ₁₈ (150 mm × 4.6 mm)	Cosmosil ₅ C ₁₈ (150 mm × 4.6 mm)	Cosmosil ₅ C ₁₈ (150 mm × 4.6 mm)	Cosmosil ₅ C ₁₈ (100 mm × 4.6 mm)
Mobile phase:	0.2 M acetate buffer (pH4)/CH ₃ CN (50:50)	0.1 M acetic acid with 1.5 mM TBAS/ CH ₃ CN(65:35)	0.2 M acetic acid/ CH ₃ CN(50:50)	0.2 M acetic acid/ CH ₃ CN(60:40)	0.2 M acetate buffer (pH4.3)/CH ₃ CN (55:45)
Flow-rate:	1 ml/min	1 ml/min	1 ml/min	1 ml/min	0.8 ml/min
Column switching: about (after injection)	10–12 min and about 24–27 min	about 6–7 min and about 18–21 min	about 8–9 min and about 15–18 min	about 6–8 min and about 16–18 min	about 15–18 min
Analytical separation					
Column:	Cosmosil ₅ C ₁₈ (150 mm × 4.6 mm)	Cosmosil ₅ C ₁₈ (150 mm × 4.6 mm)	Cosmosil ₅ C ₁₈ (150 mm × 4.6 mm)	Cosmosil ₅ C ₁₈ (150 mm × 4.6 mm)	Cosmosil ₅ C ₁₈ (150 mm × 4.6 mm)
Mobile phase:	0.2 M acetic acid/ CH ₃ CN(50:50)	0.1 M acetate buffer (pH5) with 1.5 mM TBAS/CH ₃ CN (50:50)	0.2 M acetate buffer (pH4.2)/CH ₃ CN (50:50)	0.2 M acetate buffer (pH3)/CH ₃ CN (50:50)	0.2 M acetate buffer (pH5)/CH ₃ CN (50:50)
Heartcut time:	2.6 min	1.2 min	1.5 min	1.4 min	3 min
(after valve rotation)	2.3 min	2.8 min	2.4 min	1.6 min	
Flow-rate:	1 ml/min	1 ml/min	1 ml/min	1 ml/min	0.8 ml/min
Total run-time:	50 min	30 min	35 min	30 min	30 min

stock solutions of NK-104, M-3, M-4, M-5, M-6, M-7, M-8, M-9, M-10 and M-11 were prepared in water, whereas those of M-2 and NK-104 lactone were prepared in methyl *tert*-butyl ether (MTBE). The stock solutions of NK-104 and its metabolites were diluted with water or MTBE to appropriate concentrations for standard preparation. These solutions were stored in the dark at 4°C.

2) Sample Preparation

In Methods 1, 3, 4 and 5, the internal standards were added to biological samples in a colored tube, and then 1 M potassium dihydrogenphosphate was added. These mixtures were extracted with 6 ml of MTBE and subsequently centrifuged at approximately 700 g. In the Method 1, the organic layer was transferred to another colored tube and then diazomethane-ether solution (0.5 ml) was added. After 0.5 h, excessive diazomethane was degraded by the addition of 1 M potassium dihydrogenphosphate (2 ml), and the organic layer was evaporated to dryness under a gentle stream of nitrogen at 40°C. In the Methods 3, 4 and 5, the organic layer was also transferred to another colored tube and was evaporated to dryness. In the Method 2, the unextractable fractions of biological samples were further extracted by chloroform after addition of 50 mM tetrabutylammonium hydrogen sulfate (TBAS). After the extraction, the organic layer was transferred to another colored tube and was evaporated to dryness. Each residue was dissolved in 150 μ l of the mobile phase, and an aliquot of 80 μ l was injected into an HPLC system.

3) Column Switching-HPLC

The HPLC-chromatographic system (LC-6A or 10A series, Shimadzu, Kyoto, Japan) consisted of two pumps, an automatic sample injector and a high-pressure six-port valve. A UV detector linked to a data system was used for data acquisition and storage. The chromatographic signals were monitored at 250 nm for the quantification of NK-104 and its metabolites. The column switching according to valve operation was carried out automatically by the SCL-6A or a 10A controller programmed valve rotation time. Two columns were used for the pre-separation and analytical separation, respectively. The injected samples were first separated on the pre-column, and the flow path was switched to the analytical column, transferring analytes from the pre-column to the analytical column. The chromatographic conditions of column switching are shown in Table I.

9. Calculations

The pharmacokinetic parameters were calculated from the individual plasma concentration-time curve of NK-104 and its metabolites in each animal species and humans. The plasma concentration curves of NK-104 after intravenous administration could be adequately described by an exponential equation, $C_p = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t}$. The observed data were fitted in the triexponen-

tial equation using a nonlinear least-squares curve fitting program.⁶⁾ The elimination half-life ($t_{1/2}$) value was estimated by using the least square method of actual data. The area under the plasma concentration-time curve (AUC) value was obtained by the trapezoidal rule. The plasma clearance (Clp) was estimated by the relationship of dose/AUC. After oral administration, the maximum concentration (C_{max}) of analyte was read directly from the concentration-time data. The bioavailability (BA) was calculated from the dose-normalized AUC obtained from oral and intravenous administrations. The excretion of NK-104 and its metabolites in urine, feces and bile was corrected to the % of dose. Each value represents the mean $\pm$ S.E. of 3-6 animals or humans.

Results

Pharmacokinetics Studies

1) Intravenous Administration in Animals (Fig. 2, Table II)

The plasma concentration of NK-104 after intravenous administration of 1 mg/kg to rats, 0.1 mg/kg to rabbits and dogs, and 0.3 mg/kg to monkeys showed a triexponential elimination with half-life values for the terminal phase ($t_{1/2}$) of 3.97, 5.06, 4.60 and 5.31 h, respectively. The plasma clearance (Clp) values in rats, rabbits, dogs and monkeys were 11.6, 1.0, 5.8 and 10.3 ml/min/kg, and the distribution volume of steady-state (V_{ss}) values were 1.34, 0.38, 0.97 and 1.53 L/kg, respectively. The ratios of blood to plasma concentration

Fig. 2 Mean plasma concentration of NK-104 in different species after intravenous administration

NK-104 was given to rats (○) at a dose of 1 mg/kg, dogs (●) at a dose of 0.1 mg/kg, rabbits (△) at a dose of 0.1 mg/kg and monkeys (▲) at a dose of 0.3 mg/kg.

Each point represents the mean $\pm$ S.E. of three or four animals.

Table II Pharmacokinetic parameters obtained from a three-compartment model after intravenous administration of NK-104 in different species

Parameter	Rat (n=4)	Rabbit (n=4)	Dog (n=4)	Monkey (n=3)
Dose (mg/kg)	1	0.1	0.1	0.3
B.W. (kg)	0.20±0.00	2.51±0.06	10.13±0.27	3.99±0.04
P (ng/ml)	12700±2300	3924±1926	5175±2418	4868±746
π (min ⁻¹)	0.507±0.033	0.91±0.10	1.09±0.26	0.325±0.030
A (ng/ml)	1180±80	343±59	251±49	186±56
α (min ⁻¹)	0.0328±0.004	0.0344±0.0083	0.0433±0.0087	0.0268±0.0059
B (ng/ml)	93.5±24.1	194±19	19.4±5.2	17.8±3.0
β (min ⁻¹)	0.0036±0.001	0.0024±0.0003	0.0026±0.0003	0.0024±0.0005
$t_{1/2}$ (h)	3.97±1.10	5.06±0.61	4.60±0.41	5.31±1.00
AUC ($\mu\text{g}\cdot\text{h}/\text{ml}$)	1.46±0.10	1.62±0.08	0.29±0.02	0.50±0.06
Clp (ml/min/kg)	11.6±0.8	1.04±0.05	5.79±0.41	10.25±1.18
MRT (h)	1.94±0.55	6.22±0.65	2.79±0.08	2.39±0.74
Vss (L/kg)	1.34±0.39	0.38±0.03	0.97±0.08	1.53±0.52
Lactone				
C _{max} (ng/ml)	n.d.	6.9±1.1	11.8±2.6	118±33
T _{max} (h)	—	0.38±0.21	2.00±0.35	0.03±0.00
$t_{1/2}$ (h)	—	9.69±1.03	6.93±1.16	1.95±0.22

The data are expressed as mean±S.E. of three or four different species.
n.d., not detected.

(RBP) up to 2 h after administration showed an almost constant value of approximately 0.6 in all species, indicating that the distribution of NK-104 into erythrocytes was small. Although the NK-104 lactone in plasma after administration was not found in rats, this metabolite was detected in the other three species. The species difference in pharmacokinetic parameters of

NK-104 lactone was also observed not only in the T_{max} values (0.03–2.00 h) but also C_{max} values (7, 12 and 118 ng/ml in rabbits and dogs at 0.1 mg/kg and monkeys at 0.3 mg/kg, respectively).

2) Oral Administration in Animals (Fig. 3, Table III)

After oral administration at a dose of 0.3, 1 and 3 mg/

Fig. 3 Mean plasma concentration of NK-104 in different species after oral administration at a dose of 0.1 mg/kg (○), 0.3 mg/kg (●), 1 mg/kg (△) and 3 mg/kg (▲). Each point represents the mean±S.E. of three or four animals.

Table III Pharmacokinetic parameters after oral administration of NK-104 in different species

Animal	Dose (mg/kg)	T _{max} (h)	C _{max} (ng/ml)	t _{1/2} (h)	AUC (μg·h/ml)	BA (%)	T _{max} (h)	Lactone C _{max} (ng/ml)	t _{1/2} (h)
Rat (n=4)	0.3	0.67±0.17	22±2	7.68±0.47	0.14±0.01	31	—	n.d.	—
	1.0	0.88±0.38	231±35	6.73±1.05	1.17±0.22	80	—	n.d.	—
	3.0	0.50±0.00	911±210	6.47±0.94	3.95±0.65	91	—	n.d.	—
Rabbit (n=4)	0.1	6.00±1.40	69±5	4.24±0.56	1.03±0.07	64	8.50±1.50	1.0±0.2	*
	0.3	4.50±1.30	314±33	4.67±1.25	4.34±0.19	89	5.50±0.96	4.8±0.4	25.4±4.3
	1.0	1.50±0.29	1184±154	3.91±0.73	15.67±1.55	97	4.25±2.02	21.1±3.8	16.9±5.9
Dog (n=4)	0.1	1.94±0.66	29±9	3.95±0.41	0.17±0.03	58	4.00±0.70	7.1±1.2	5.51±0.46
	0.3	2.50±1.19	93±24	4.26±0.50	0.63±0.13	71	2.75±0.63	26.6±3.7	5.55±0.39
	1.0	0.56±0.16	724±131	4.32±0.27	2.57±0.27	88	2.50±0.29	117±12	7.16±0.97
Monkey (n=3)	0.3	2.67±1.69	17±5	4.50±0.54	0.16±0.06	31	6.67±1.76	2.6±1.7	11.21**
	1.0	3.17±2.42	61±11	4.54±0.76	0.31±0.07	18	2.33±0.88	9.4±3.1	5.24±1.56
	3.0	2.17±0.93	165±43	3.66±0.37	0.85±0.16	17	2.67±0.67	21.3±6.2	4.87±1.03

The data are expressed as mean±S.E. of three or four different species.

n.d., not detected.

*: unable to calculate.

** : n=2.

kg to rats, NK-104 was rapidly absorbed and reached maximum levels within 1h independent of the dosage. The C_{max} values increased more than dose-proportionally in the dose range. The increase in AUC was largely dose-proportional up to a dose of 1 mg/kg, but dose-proportional from 1 to 3 mg/kg. The plasma concentrations of NK-104 exhibited a biexponential decrease, and the t_{1/2} in the last phase was about 7 h at a dose of 1 mg/kg. The BA in rats was 80% at a dose of 1 mg/kg. In addition, the NK-104 lactone in the rat plasma was not detected.

In rabbits, the C_{max} and AUC values of NK-104 increased linearly in the tested dose range of 0.1 to 1 mg/kg. Although the plasma concentrations of NK-104 were declined moderately up to 10 h after administration, thereafter its elimination t_{1/2} was about 4 h at a dose of 1 mg/kg. The BA in rabbits was 97%. The C_{max} of NK-104 lactone as a metabolite after administration at 1 mg/kg was 21.1 ng/ml, only 1/56 of the C_{max} of NK-104.

In dogs, the C_{max} and AUC values of the parent drug increased dose-proportionally as they did in rabbits. The plasma concentrations of NK-104 exhibited an exponential decrease, and its t_{1/2} was about 4 h in the 1 mg/kg administration. The BA in dogs was 88% at a dose of 1 mg/kg. The C_{max} of NK-104 lactone after administration at 1 mg/kg was 117 ng/ml, about 1/6 compared with the C_{max} of NK-104.

In monkeys, the C_{max} and AUC values of the parent drug also increased linearly in the dose range of 0.3 to 3 mg/kg, and the t_{1/2} values were similar to those of the rabbits and dogs. However, the BA in monkeys was 18%, lower than those of the other animal species. The C_{max} of NK-104 lactone after administration at 1 mg/kg

was very low (9.4 ng/ml).

3) Urinary and Fecal Excretion in Animals

Fig. 4 shows the excretion ratio of NK-104 in the urine and feces up to 72 or 96 h after intravenous administration at a dose of 0.1–1.0 mg/kg, and after oral administration at a dose of 1 mg/kg to the four animal species. The excretion was nearly complete in the first 24 h after administration (data not shown). After oral administration, the drug was excreted mainly into feces in rats and dogs as the unchanged form, accounting for 61.6 and 46.2% of the dose, respectively. The urinary excretions were practically negligible in both species, indicating less than 1% of the dose. The NK-104 was excreted mainly into urine in rabbits (35.7% of dose), while the fecal excretion was only 13.0% of dose. The excretion of unchanged NK-104 in monkeys was slight, being 0.1 and 5.2% of the dose into urine and feces, respectively.

The NK-104 lactone and the glucuronic acid conjugates of NK-104 and NK-104 lactone were excreted at less than 5% of the dose in all species after oral administration. In addition, no essential changes were noted in the excretion ratio at any dose after oral administration of NK-104 (data not shown). After intravenous administration, the urinary and fecal excretions of NK-104, NK-104 lactone and the glucuronic acid conjugates were similar to those in the oral dose study in all species.

Biliary Metabolites in Rats (Table IV)

In bile, the unchanged NK-104 accounted for 32.3% of the dose. The metabolites of NK-104, M-3 (5-keto NK-104), NK-104 lactone, M-11 (taurine conjugate of pentenoic acid derivative), M-10 (taurine conjugate of pentadienoic acid derivative) and M-6 (pentenoic acid

Fig. 4 Urinary and fecal excretions of NK-104, NK-104 conjugate and Lactone after intravenous and oral administration of NK-104 at a dose of 0.1–1 mg/kg to different species

■ : NK-104 □ : Lactone ▨ : NK-104 conjugate

Table IV Composition of NK-104 and its metabolites in bile after oral administration at dose of 1 mg/kg to rats

	Composition (% of dose)	
	Unconjugate	Conjugate
NK-104	32.27±4.73	0.32±0.20
Lactone	5.56±1.29	0.10±0.10
M-2	0.00	0.02±0.02
M-3	7.08±0.85	1.01±0.35
M-4	0.12±0.02	0.01±0.00
M-5	0.00	0.34±0.01
M-6	1.52±0.38	11.22±1.32
M-7	0.25±0.02	0.00
M-8	0.07±0.04	0.02±0.02
M-9	0.00	0.00
M-10		1.97±0.21
M-11		3.07±0.49
Total recovery	64.96±5.97	

Each value represents the mean±S.E. of five rats.

derivative) accounted for 7.1, 5.6, 3.1, 2.0 and 1.5% of the dose, respectively. M-7 (hydroxypentenoic acid derivative) and M-8 (propenoic acid derivative) accounted for less than 0.3% of the dose. After the enzymatic hydrolysis of bile, M-6 accounted for 11.2% of the dose. The other glucuronic acid conjugate metabolites accounted for less than 1.0%. No other metabolites were determined in rat bile. The total recovery of NK-104 and its metabolites was about 65% of the dose up to 24 h.

Fig. 5 Mean plasma concentration of NK-104 and its metabolites after intravenous (0.1 mg/kg) or oral administration (1 mg/kg and 10 mg/kg) of NK-104 to dogs
Each point represents the mean±S.E of four dogs.

Table V Pharmacokinetic parameters of NK-104 and its metabolites after intravenous or oral administration of NK-104 to dogs

Parameter		$C_{\max}$ (ng/ml)	$T_{\max}$ (h)	AUC ($\mu\text{g}\cdot\text{h}/\text{ml}$)	$t_{1/2}$ (h)
I.V. 0.1 mg/kg	NK-104	—	—	0.398 ± 0.037	—
	Lactone	11.0 ± 2.2	1.0 ± 0.4	0.042 ± 0.006	—
	M-3	1.8 ± 0.6	0.5 ± 0.0	0.002 ± 0.001	—
	M-5	7.4 ± 2.3	1.9 ± 0.8	0.025 ± 0.008	—
P.O. 1 mg/kg	NK-104	526 ± 107	0.8 ± 0.2	1.59 ± 0.27	—
	Lactone	72.8 ± 2.4	2.5 ± 0.5	0.44 ± 0.03	—
	M-3	8.6 ± 2.4	0.7 ± 0.2	0.02 ± 0.01	—
	M-5	36.4 ± 9.5	4.0 ± 0.0	0.17 ± 0.03	—
P.O. 10 mg/kg	NK-104	9509 ± 2488	1.3 ± 0.3	35.48 ± 4.32	5.56 ± 0.47
	Lactone	1013 ± 177	4.0 ± 1.4	14.50 ± 1.65	5.76 ± 0.97
	M-3	96.9 ± 21.0	1.0 ± 0.0	0.46 ± 0.14	—
	M-4	6.7 ± 1.4	3.5 ± 1.5	0.11 ± 0.04	—
	M-5	400 ± 57	6.0 ± 1.2	7.57 ± 0.85	13.8 ± 0.85
	M-6	4.0 ± 1.4	2.5 ± 0.5	0.04 ± 0.03	—
	M-8	39.9 ± 10.6	4.5 ± 1.3	1.07 ± 0.32	28.4 ± 4.29

The data are expressed as mean $\pm$ S.E. of four dogs.

Plasma Metabolites in Dogs (Fig. 5, Table V)

After intravenous administration, the unchanged NK-104 was mainly found in the plasma and showed a triexponential elimination. The AUC values of NK-104, NK-104 lactone, M-5 (pentadienoic acid derivative) and M-3 were 0.398, 0.042, 0.025 and 0.002 $\mu\text{g h}/\text{ml}$, respectively. After oral administration at a dose of 1 mg/kg, the parent drug appeared rapidly in plasma, and the $C_{\max}$ was 526 ng/ml. The AUC values of NK-104, NK-104 lactone, M-5 and M-3 were 1.59, 0.44, 0.17 and 0.02 $\mu\text{g h}/\text{ml}$, respectively. At a dose of 10 mg/kg, the AUC of NK-104, lactone and M-5 were 35.5, 14.5 and 7.6 $\mu\text{g h}/\text{ml}$, respectively. Moreover, the $t_{1/2}$ of M-5 was 13.8 h. The concentrations of M-3, M-4, M-6 and M-7 were also detected as minor metabolites. Although M-8 was also a minor metabolite, the $t_{1/2}$ was 28.4 h. No other metabolites were found in dog plasma at any dosing.

Urinary and Fecal Metabolites in Dogs (Table VI)

The unchanged NK-104 was excreted mainly in feces after intravenous and oral administrations to dogs. After intravenous administration at a dose of 0.1 mg/kg, the unchanged NK-104 in feces was 31.1% of the dose. The excretions of M-5 and NK-104 lactone were 2.2 and 0.3% of the dose, respectively. The total recovery of NK-104 and metabolites reached about 34% of the dose. The excretions of NK-104 after oral administration at 1 mg/kg and 10 mg/kg were 49.9 and 38.7%; those of NK-104 lactone were 2.6 and 2.3%, and those of M-5 were 1.7 and 1.8% of the dose. M-2 (NK-104 dehydrolactone), M-3, M-4, M-6, M-7 and M-8 were slightly detected in feces; the excretion ratios were less than 0.3% of the dose. No other metabolites were detected in the feces. The total recovery of NK-104 and its metabolites at 1 mg/kg and 10 mg/kg was about 55 and

44% of the dose, respectively. The urinary excretion of NK-104 at both doses was less than 0.2% of the dose, and the excretions of metabolites were negligible. After enzymatic hydrolysis, no marked difference was noted in the relative amounts of NK-104 and its metabolites in the urine and feces (data not shown).

Repeated Administration of NK-104 to Humans (Fig. 6, Table VII)

After repeated oral administration of NK-104 to humans, the unchanged NK-104 appeared rapidly in the plasma. The $C_{\max}$ value of NK-104 was 36.3 ng/ml on day 1, and slightly increased to the $C_{\max}$ of 41.4 ng/ml on day 5. Subsequently, the concentration of NK-104 declined with the $t_{1/2}$ of 13.1 h. NK-104 lactone was found in plasma as a major metabolite of NK-104. The $C_{\max}$ value of NK-104 lactone was 19.5 ng/ml on day 1, and the $C_{\max}$ of 21.5 ng/ml was observed on day 5. Subsequently, the concentration of NK-104 lactone decreased with the $t_{1/2}$ of 12 h on day 5. Due to the repeated administration, the AUC values of NK-104 and NK-104 lactone on day 5 were increased by about 20% compared to day 1. M-9 was slightly detected at 6 and 24 h on day 5, whereas the level of M-9 was negligible. No other metabolites were detected in human plasma during the repeated administration (data not shown). Moreover, no remarkable difference was observed in the other pharmacokinetic parameters during the repeated oral administration.

Urinary Metabolites in Humans (Table VIII)

The urinary excretions of NK-104 and NK-104 lactone were 0.7% and 1.7% of the dose, respectively. The excretion of M-2 was slight in urine (0.1% of the dose). No other metabolites were detected in human urine dur-

Table VI Mean urinary and fecal excretion ratios of NK-104 and its metabolites after intravenous or oral administration of NK-104 at a dose of 0.1–10 mg/kg to dogs

Metabolite		Mean excretion ratio (% of dose)		
		Urine	Feces	Total
0.1 mg/kg (i.v.)	NK-104	0.00±0.00	31.13±0.70	31.13
	Lactone	0.00±0.00	0.31±0.31	0.31
	M-5	0.00±0.00	2.22±1.28	2.22
	Total recovery			33.65
1 mg/kg (p.o.)	NK-104	0.18±0.06	49.92±5.69	50.10
	Lactone	0.00±0.00	2.59±0.34	2.59
	M-2	0.00±0.00	0.03±0.03	0.03
	M-3	0.00±0.00	0.19±0.05	0.19
	M-5	0.00±0.00	1.73±0.11	1.73
	M-8	0.00±0.00	0.07±0.04	0.07
	Total recovery			54.71
10 mg/kg (p.o.)	NK-104	0.16±0.05	38.71±3.36	38.86
	Lactone	0.00±0.00	2.28±0.07	2.28
	M-2	0.00±0.00	0.19±0.02	0.19
	M-3	0.01±0.01	0.25±0.10	0.26
	M-4	0.00±0.00	0.03±0.03	0.03
	M-5	0.02±0.02	1.76±0.11	1.78
	M-6	0.00±0.00	0.09±0.03	0.09
	M-7	0.00±0.00	0.01±0.01	0.01
	M-8	0.00±0.00	0.29±0.06	0.29
	Total recovery			43.78

Each value represents the mean ± S.E. of four dogs.

Fig. 6 Mean plasma concentration of NK-104 (○) and NK-104 lactone (●) after repeated oral administrations of NK-104 at a dose of 2 mg once a day for 5 days to human subjects
Each point represents the mean ± S.E of six male subjects.

Table VII Pharmacokinetic parameters of NK-104 and lactone after repeated oral administration of NK-104 at a dose of 2 mg once a day for five days to subjects

		Day-1	Day-5
NK-104	T_{max} (h)	1.2±0.1	1.2±0.2
	C_{max} (ng/ml)	36.3±8.5	41.4±9.0
	AUC(ng. h/ml)	99.8±19.3	123.4±22.2
	$t_{1/2}$ (h)	—	13.1±1.4
Lactone	T_{max} (h)	1.7±0.1	1.6±0.2
	C_{max} (ng/ml)	19.5±2.6	21.5±2.7
	AUC(ng. h/ml)	147.0±19.0	166.1±23.2
	$t_{1/2}$ (h)	—	12.0±1.1

Each value represents mean ± S.E. of five human.

Table VIII Urinary excretion of NK-104 and its metabolites after repeated oral administration of NK-104 2 mg once a day for five days to subjects

	Cumulative excretion (% of dose)
NK-104	0.67 ± 0.13
Lactone	1.67 ± 0.35
M-2	0.13 ± 0.07
M-3	0.00 ± 0.00
M-4	0.00 ± 0.00
M-5	0.00 ± 0.00
M-6	0.00 ± 0.00
M-7	0.00 ± 0.00
M-8	0.00 ± 0.00
M-9	0.00 ± 0.00
M-10	0.00 ± 0.00
M-11	0.00 ± 0.00
Conjugate*	4.01 ± 0.33

Each value represents mean ± S.E. of five human.

*: Glucuronic acid conjugate of NK-104 and NK-104 lactone.

ing the repeated administration. After enzymatic hydrolysis, the total excretion ratio of the glucuronic acid conjugate of NK-104 and NK-104 lactone was about 4% of the dose.

Discussion

In the present study, the interspecies variation on the pharmacokinetics and metabolism of NK-104 was investigated after intravenous and/or oral administration to animals and humans.

The plasma concentration of unchanged NK-104 and its lactone form after an intravenous administration to rats, rabbits, dogs and monkeys decreased triexponentially, and the terminal half-life ($t_{1/2}$) values were about 5 h in all species. The distribution volumes of steady-state (V_{ss}) and the plasma clearance (Cl_p) values showed a large difference. Therefore, marked species differences were observed in the distribution into systemic tissues, and in the systemic clearance.

After oral administration, NK-104 was well absorbed and the $t_{1/2}$ of the terminal phase in each species was similar to that after intravenous administration. Moreover, high bioavailability (BA) was observed in all species except monkeys. The relationship between oral dosage and AUC was apparent linear in the relatively high dose range in all species; however, the correlation lines were not extrapolated through the origin. Moreover, the improvement of BA was observed as the increase of dose in all species except monkeys. These results indicated that the saturated metabolism might affect the plasma concentration of minimum dose after oral administration. On the other hand, the results previously reported for humans,⁷⁾ are presented in **Fig. 7** for comparison with these animal data. The correlation line for humans was well extrapolated through the origin, even at the low dosage. The correlation line for humans was located between those of dogs and rats. In addition, NK-104 lactone was found in the plasma of dogs and humans as the major metabolite of this drug, suggesting that the pharmacokinetics of NK-104 in humans resemble those in dogs. We expected that the monkeys would show the pharmacokinetic model most similar to that of humans. In the monkeys, however, that NK-104 may be

Fig. 7 Relationships between area under the curve of the plasma concentration of NK-104 and dose in rats (Δ), rabbits ($\circ$), dogs ($\blacktriangle$), monkeys ($\bullet$) and humans ($\diamond$). Each point represents the mean ± S.E. of 3-6 animals or humans. Human data were quoted from a phase-one clinical study⁷⁾.

most markedly metabolized compared with the other animal models. In particular, the pharmacokinetic parameters of the AUC and BA in monkeys showed significantly lower values than in other species.

It has been reported that the BA of other HMG-CoA reductase inhibitors (statins), i.e. pravastatin, fluvastatin, and cerivastatin, was 37, 41 and 67%, respectively,⁸⁻¹⁰ in dogs. The BA of fluvastatin and cerivastatin in rats was 46% and 26%, respectively.^{10,11} However, the BA of NK-104 at 1 mg/kg was considerably high in dogs and rats of the present study (80% or more) compared with other statins. We suspect that NK-104 was excreted mainly into bile as the unchanged form and was reabsorbed by the entero-hepatic circulation, suggesting that the apparent BA of NK-104 in animals was high.

The lactone form of NK-104 was not detected in plasma after intravenous and oral administrations of NK-104 to rats; however, this metabolite was determined although at significantly lower levels than the parent drug in plasma of rabbits, dogs, and monkeys. In humans, the $C_{\max}$ of NK-104 lactone showed a level of approximately half in comparison with the parent drug, indicating that lactonization was the major metabolic pathway. Interestingly, the lactonization of NK-104 is a dehydrated reaction unparticipating with cytochrome P-450 enzymes. Since the HMG-CoA reductase inhibitors, lovastatin and simvastatin are lactone prodrugs that become active inhibitors upon hydrolytic conversion,¹² NK-104 lactone would be reversibly converted to NK-104 in humans.

The total excretion ratio of NK-104 and its lactone reached more than 40% of the dose independently of on the administration route in all animal species tested here except monkeys. In addition, the main excretion pathway of NK-104 in rabbits was the renal route. It has been also reported that several other statins are mainly excreted *via* bile in most animal species.^{11,13} However, the urinary excretion of ¹⁴C-fluvastatin in rabbits reached about 32% of the total radioactivity.¹⁴ These results suggest that the renal route may be a major excretion pathway of statins in rabbits. In rats and dogs, the unchanged NK-104 was excreted mainly in feces. The urinary excretion rates of NK-104 and NK-104 lactone in both species were low (less than 2% of the dose), indicating that the urinary excretion pattern in both rats and dogs resembles that in humans, suggesting that NK-104 may be excreted in feces *via* biliary excretion in humans.

We have previously reported that the biliary excretion of ¹⁴C-NK-104 was 75% up to 48 h after oral administration in rats and that most of this drug was subjected to entero-hepatic circulation.² The remarkable inhibitory effect of sterol synthesis in rat was observed until 6 hr after administration of NK-104 and the concentration of unchanged NK-104 in liver was about 70-fold higher than that in plasma.¹ These results suggested that the

entero-hepatic circulation of NK-104 related to the duration of drug effect. The metabolic pattern in rat bile was further examined in the present study. The unchanged NK-104 accounted for 32% of the dose in bile until 24 h after administration. On the other hand, the metabolites of NK-104 were also excreted in bile. Moreover, NK-104 and its metabolites were detected in dog feces, indicating that elimination pathway of this drug and metabolites were hepatobiliary excretion. It was recently reported that the carrier-mediated transporter contributes to the hepatobiliary excretion of many drugs.¹⁵ In particular, a primary active transport mechanism has been shown to be responsible for the biliary excretion of organic anions including glucuronic acid conjugates.^{16,17} Therefore, some metabolites of NK-104 are probably eliminated by the hepatobiliary transport mechanism because these metabolites are also organic anions as well as NK-104.

We also investigated the metabolic patterns of NK-104 in plasma of dogs; these patterns resemble the NK-104 pharmacokinetics in humans. No marked difference was observed in the ratios of metabolites between intravenous and oral administrations to dogs. The ratios occupied by the unchanged NK-104 in plasma after administration to dogs were much higher than those of every metabolite. NK-104 lactone and M-5 were detected as minor metabolites. In addition, M-5 and M-8, which were formed at the latter stage of the metabolic pathway, were maintained up to 8 h, and their elimination was also considerably slow at a dose of 10 mg/kg. These results suggest that although NK-104 was scarcely metabolized in dogs, the elimination rates of minor metabolites (M-5 and M-8) were lower than that of NK-104.

The M-5 and M-8 metabolized from NK-104 are products of β -oxidation of the heptenoic side chain, and they possess a mono-carboxylate moiety. It has been reported that the mono-carboxylate transport system plays important roles in the transport of short-chain mono-carboxylic acids at the plasma membrane of the heart and skeletal muscle.^{18,19} Since the retention of M-6 and M-8, β -oxidation products of NK-104, has been previously observed in these tissues of rats,² we suggest that M-5 and M-8 in plasma of dogs also may be transported across the plasma membrane *via* the mono-carboxylate transporter in the heart and skeletal muscle.

In the present study, we also determined NK-104 and its metabolites in plasma and urine after a repeated oral administration to healthy human volunteers at 2 mg/subject once a day for 5 days. The variations of inter-AUC and inter- $C_{\max}$ of NK-104 in humans were about 3 folds. However, no marked difference was noted between the humans and animals in the variation of inter-plasma concentration. After this oral administration to humans, the unchanged NK-104 and its lactone were mainly observed in plasma. On the other hand, a slight amount of metabolites were detected. These findings suggest that

NK-104 is hardly metabolized in the human liver more than in each animal species. The $t_{1/2}$ (13.1 h) of the terminal phase in humans was thus longer than those in the animal species studied (4–7 h) and these results indicated that NK-104 was subjected to entero-hepatic circulation in human.

Compared with the biotransformation of other statins, lovastatin was extensively metabolized by two major pathways, namely the cytochrome P-450-mediated oxidation of the fused-ring system and the β -oxidation of the heptanoic acid side chain after hydrolysis of the lactone ring.²⁰⁾ Fluvastatin was completely metabolized by the hydroxylation of the indole ring, the loss of the 1-isopropyl group, β -oxidation, lactone formation, the formation of the threo-isomer and conjugation with glucuronic acid or sulfate.²¹⁾ In contrast, pravastatin was metabolized by β -oxidation, lactone formation, isomerization and conjugation with glutathione.¹³⁾ It has been reported that cerivastatin is also susceptible to being metabolized by cytochrome P-450-mediated hydroxylation and β -oxidation.²²⁾ A remarkable difference was observed in the metabolic pathway between NK-104 and other statins in the ring hydroxylation, which is the major metabolic pathway of other statins.

We therefore find that the most interesting features of NK-104 is that it is hardly metabolized *via* cytochrome P-450 mediated oxidation and/or β -oxidation in humans. The pharmacokinetic properties of NK-104 in humans are good absorption, a relatively long $t_{1/2}$ and possibly high bioavailability. We speculated that these favorable metabolic and pharmacokinetic characteristics of this drug support efficacy in clinical studies^{23,24)} as a hypolipidemic and antiatherosclerotic agent in humans.

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正誤表

Vol. 14, No. 2(1999)掲載論文中の“NK-104の体内動態(4), (PP79-91)”に誤りがありました。お詫びして訂正いたします。

	正	誤
Vol. 14, No. 2		
81 頁 Table I 中 20 行目 Method-3 Mobile phase	(35 : 65)	(50 : 50)
83 頁 Table II 中 8 行目 Rat β (min^{-1})	0.0038 ± 0.0012	0.0036 ± 0.0011
84 頁 Table III 中 8 行目 Rat 0.3 mg/kg	n=3	n=4
87 頁 Fig. 6 文章中 5 行目	five	six
88 頁 右段本文中 1 行目	NK-104	NK-104 and its lactone form