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PHARMACOKINETICS OF NIPRADILOL (K-351), A NEW ANTIHYPERTENSIVE AGENT. I. STUDIES ON INTERSPECIES VARIATION IN LABORATORY ANIMALS

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The pharmacokinetics, plasma protein binding and metabolism of nipradilol (K-351: NIP), a new potent antihypertensive and antianginal agent, were compared in dogs, monkeys, rabbits and rats.

In all species studied, NIP did not appreciably bind plasma protein (less than 30%) and was extensively distributed in tissues. There was a good correlation between the volume of distribution at steady state (V_{ss}, l) and body weight (B, kg) of the animal species as follows: $V_{ss} = 4.42\ B^{0.805}$. In addition, V_{ss} increased as a function of the plasma free fraction. Intrinsic clearance of unbound drug (CLu_{int}, l/h) also correlated with body weight as follows: $CLu_{int} = 4.78\ B^{0.722}$, but blood clearance in rabbits exceeded hepatic blood flow, suggesting extrahepatic metabolism.

Following oral administration, the systemic availability for all species increased with the oral dose, while the half-life was about 2 h, and was independent of dose. The apparent threshold dose (ATD, mg/kg) was observed to vary inversely with body weight of the animal species as follows: $ATD = 4.33\ B^{-0.472}$.

Less than 2% of the dose was excreted into the urine as unchanged NIP in all species. The metabolic profile for all species was similar, but pronounced quantitative differences among species was observed for aliphatic and aromatic hydroxylation of the 3,4-dihydro-2*H*-1-benzopyran ring.

Keywords — nipradilol; antihypertensive agent; antianginal agent; pharmacokinetics; bioavailability; plasma protein binding; metabolism; interspecies variation; laboratory animal

INTRODUCTION

Recently, considerable attention has been paid to interspecies variation in the quantitative aspects of pharmacokinetics. Dedrick *et al.*[1] was first in treating pharmacokinetic parameters as functions of animal body weight, using the heterogonic relationship described by Adolph.[2] Thereafter, Boxenbaum[3–5] and Sawada *et al.*[6] reported good correlations between body weight and various pharmacokinetic parameters such as disposition half-life, intrinsic clearance and volume of distribution for many drugs, when data were plotted on a double logarithmic grid. Attempts were made extrapolations from animal data to humans. These methods may be of use to quantitatively predict the fate of a new drug in humans.

Nipradilol (K-351: 3,4-dihydro-8-(2-hydroxy-3-isopropylamino)propoxy-3-nitroxy-2*H*-1-benzopyran, NIP, Fig. 1) which is synthesized in our laboratories, is a new and potent antihypertensive and antianginal agent having beta-adrenoceptor blocking and vasodilating properties.[7,8]

Concerning the metabolic fate of NIP, recent studies on the structural determination of the urinary metabolites in dogs and humans have shown that NIP was metabolized by four principal metabolic pathways, *i.e.* denitration, hydroxylation of the ring system, degradation of the

side chain and glucuronidation.[9]

The present investigations were undertaken to compare various pharmacokinetic parameters of NIP in dogs, monkeys, rabbits and rats by the method described above. Moreover, we investigated urinary metabolic patterns in mice in addition to the four animal species noted elsewhere in this paper.

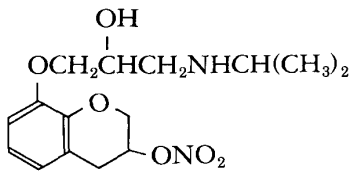

FIG. 1. *The Structure of Nipradilol (NIP)*

MATERIALS AND METHODS

Materials — NIP synthesized by Kowa Co., Ltd.,[10] was used. Various metabolites and internal standards used for identification and determination were prepared in our laboratories. All other chemicals used in this study were of analytical grade.

Animals — As experimental animals, male dogs (HRA-Beagles, Hazleton Research Animals Inc., U.S.A., 10—14 kg), male monkeys (Cynomolgus monkey, Shizuoka Laboratory Animal Center, Hamamatu, Japan, 4.2—4.6 kg), male rabbits (JW-NIBS, Nippon Institute for Biological Science, Tokyo, Japan, 2.3—2.8 kg), male rats (Jla: Wistar, Japan Laboratory Animal Ins., Tokyo, Japan, 245—260 g) and male mice (Std: ddY, Shizuoka Laboratory Animal Center, Hamamatu, Japan, 28—32 g) were used. They were maintained on a laboratory chow diet.

Drug Administration — i) *In Situ* Gastrointestinal Loop: Rat abdomen was opened under anesthesia with pentobarbital sodium (25—30 mg/kg) after 16 h-fasting, and multiple loops of about 10 cm long were prepared from stomach, duodenum, jejunum and ileum according to the method of Taniguchi *et al.*[11] Bile duct was ligated, and then 1 ml of NIP, aqueous solution (100 μg/ml), was injected into each loop. After 0.5 or 1 h, the loop was removed, and the interior of the loop was washed with 10 ml cold saline.

ii) Intragastric and Intraduodenal Administration: NIP was given intragastrically or intraduodenally at a dose of 10 mg/kg as an aqueous suspension in 0.5% carboxymethyl cellulose (CMC) in a volume of 0.2 ml/100 g to pylorus-ligated rats under anesthesia with pentobarbital sodium (25—30 mg/kg). Blood (0.5 ml) was obtained from the femoral artery through cannulation, and the lost blood was replenished by infusion from another rat's blood containing sodium citrate *via* a cannula into the femoral vein.

iii) Oral Administration: Dogs, monkeys, rabbits, rats and mice were given NIP in single oral doses of 1—30 mg/kg under unanesthesia. The oral dose was administered to dogs and monkeys as a solid material in gelatin capsules, or to rabbits, rats and mice as a suspension in a volume of 1, 0.2 ml/kg and 0.2 ml/10 g, respectively. The animals were kept individually in stainless steel metabolic cages, and fasted for 18 h prior to the experiments, but had free access to water. Blood was collected from the front leg vein in dogs, from the femoral vein in monkeys, from the ear vein in rabbits and from the jugular vein in rats. Plasma was separated from the cells after centrifugation of the blood samples and frozen. Urine was collected at 24 h intervals for 2 d and frozen. The frozen specimens were subsequently assayed.

iv) Intravenous Administration: Dogs, monkeys and rabbits were given intravenously 1 mg/kg NIP as an aqueous solution (volume: 0.1, 0.2 and 0.4 ml/kg, respectively) containing equimolar hydrochloride because of poor solubility. In order to reduce the volume of the blood sample, higher dose (3 mg/kg) was given to rats in a volume of 0.2 ml/100 g. Intravenous injection was made in the front leg vein in dogs, in the femoral vein in monkeys, in the ear vein in rabbits and *via* the tail vein in rats for 30 s.

Protein Binding Studies — The protein binding of NIP was determined in monkeys, rabbits and rats by an equilibrium dialysis method.[12] Plasma was obtained from the above oral administration study and plasma concentrations of

50—400 ng/ml were used. Dialysis was performed against 1/15 M phosphate buffer (pH 7.4) for overnight at 37 °C.

Analytical Methods—NIP and Denitro Nipradilol (DNIP), one of major metabolites, in blood or plasma were determined by a selected ion monitorning method (SIM) using gas chromatography-mass spectrometry (GC-MS) after separative extraction and derivatization. Aliquots of blood or plasma (0.1—1.0 ml) were adjusted to pH 8 with 0.5 M phosphate buffer, and NIP was extracted with benzene (6 ml). The benzene layer was then extradcted with 1.5 ml of 3 M acetic acid containing 20 ng of 3,4-dihydro-3-hydroxymethyl-8-(2-hydroxy-3-isopropylamino)propoxy-2*H*-1-benzopyran as an internal standard (I.S.). The nitroxy group in NIP was reduced to a hydroxy group with zinc dust (50 mg) in the acetic acid solution. The reduction product was extracted with benzene under a strong basic condition and derivatized to the tri-acylated compound with heptafluoro-*n*-butyric anhydride and trimethylamine for SIM, as described previously.[9] The selected ion used was *m/z* 466 for both NIP and I.S. as the derivatized isopropylaminopropanol side chain.

To the aqueous layer that remained after the extraction at pH 8, the aqueous solution of the above I.S. (20 ng/0.2 ml) was added, and DNIP

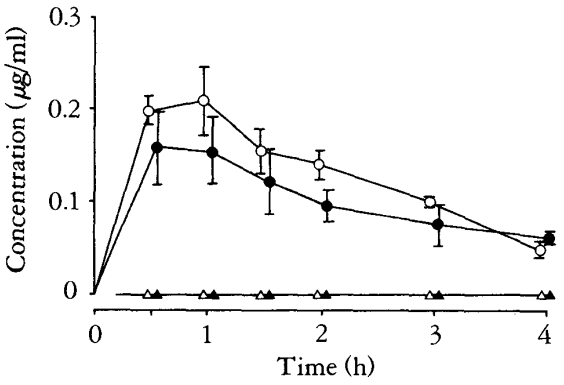

FIG. 2. *Mean Plasma Levels of NIP (○, △) and DNIP (●, ▲) after Intraduodenal or Intragastric Injection of NIP Suspension to Pylorus-Ligated Rats at a Dose of 10 mg/kg*

The circles and triangles represent plasma levels after intraduodenal and intragastric injection, respectively. Each point represents mean ± S.E. (n=3).

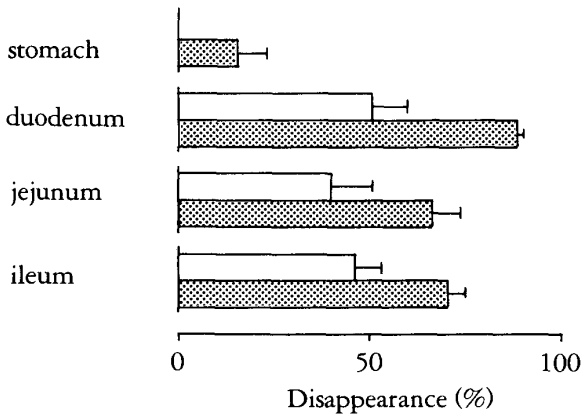

FIG. 3. *Disappearance of NIP from Different Regions of Rat Gastrointestinal Loop*

Clear and cross areas represent fraction of disappearance in 30 and 60 min, respectively. The data are expressed as mean ± S.E. (n=4).

TABLE I. *Plasma Unbound Fraction (fu) and Blood-to-Plasma Concentration Ratio (R_B) of NIP in Different Species*

Species	fu (%)	R_B
Dog	70.7±1.0 (*n*=12)[a]	0.94±0.04 (*n*= 6)
Monkey	74.1±1.3 (*n*= 6)	1.13±0.04 (*n*= 6)
Rabbit	83.2±1.6 (*n*= 7)	1.30±0.03 (*n*= 6)
Rat	79.1±1.2 (*n*= 7)	1.08±0.04 (*n*=14)

a) Data from Ohshima et al. of the Tokyo Research Laboratories of this Division (unpublished data). Data are expressed as mean ± S.E.

was extracted with benzene (6 ml) under a strong basic condition. DNIP was directly derivatized and determined with the above method.

Plotting peak height ratio to I.S. as ordinate *vs.* concentration of NIP or DNIP as abscissa gave linear calibration curves, and the detection limit after extraction from 1 ml blood or plasma was less than 0.5 ng for both compounds. The coefficients of variation of the peak height ratio corresponding to concentration of 1 ng/ml NIP and DNIP in plasma were 4.7% and 6.2%, respectively.

Urinary metabolites were separated into basic and acidic fractions and were determined by SIM after derivatization into the above acyl compound and methylester-trimethylsilyl ether, respectively. The details of these procedures were described in a previous report.[9]

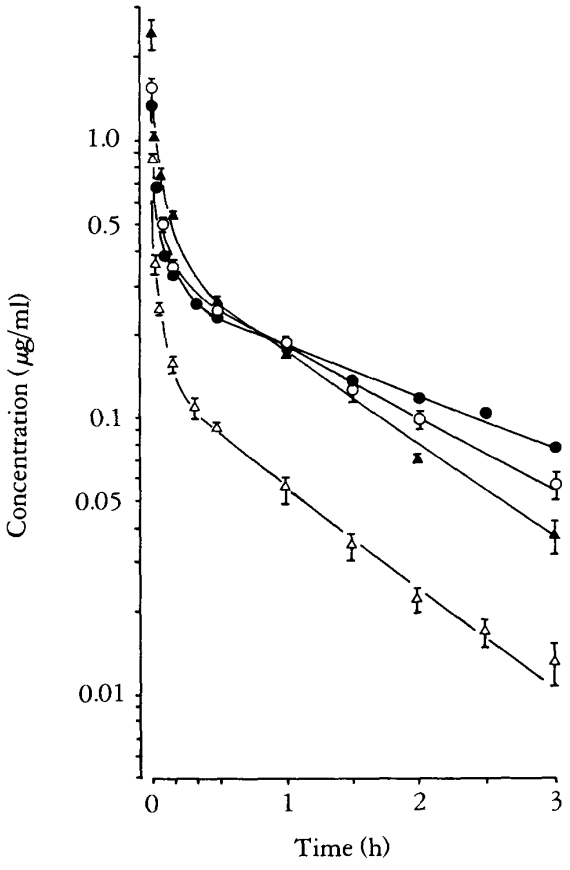

FIG. 4. *Mean Plasma Levels of NIP in Different Species after Intravenous Administration of NIP Solution*

NIP was given to dogs (○, n=5), monkeys (●, n=2), rabbits (△, n=4) at a dose of 1 mg/kg, or to rats (▲, n=4) at a dose of 3 mg/kg. Each point represents the mean ± S.E.

Gas Chromatography-Mass Spectrometry (GC-MS) — GC-MS was performed on a JEOL model JMS-DX300 mass spectrometer coupled to a JEOL model G05 gas chromatograph. Silanized glass columns, 1 m × 2 mm i.d., were packed with 3% OV-17 and 3% OV-1 on Gas Chrom Q (80–100 mesh) and maintained at 210 and 245 °C for determination of the basic and acidic metabolites, respectively. The accelerating voltage, ionization voltage and trap current were 3.0 *k*V, 70 eV and 300 μA, respectively.

Pharmacokinetic Calculations — The plasma concentration-time curves of NIP after intravenous administration could be adequately described by a biexponential equation, $C_p = Ae^{-\alpha t} + Be^{-\beta t}$. The observed data was fitted the biexponential equation using a nonlinear least-squares curve fitting program[13] with the weighting factor of C_p^{-2}. Pharmacokinetic parameters were calculated using standard equations[14] for a two-compartment open model with elimination from the central compartment. The elimination half-life ($T_{1/2}$) for oral data was determined by least-squares regression of the log-linear portion of the curve. Area under the plasma level-time curve (AUC) value was obtained by the trapezoidal rule with extrapolation to infinity using the calculated $T_{1/2}$ value. Systemic availability was calculated from the ratio of the AUC values obtained after oral and intravenous administration.

RESULTS

Gastrointestinal Absorption

The absorption site was studied on pylorus-ligated rats after intragastric or intraduodenal administration (Fig. 2). Plasma levels of NIP after intraduodenal administration rose rapidly and reached a peak within 1 h. On the other hand, no drug could be detected in the plasma after intragastric administration. Comparing the

absorption of NIP in different regions of the rat gastrointestinal tract *in situ*, it was found that NIP is easily absorbed in all regions of small intestine except the stomach (Fig. 3). The fraction of disappearance from the duodenum loop was the highest, about 90% for 1 h.

Plasma Protein Binding

The binding of NIP to plasma proteins was studied using the plasma obtained from oral dose-ranging studies. The resulting unbound fraction (fu) in different species is listed in Table I. The mean value of fu was more than 0.7 in all species studied, indicating little binding to plasma proteins.

Plasma Levels

After intravenous administration of NIP to dogs, monkeys, rabbits and rats, plasma levels of NIP showed a biexponential decline as illustrated in Fig. 4. In all species studied, the distribution was quite rapid ($T_{1/2,\alpha}$=1−2 min), and the volume of distribution at steady state (V_{ss}) was extremely large (more than 2.4 l/kg, Table II). Rabbits and rats had lower plasma levels and shorter half-life of NIP in the terminal phase ($T_{1/2,\beta}$) and therefore the plasma clearance (Cl_p) was about 3 times greater than the Cl_p for those of dogs and monkeys.

After oral administration of 1−30 mg/kg NIP, maximum concentration (C_{max}) in dogs, rabbits and rats was achieved within 1 h, independent of dose, whereas the peak time (T_{max}) in monkeys tended to be later with increasing dose (Fig. 5). The plasma levels of NIP in rabbits were the lowest among the species studied, because of a considerable first-pass metabolism which will be described later. The half-time ($T_{1/2}$) for the elimination phase was about 2 h in all species studied.

Partition of NIP between plasma and red blood cell was measured in the oral study. The blood-to-plasma concentration ratios (R_b) in four species is shown in Table I and NIP was found to be nearly equally distributed between the plasma and the red blood cell in all species studied.

Upon oral administration of NIP, DNIP as major metabolite was detected in plasma as shown in Fig. 6. DNIP appeared rapidly in

TABLE II. *Pharmacokinetic Parameters Obtained from a Two-Compartment Open Model after Intravenous Administration of NIP in Different Species*

Parameter		Dog (n=5)	Monkey No. 1	Monkey No. 2	Rabbit (n=4)	Rat (n=4)
B.W.	(kg)	13.0 ±1.5	4.5	4.2	2.38±0.06	0.25±0.0
Dose	(mg/kg)	1.0 ±0.0	1.0	1.0	1.0 ±0.0	3.0 ±0.0
A	(μg/ml)	2.19±0.28	1.06	1.33	0.96±0.31	2.63±0.55
B	(μg/ml)	0.34±0.03	0.24	0.32	0.14±0.01	0.40±0.03
α	($min^{-1}\times10^{-1}$)	6.80±1.24	4.16	2.96	4.42±0.46	4.14±0.30
β	($min^{-1}\times10^{-1}$)	0.11±0.00	0.06	0.08	0.16±0.05	0.14±0.01
$T_{1/2,\alpha}$	(min)	1.14±0.17	1.67	2.34	1.62±0.16	1.70±0.13
$T_{1/2,\beta}$	(min)	65.97±2.91	109.68	86.97	44.81±1.51	51.19±4.79
k_{12}	($min^{-1}\times10^{-1}$)	5.19±1.08	3.08	2.03	2.83±0.30	2.73±0.20
k_{21}	($min^{-1}\times10^{-1}$)	1.00±0.13	0.82	0.64	0.75±0.09	0.70±0.07
k_{el}	($min^{-1}\times10^{-1}$)	0.71±0.08	0.32	0.37	1.00±0.25	0.85±0.14
V_c	(l/kg)	0.43±0.05	0.77	0.61	1.07±0.19	1.12±0.20
V_{ss}	(l/kg)	2.45±0.17	3.64	2.55	4.88±0.36	5.27±0.70
AUC	(μg·min/ml)	35.85±2.78	40.74	44.38	11.00±0.90	35.31±0.95
Cl_p	(ml/min/kg)	28.58±2.26	24.55	22.53	93.09±8.99	85.14±2.24

Data are expressed as mean ± S.E.

plasma and declined with $T_{1/2}$ of 2–3 h. In all species, except for dogs, the levels of DNIP were higher than the levels of the unchanged drug, and were independent of dose.

Bioavailability

The pharmacokinetic parameters calculated from the oral data in different species are summarized in Table III. Marked interspecies variations were noted in *AUC* and the systemic availability (*F*), *i.e.* the fraction of the dose available for systemic circulation.

A graph of the *AUC vs.* dose for each animals is provided in Fig. 7. In all species studied, a relationship between *AUC* and oral dose was linear in the range of high dose, but the straight line showing the correlation was not extrapolated through the origin. Species differences existed with apparent threshold dose (ATD), which was estimated with the zero-*AUC* intercept obtained by extrapolation of the linear portion of the curve (the solid lines in Fig. 7). ATD was 1.09 mg/kg for dogs, 2.46 mg/kg for monkeys, 2.94 mg/kg for rabbits and 7.59 mg/kg for rats, indicating that the larger animals in terms of body weight tend to possess smaller ATD values.

The systemic availability was varied with the dose in all species, and its characteristics were different: in rabbits, the systemic availability was the lowest among the species and increased slightly with the dose, while in dogs and monkeys, high availability and considerable increments with the dose were observed.

Urinary Metabolite Patterns

The isolation and characterization of the urinary metabolites in dogs have been reported earlier.[9] The identification of urinary metabolites in monkeys, rabbits, rats and mice was carried out by comparison of their mass spectral fragmentation with those of authentic samples synthesized or isolated from dog urine. Table IV lists the quantities of metabolites identified in the 0–48 h urinary fraction after oral adminis-

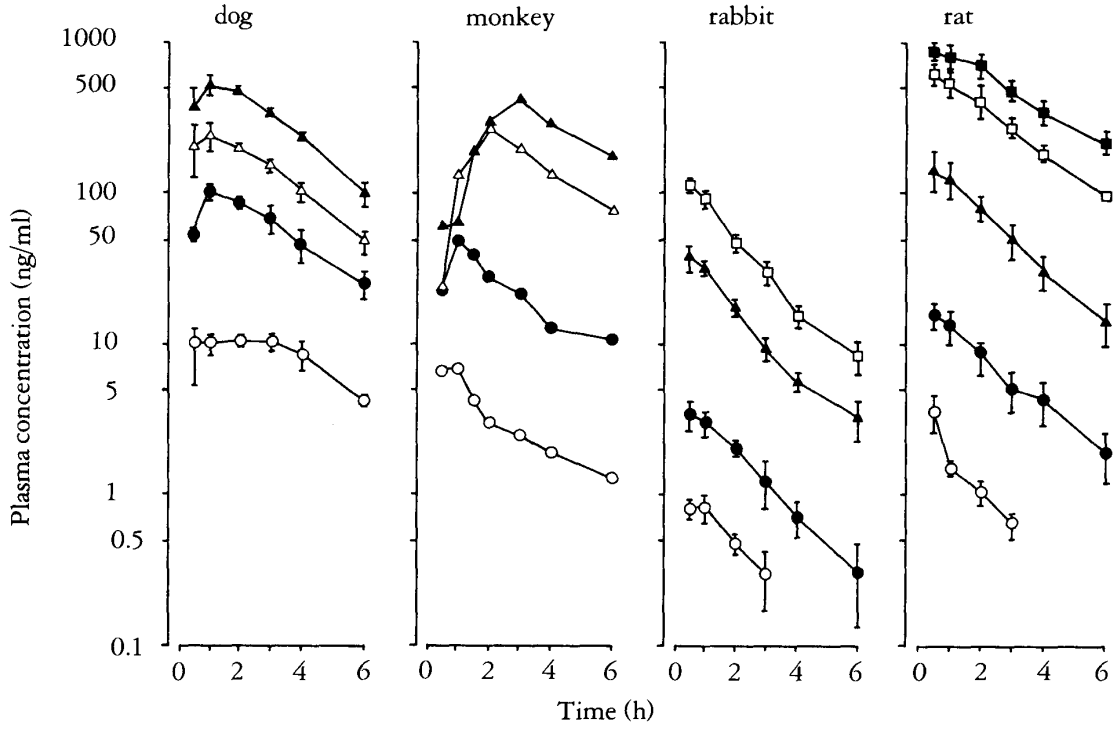

FIG. 5. *Mean Plasma Levels of NIP in Different Species after Oral Administration of NIP at Dose of 1 mg/kg (○), 3 mg/kg (●), 6 mg/kg (△), 10 mg/kg (▲), 20 mg/kg (□) and 30 mg/kg (■) Each point represents the mean ± S.E. Number of experiments are indicated in Table III.*

tration of 1 mg/kg NIP. In five species, less than 2% of the dose was excreted as unchanged NIP in the urine. NIP-glucuronide was also detected in the urine, but the amount in the animal species, except monkeys (7.1%), was less than 2%. As estimated by summation of free and conjugated DNIP, approximately 2% of the dose was excreted in rabbits and rats, whereas larger amounts were observed in monkeys (13.6%), dogs (8.5%) and mice (8.1%). Monohydroxylated derivatives, one of the major urinary metabolites in the five animals, were excreted mainly as 4-hydroxy metabolites by monkeys and rabbits and as 5-hydroxy metabolite by dogs and rats. In mice, almost equivalent amounts of both metabolites were excreted. Among the five species, dogs excreted the largest amount of N-deisopropyl and N-methyldeisopropyl metabolites, products of the dealkylation and subsequent N-methylation of the side chain. On the other hand, rabbits excreted the highest amount of DNIP-lactic acid which is produced by oxidative deamination of the side chain.

DISCUSSION

This paper contains data on certain basic pharmacokinetics of NIP in four species, dogs, monkeys, rabbits and rats.

NIP was found to be completely absorbed in all regions of the small intestine, in particular from the duodenum (Figs. 2 and 3), and peak plasma levels of NIP were seen within 2 h after oral dosing in all species studied.

In spite of complete absorption, orally administered NIP underwent first-pass metabolism leading to low bioavailability (Table III). In regards to the presystemic metabolism of NIP, recent studies on bioavailability in dogs with implanted cannula in the portal vein have shown that the reduction in the systemic availability could be partly attributed to denitration and glucuronidation of NIP. This metabolism may occur in the liver and/or the intestinal tract.[15]

Marked species differences were observed in the plasma levels after oral administration.

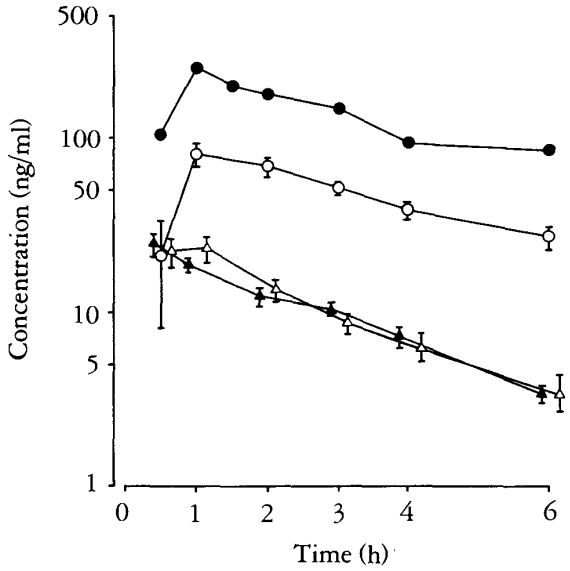

FIG. 6. *Mean Plasma Levels of DNIP in Different Species after Oral Administration of NIP*

NIP was given to dogs (○, n=3), monkeys (●, n=2), rabbits (△, n=5) and rats (▲, n=3) at a dose of 3 mg/kg. Each point represents the mean ± S.E.

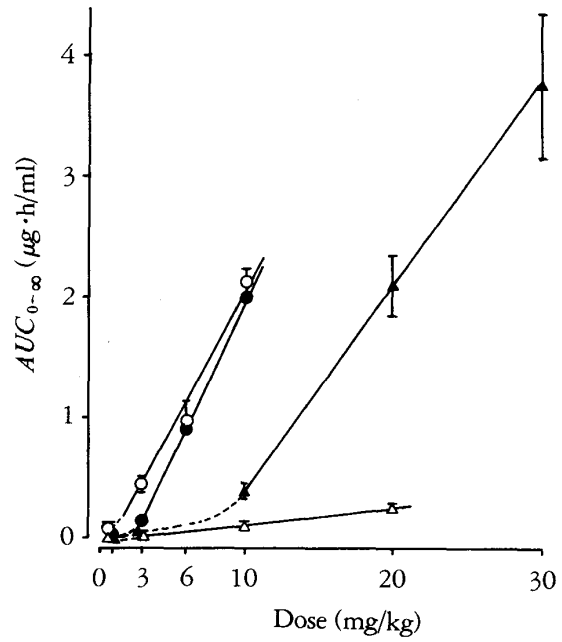

FIG. 7. *Relationships between Area under Curve of the Plasma Levels of NIP vs. Time ($AUC_{0-\infty}$) and Dose in Dogs (○), Monkeys (●), Rabbits (△) and Rats (▲)*

Each point represents the mean ± S.E. Number of experiments are indicated in Table III.

TABLE III. *Pharmacokinetic Parameters after Oral Administration of NIP in Different Species*

Animal	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/ml)	$T_{1/2}$ (h)	*AUC* (μg.h/ml)	*F*[a] (%)
Dog	1 (n=4)	1.5±0.6	17± 1	1.9±0.2	0.064±0.004	10.7± 1.0
	3 (n=3)	1.7±0.7	113± 14	2.0±0.2	0.428±0.048	23.9± 2.7
	6 (n=3)	1.3±0.3	255± 40	1.8±0.1	0.960±0.156	26.8± 4.4
	10 (n=3)	1.3±0.3	554± 61	1.9±0.1	2.140±0.117	35.9± 3.8
Monkey	1 (n=2)	0.8	8	2.2	0.022	3.1
	3 (n=2)	1.0	49	2.1	0.162	7.6
	6 (n=2)	1.8	265	2.2	0.917	21.6
	10 (n=2)	3.0	431	2.4	2.032	28.7
Rabbit	1 (n=5)	0.8±0.1	1± 0	1.4[b]	0.002[b]	1.2[b]
	3 (n=5)	0.9±0.3	4± 1	1.2±0.2	0.010±0.002	2.2± 0.5
	10 (n=5)	0.6±0.1	42± 7	1.3±0.1	0.088±0.010	4.8± 0.5
	20 (n=5)	0.5±0.0	116± 14	1.4±0.1	0.255±0.027	7.0± 0.7
Rat	1 (n=3)	0.8±0.2	4± 1	1.7[b]	0.006[b]	3.0[b]
	3 (n=3)	0.5±0.0	16± 3	1.8±0.0	0.045±0.012	7.6± 2.0
	10 (n=4)	1.1±0.3	170± 37	1.6±0.3	0.402±0.071	20.5± 3.6
	20 (n=4)	0.6±0.1	687± 90	2.1±0.2	2.112±0.266	53.8± 6.8
	30 (n=4)	1.0±0.4	969±121	2.3±0.1	3.786±0.592	64.3±10.0

a) Systemic availability. b) Parameter was calculated from the mean plasma level-time course. Data are expresed as mean ± S.E.

TABLE IV. *Mean Urinary Excretion of NIP and Its Metabolites during 48 h after Oral Administration of 1 mg/kg NIP in Different Species*

Metabolites	Percentage of dose									
	Dog		Monkey		Rabbit		Rat		Mouse	
	F.[a]	Sum[b]	F.	Sum	F.	Sum	F.	Sum	F.	Sum
A) NIP	1.9	3.5	0.8	7.8	0.7	0.8	0.3	0.3	1.1	2.8
B) DNIP	4.4	8.5	8.5	13.6	2.1	2.2	1.0	1.5	8.1	8.1
C) 4-Hydroxy NIP	0.7	1.4	1.7	5.6	1.3	1.7	n.d.	n.d.	n.d.	n.d.
D) 4-Hydroxy DNIP	1.6	1.6	5.7	6.4	5.7	6.1	1.6	1.8	5.7	7.0
E) 5-Hydroxy NIP	0.9	3.7	n.d.	0.2	n.d.	0.9	0.5	1.6	n.d.	n.d.
F) 5-Hydroxy DNIP	3.0	3.4	0.5	0.5	0.4	3.2	0.6	3.3	n.d.	7.1
G) *N*-Deisopropyl NIP	3.5	4.4	1.1	1.1	0.7	0.7	0.6	0.6	n.d.	0.7
H) *N*-Deisopropyl DNIP	2.8	5.1	1.2	1.4	1.3	1.3	0.8	0.8	1.7	1.7
I) *N*-Methyldeisopropyl NIP	0.5	1.6	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
J) *N*-Methyldeisopropyl DNIP	1.5	2.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
K) NIP glycol	0.6	1.0	0.2	1.7	n.d.	n.d.	0.2	0.2	n.d.	n.d.
L) DNIP glycol	1.9	3.8	0.8	2.9	n.d.	n.d.	0.4	0.9	n.d.	n.d.
M) DNIP lactic acid	3.7	4.3	5.9	5.9	15.0	15.0	1.3	1.3	0.9	0.9
N) DNIP acetic acid	1.6	1.6	0.7	0.7	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total	28.6	46.1	27.1	47.8	27.2	31.9	7.3	12.3	17.5	28.3

a) Free metabolites. b) Summation of free and conjugated metabolites.

In rabbits, the plasma concentration and the systemic availability were the lowest among the species studied. This might be attributed to the large value of plasma clearance (Cl_p) and volume of distribution (V_{ss}) in rabbits, when compared with those observed in dogs and in monkeys (Table II). Furthermore, protein binding probably affects Cl_p and V_{ss} values as discussed by Evans *et al.* [16] in the case of a basic drug, propranolol. Relatively little NIP was found bound to the plasma proteins (less than 30%) in all species studied, particularly in rabbits where the bound NIP (17%) was the smallest among the species studied (Table I). In order to solve the above complexities of interspecies variation, we attempted to treat these pharmacokinetic parameters as a function of animal body size.[1,3-6] Details of the quantitative aspects of pharmacokinetics will be discussed later, but for the present, these aspects are summarized as follow: the large value of V_{ss} in rabbits expresses interspecies variation in the plasma free fraction. The reason for the low bioavailability in rabbits may be that clearance in rabbits, presumably by presystemic metabolism in the intestinal tract, is different from those of the other species.

The $T_{1/2}$ of orally administered NIP averaged 2 h in all species and was longer than that of intravenous administration. The longer $T_{1/2}$ may be due to continuous absorption which presumably occurs under the following conditions: NIP reduces cardiac output[7] and consequently causes significant reduction of mesenteric blood flow.[17]

After intravenous administration, NIP was rapidly and extensively distributed in tissues and then declined with $T_{1/2,\beta}$ of 0.7—1.6 h. V_{ss} of NIP was about 6-times greater than total body water[3] in all species studied. This extensive tissue distribution of NIP was consistent with the results of radiochemical analysis in rats. Unchanged ^{14}C-NIP showed high affinity in various tissues except the brain, and the tissue-to-blood concentration ratios of NIP was 30 in

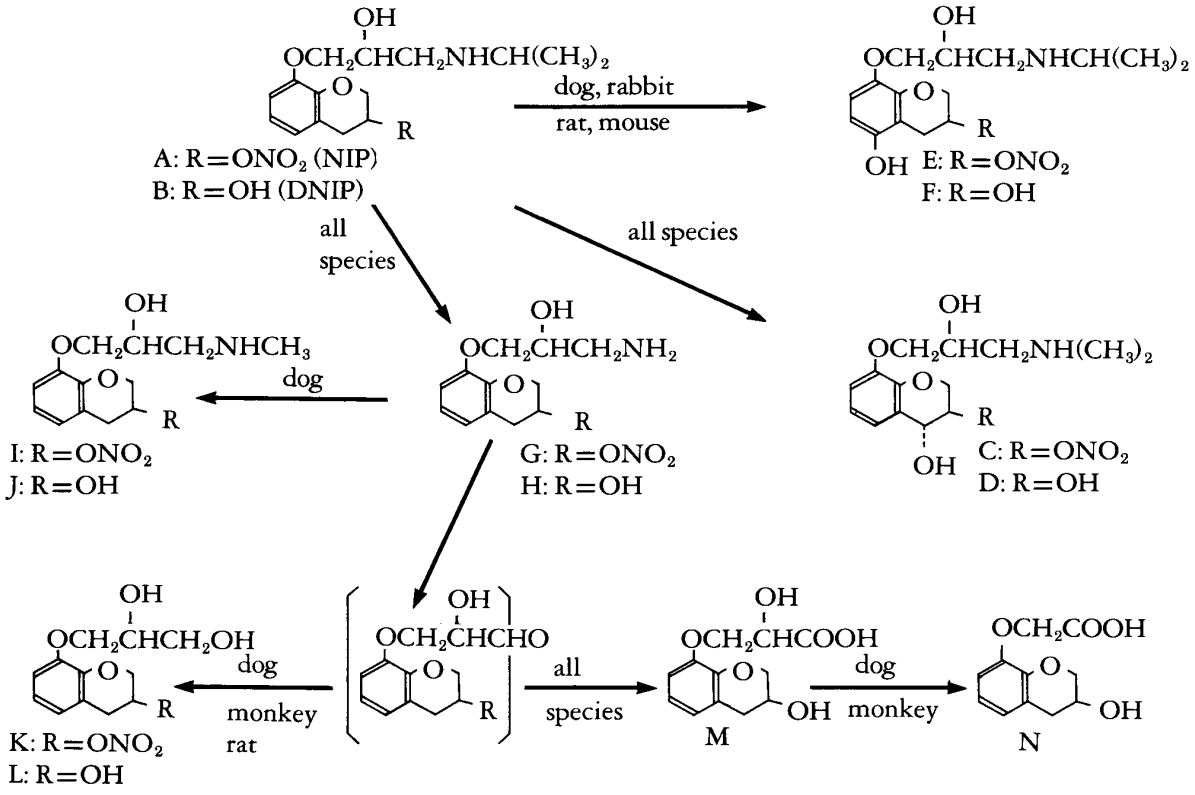

FIG. 8. *Possible Metabolic Pathways of NIP in Different Species*

kidney, 25 in lung, 8 in heart, and 6 in inferior vena cava, which are considered target organs (unpublished data).

In pharmacodynamic and pharmacokinetic studies of NIP after intravenous administration (1 mg/kg) in rabbits, Kawashima *et al.*[18] reported that $T_{1/2,\beta}$ was 1.2 h, and that NIP caused a significant reduction of the resting heart rate, but had no significant effect on blood pressure. However, Uchida *et al.*[8] reported that in dogs, NIP lowered blood pressure after intravenous dose of 10 μg/kg. Based on present studies, these inerspecies variations in pharmacological effects can be partly attributed to the differences in the plasma levels, namely, in clearance (Table II).

In all species studied, less than 2% of the dose was excreted into the urine, suggesting that NIP was eliminated mainly by metabolism; renal clearance was minimal. The total urinary excretion was less than 50% of the dose (Table IV). The reason for the relatively low total urinary recovery may be due to biliary excretion, since 36 and 63% of the oral administration of ^{14}C-NIP in rats (unpublished data) were excreted in urine and *via* the bile in faces, respectively.

Many metabolites identified in urine were found in all species, showing quantitative rather than qualitative differences. A summary of a proposed metabolic pathway of NIP in several animals is shown in Fig. 8. The major urinary metabolites arise from four metabolic pathways, namely, denitration by glutathione (GSH)-dependent organic nitrate reductase, hydroxylation of the ring system by cytochrome P-450, degradation of the isopropylaminopropanol side chain and glucuronidation. The side chain is metabolized through a variety of metabolic process including oxidative dealkylation, deamination and N-methylation. Dealkylation by cytochrome P-450 and/or amine oxidase[19] produces primary amines, which may be oxidatively deaminated by monoamine oxidase[20] to yield aldehyde intermediates. Subsequently, the intermediate is reduced to glycols (metabolites K and L in Fig. 8) or oxidized to an acidic metabolite (M in Fig. 8). N-Methylation of the primary amines, which was found only in dogs, as reported for most beta-blockers,[20] seems likely to be catalyzed by phenylethanolamine N-methyltransferase or by a nonspecific N-methyltransferase.[21] A detailed description of these biotransformation steps in NIP is reported in a previous paper.[9]

Quantitatively, pronounced species dif-

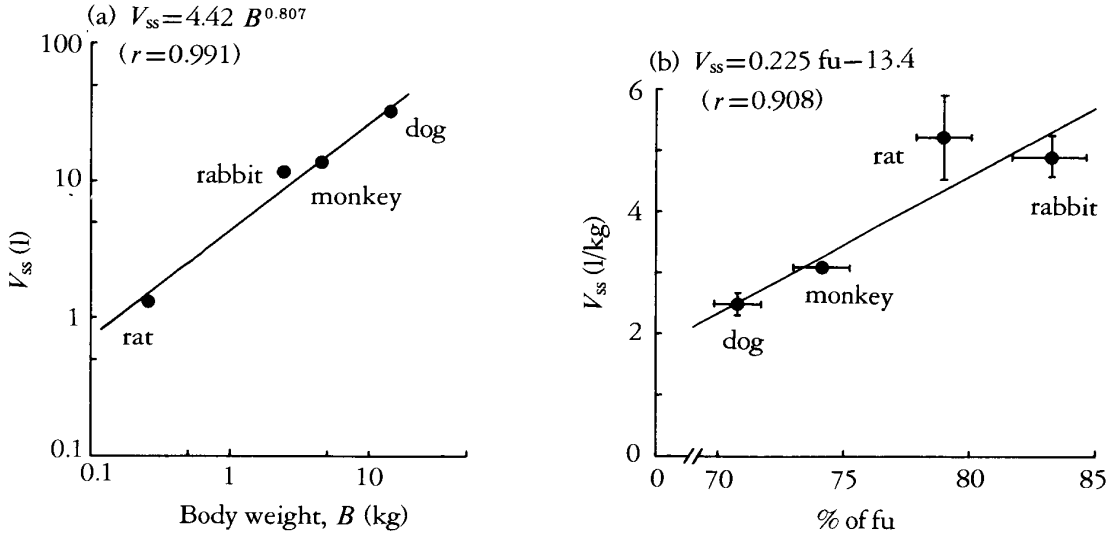

FIG. 9. *Correlation between Volume of Distribution at Steady State (V_{ss}) of NIP and Body Weight (B, panel a) or Plasma Unbound Fraction (fu, panel b) in Mammals*

ference was observed in the aliphatic and aromatic hydroxylation of the 3,4-dihydro-2*H*-1-benzopyran ring. The former was a major pathway in monkeys and rabbits whereas the latter was favored in dogs and rats (Table IV). In an early study,[9] only aliphatic hydroxylation was observed in humans, and its product was found to show pseudo-axial *trans*-conformation. For the aromatic hydroxylation process, an arene oxide-NIH shift pathway may be operative, as in the hydroxylation of the aryloxy beta-blocking drugs.[22,23] These biotransformations, that proceed by different mechanisms and illustrate species difference are of particular interest.

Using the linear relationship between physiological parameters and animal body weight on a double logathmic grid,[2] interspecies variations in the quantitative aspects of pharmacokinetics, namely, correlations between pharmacokinetic parameters and animal body size, will be discussed below.

When V_{ss}, obtained after intravenous administration, was plotted against body weight (B) on a double logarithmic grid, it was found that V_{ss} (l) was related to body weight (kg) with the correlation coefficient of 0.991. The relationship is as follows (Fig. 9 (a)):

$$V_{ss}=4.42\ B^{0.807} \quad (1)$$

The value of V_{ss} in rabbits was observed to deviate considerably from the regression line. However, that may be explained mainly by the difference in the plasma free fraction (fu) among animal species, since V_{ss} increased as a function of fu in all species studied (Fig. 9 (b)). It can be considered that distribution of NIP into tissues is dependent on free drug concentration in the circulation because the blood-to-plasma concentration ratio (R_b) also increased as free drug in the plasma increased (Table I). Thus, these findings show that binding of NIP to plasma protein determines the relative amounts of drug in blood and tissues.

The intrinsic clearance of unbound drug (Clu_{int}) can be expressed as follows:[5,24]

$$Clu_{int}=D_{i.v.}/AUC_{i.v.,p}/fu=Cl_p/fu \quad (2)$$

where $D_{i.v.}$ is intravenous dose, and $AUC_{i.v.,p}$ is the area under the plasma curve ($0-\infty$) following *i.v.* administration. Figure 10 (a) illustrates

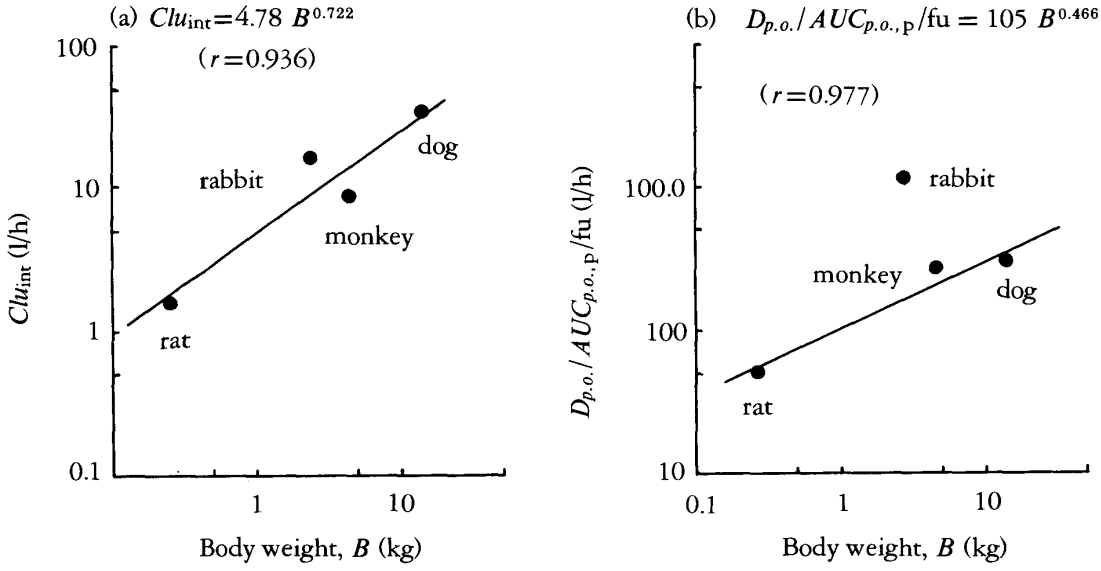

FIG. 10. *NIP Intrinsic Clearance of Unbound Drug (Clu_{int}, panel a) and $D_{p.o.}/AUC_{p.o.,p}/fu$ (panel b) in Mammals as a Function of Body Weight (B)*

The value of $D_{p.o.}/AUC_{p.o.,p}/fu$ was obtained after the oral administration at the dose of 1 mg/kg. Regression line in panel b dose not utilized rabbits data point (see text for Discussion).

the log-log relationship between Clu_{int} and body weight. Clu_{int} was proportional to the 0.722 power of body weight as follows:

$$Clu_{int}=4.78\ B^{0.722} \quad (3)$$

The value of Clu_{int} in rabbits did not fit this heterogonic relationship. This could be ascribed to extrahepatic metabolism because the total blood clearance (Cl_b, $Cl_b=Cl_p/R_b$) and Cl_p in rabbits exceeded the hepatic blood flow (Q_h) reported for rabbits.[3] The intrinsic clearance obtained after oral administration ($Clu_{int,p.o.}$) can be estimated from the equation, $Clu_{int,p.o.}=D_{p.o.}/AUC_{p.o.,p}/fu$. The double logarithmic relationship between $Clu_{int,p.o.}$ at the dose of 1 mg/kg and body weight gave a good coefficient ($Clu_{int,p.o.}=105\ B^{0.466}$, $r=0.977$) if the rabbit data point is not utilized (Fig. 10 (b)). In rabbits $Clu_{int,p.o.}$ deviated considerably from the regression line compared in the case of Clu_{int}. Therefore, the low systemic availability in rabbits could be attributed to a large value of $Clu_{int,p.o.}$, which might be caused by an extensive presystemic metabolism in the intestinal tract. Relationships between Clu_{int} or $Clu_{int,p.o.}$ and body weight shows that as the body weight increased, the above parameters per unit body weight decreased. These findings may be explained by interspecies variation in the hepatic enzyme activity, since GSH-dependent organic nitrate reductase which catalyzed the denitration of NIP (unpublished data) or microsomal monooxygenase[25] varied inversely with body weight.

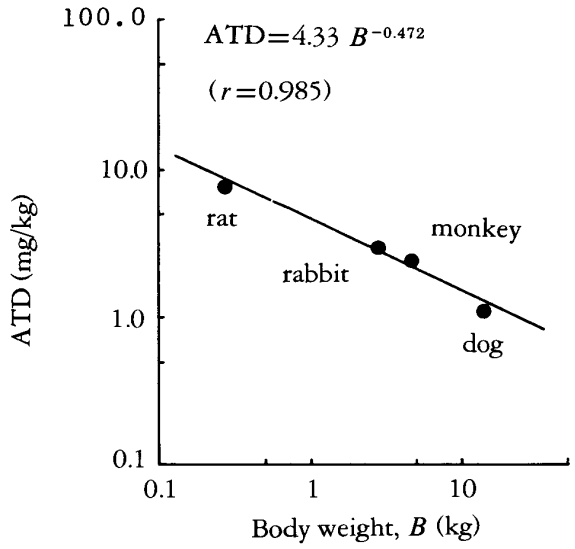

FIG. 11. *Apparent Threshold Dose (ATD) of NIP in Mammals as a Function of Body Weight (B)*

Systemic availability was increased with the oral dose in all species studied (Table IV). Marked species difference was seen in the apparent threashold dose (ATD, mg/kg), with the larger animals exhibiting the smaller ATD as follows (Fig. 11):

$$ATD=4.33\ B^{-0.472} \quad (4)$$

These findings suggest that as the body weight increased, presystemic drug metabolism saturated at lower doses. Although data on the hepatic metabolism *in vitro* are not shown here, we observed that larger animals possessed smaller values of Michaelis constant in the denitration of NIP. Therefore, it seems likely that interspecies variation in ATD is closely related to the differences in the enzyme activity and affinity in the eliminating organ, liver.

Thus, analysis of pharmacokinetic parameters as a function of body size of animal species will provide useful clues for solving interspecies variation in pharmacokinetics. The prediction of pharmacokinetics in man will be described in a succeeing paper.

REFERENCES

1) R. L. Dedrick, D. D. Forester, J. N. Cannon, S. M. ElDarren and L. B. Mellett: Pharmacokinetics of 1-β-D-arabino-furanosylcytosine (Ara-c) deamination in several species, *Biochem. Pharmacol.*, **22**, 2405–2417 (1973).
2) E. F. Adolph: Quantitative relationships in the physiological constitutions of mammals, *Science*, **109**, 579–585 (1949).
3) H. Boxenbaum: Interspecies variation in liver weight, hepatic blood flow, and antipyrine intrinsic clearance: Extrapolation of data to benzodiazepines and phenytoin, *J. Pharmacokinet. Biopharm.*, **8**, 165–176 (1980).
4) H. Boxenbaum: Interspecies scalling, allometry, physiological time, and the ground plan of pharmacokinetics, *J. Pharmacokinet. Biopharm.*, **10**, 201–227 (1982).
5) H. Boxenbaum: Comparative pharmacokinetics of

benzodiazepines in dog and man, *J. Pharmacokinet. Biopharm.*, **10**, 411–426 (1982).
6) Y. Sawada, M. Hanamo, Y. Sugiyama and T. Iga: Prediction of the disposition of β-lactam antibiotics in humans from pharmacokinetic parameters in animals, *J. Pharmacokinet. Biopharm.*, **12**, 241–260 (1984).
7) Y. Uchida: Cardiovascular effect of 3,4-dihydro-8-(2-hydroxy-3-isopropylamino)propoxy-3-nitrato-2*H*-1-benzopyran (K-351), *Jpn. Heart J.*, **23**, 981–988 (1982).
8) Y. Ucida, M. Nakamura, S. Shimizu, Y. Shirasawa and M. Fujii: Vasoactive and β-adrenoceptor blocking properties of 3,4-dihydro-8-(2-hydroxy-3-isopropylamino) propoxy-3-nitroxy-2*H*-1-benzopyran (K-351), a new antihypertensive agent, *Arch. Int. Pharmacodyn. Ther.*, **262**, 132–149 (1983).
9) M. Yoshimura, J. Kojima, T. Ito and J. Suzuki: Structural determination of dog and human metabolites of Nipradilol (K-351), a new antihypertensive agent, *Chem Pharm. Bull.*, **33**, 3456–3468 (1985).
10) Kowa Co., Ltd., Laid open patent No. 57-7481 (1982). [*Chem. Abstr.*, **96**, 181147*d* (1982)].
11) M. Taniguchi, M. Nakano and T. Arita: Hydrolytic behaviors of trichloroethyl phosphate in rat gastrointestinal tract, *Yakugaku Zasshi*, **98**, 129–132 (1978).
12) A. Etoh, H. Yamakita, K. Nagano and T. Ikeo: Studies on the binding interaction of diltiazem. III. Mechanism of the absorption enhancing effect of diltiazem on the co-administration propranolol, *Yakugaku Zasshi*, **103**, 573–580 (1983).
13) K. Yamaoka, Y. Tanigawara, T. Nakagawa and T. Uno: A pharmacokinetic analysis program (MULTI) for microcomputer, *J. Pharmacobio-Dyn.*, **4**, 879–885 (1981).
14) M. Gibaldi, P. Nagashima and G. Levy: Relationship between drug concentration in plasma or serum and amount of drug in the body, *J. Pharm. Sci.*, **58**, 193–197 (1969).
15) M. Yoshimura, J. Kojima, T. Ito, M. Fujii and J. Suzuki: Pharmacokinetics of nipradilol (K-351), a new antihypertensive agent. II. Influence of the route of administration on bioavailability in dogs, *J. Pharmacobio-Dyn.*, **8**, 503–512 (1985).
16) G. H. Evans, A. S. Nies and D. G. Shand: The disposition of propranolol. III. Decreased half-life and volume of distribution as a result of plasma binding in man, monkey, dog and rat, *J. Pharmacol. Exp. Ther.*, **186**, 114–122 (1973).
17) H. Hisa, M. Suzuki, T. Kamijo and S. Satoh: Effects of K-351 on hemodynamic and renin release in the anesthetized dogs, *Arch. Int. Pharmacodyn. Ther.*, **271**, 169–176 (1984).
18) K. Kawashima, T. X. Watanabe and H. Sokabe: Pharmacodynamic and pharmacokinetic studies on prizidilol and nipradilol (K-351), antihypertensive drugs with combined vasodilator and β-adrenoceptor blocking actions, in rabbits, *Jpn. J. Pharmacol.*, **36**, 519–526 (1984).
19) D. M. Ziegler and C. H. Mitchell: Microsomal oxidase IV. Properties of a mixed-function amine oxidase isolated from pig liver microsomes, *Arch. Biochem. Biophys.*, **150**, 116–125 (1972).
20) U. K. Walle, M. J. Wilson and T. Walle: Propranolol, alprenolol and oxprenolol metabolism in the dog. Identification of N-methylated metabolites, *Biomed. Mass Spectrom.*, **8**, 78–84 (1981).
21) C. A. Leeson, D. A. Garteiz, W. C. Knapp and G. J. Wright: N-methyation, a newly identified pathway in the dog from the metabolism of oxprenolol, a β-receptor blocking agent, *Drug Metab. Dispos.*, **1**, 565–568 (1973).
22) W. L. Nelson and M. L. Powell: Evidence for an arene oxide-NIH shift pathway in the metabolic conversion of propranolol to 4'-hydroxypropranolol in the rat and in man, *Drug Metab. Dispos.*, **7**, 351–355 (1979).
23) W. L. Nelson and T. R. Burke, Jr.: Metabolism of β-adrenergic antagonists. Evidence for an arene oxide-NIH shift pathway in the aromatic hydroxylation of oxprenolol, *J. Med. Chem.*, **22**, 1088–1092 (1979).
24) Y. Igari, Y. Sugiyama, Y. Sawada, T. Iga and M. Hanamo: Prediction of diazepam disposition in the rat and man by a physiological based pharmacokinetic model, *J. Pharmacokinet. Biopharm.*, **11**, 577–593 (1983).
25) C. H. Walker: Species differences in microsomal monooxygenase activity and their relationship to biological half-lives, *Drug Metab. Rev.*, **2**, 295–323 (1978).