



## Milk Transfer and Toxicokinetics of Valproic Acid in Lactating Cynomolgus Monkeys

Jong-Hwa Lee, Wook-Joon Yu, Eun Ju Jeong and Moon-Koo Chung

*Korea Institute of Toxicology, KRICT, Daejeon, Korea*

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Studies on milk transfer of drugs in non-human primates (NHPs) are among the crucial components in the assessment of peri- and postnatal toxicity because of the similarity between NHPs and humans. To evaluate the milk transfer of valproic acid (VPA) in NHPs, the toxicokinetics of VPA, an antiepileptic drug, were studied in pregnant cynomolgus monkeys. VPA was administered once daily to pregnant cynomolgus monkeys at doses of 0, 30, 90, and 270 mg/kg by oral gavage from Day 100 of gestation (GD 100) to Day 31 of lactation (LD 31). Concentrations of VPA and its metabolite, 4-ene-VPA, in the maternal plasma on GD 100, GD 140, and LD 30, and concentrations of VPA and 4-ene-VPA in the offspring plasma and milk on LDs 30 and 31, respectively, were quantified using liquid chromatography tandem mass spectrometry (LC/MS/MS). After administration of a single oral dose of VPA to pregnant monkeys on GD 100, the concentrations of VPA and 4-ene-VPA were generally quantifiable in the plasma of all treatment groups up to 24 hr after administration, which showed that VPA was absorbed and that the monkeys were systemically exposed to VPA and 4-ene-VPA. After administration of multiple doses of VPA to the monkeys, VPA was detected in the pup's plasma and in milk taken on LD 30 and LD 31, respectively, which showed that VPA was transferred via milk, and the pup was exposed to VPA. Further, the concentration of VPA in the milk increased with an increase in the dose. Extremely low concentrations of 4-ene VPA were detected in the milk and in the pup plasma. In conclusion, pregnant monkeys were exposed to VPA and 4-ene-VPA after oral administration of VPA at doses of 30, 90, and 270 mg/kg/day from GD 100 to LD 31. VPA was transferred via milk, and the VPA exposure to the pup increased with an increase in the dose of VPA. The metabolite, 4-ene VPA, was present in extremely low concentrations ( $< 0.5 \mu\text{g/ml}$ ) in the milk and in the pup plasma. In this study, we established methods to confirm milk transfer in NHPs, such as mating and diagnosis of pregnancy by examining gestational sac with ultrasonography, collection of milk and pup plasma and determination of toxicokinetics, using cynomolgus monkeys.

**Key words:** Non-human primate, Cynomolgus monkey, Milk transfer, Toxicokinetics, Valproic acid, 4-ene valproic acid, LC/MS/MS

### INTRODUCTION

Investigation of milk transfer and exposure of milk and pup plasma by potential toxicants in non-human primate

Correspondence to: Moon-Koo Chung, Korea Institute of Toxicology, KRICT, 100 Jang-dong Yuseong-gu, Daejeon 305-343, Korea  
E-mail: [mkchung@kitox.re.kr](mailto:mkchung@kitox.re.kr)

List of abbreviation: NHP: non-human primate, VPA: valproic acid, 4-ene VPA: 4-ene valproic acid, GD: gestation day, LLOQ: Lower limit of quantification

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(NHP) is one of the important components in the assessment of peri- and postnatal toxicity because of the similarity between NHP and humans and potential species differences in teratogenic manifestations.

Valproic acid (2-propyl-pentanoic acid, VPA) has been widely used as an anticonvulsant and mood-stabilizing drug, primarily in the treatment of epilepsy, bipolar disorder and less commonly in major depression. The daily dose for seizure control is 300~2000 mg for aiming to achieve plasma concentration level of 50~100  $\mu\text{g/ml}$  (1). These effects of VPA are shown through the inhibition of GABA transaminase and indirect action as GABA agonist. In addition, it was reported that VPA also blocks the voltage-gated sodium channels and T-type calcium channel. These mechanisms make VPA a broad spectrum anticonvulsant drug (2). Recently,

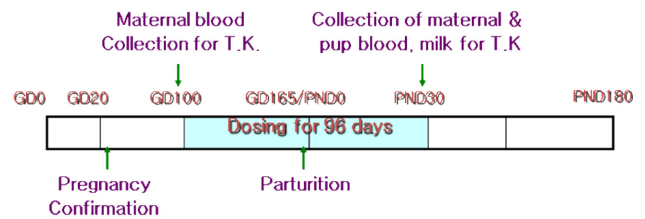
it was reported that VPA has an anti-cancer effects on various tumors such as multiple myeloma, glioma and melanoma through inhibition of histone deacetylases and induction of DNA-methylation (2). VPA treatment with an intensified antiviral therapy could reduce CD4+ T-cell infection of human immunodeficiency virus (HIV) through the inhibition of histone deacetylase HDAC1 which is needed for HIV to remain in infected cells. Therefore, the scope of VPA application on diseases is gradually getting wider. However, in spite of its usefulness on various diseases, there have been a lot of concerns because of the various side effects, especially such as teratogenesis and hepatotoxicity. In human, prospective and retrospective epidemiological studies suggested that medication of VPA during early pregnancy may be associated with an increased incidence of spina bifida and autism (3,4).

A lot of studies on peri- and postnatal toxicity and toxicokinetics of VPA have been conducted using rats and monkeys (5-8), and it has been reported that there are species differences in pharmacokinetics and teratogenesis of VPA (9,10). It was pointed out that the half-life VPA in experimental animals (0.3~4 hr) is much shorter than that in humans (9~18 hr).

However, there was no information on the milk transfer and toxicokinetics of VPA in cynomolgus monkey, which is one of the mostly used animal strains for reproductive toxicity studies using NHP. In a physiologic point of view, there may be chances of VPA transferring to the fetuses in pregnant women or to the infants by breast-feeding in lactating women after intake of VPA-contaminated foods. However, the probability for VPA crossing the placental barrier and mammary gland has not been studied so far. This study was carried out in order to examine the effect of gestational and lactational transfer of VPA from pregnant and lactating monkeys to the fetuses and the infants, respectively. Therefore, this study was conducted the first time to establish the method and to determine milk transfer and toxicokinetics of VPA and its major toxic metabolite, 4-ene valproic acid (2-n-propyl-4-pentanoic acid, 4-ene-VPA) in lactating cynomolgus monkeys.

## MATERIALS AND METHODS

**Animals.** The cynomolgus monkey (*Macaca fascicularis*) was used in this study. Six healthy males, aged 6~7 years, weighing 4~6 kg and 18 healthy females showing regular menstrual cycle, aged 5~7 years, weighing 2.5~4.5 kg were selected. Animal room was maintained at 20~26°C, with a relative humidity of 45~65%, under a controlled 12-hourlight/dark cycle, with a ventilation rate of 10~20 air changes/hour, and were housed individually, except for mating. The monkeys were fed 120 g/day of diet (Oriental Yeast Co., Ltd., Japan). Tap water was given to rat ad libitum, following the UV-irradiation and filtration. This study has been



**Fig. 1.** Schematic representation of the milk transfer study in cynomolgus monkeys.

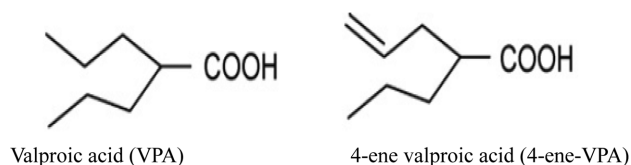
approved by the Institutional Animal Care and Use Committee of Korea Institute of Toxicology (KIT) and performed in accordance with the ethics criteria contained in the bylaws of the committee of KIT.

**Mating and diagnosis of pregnancy.** Pair of adult male and female monkey was cohabitated for mating. The visual confirmation of copulation and/or the presence of sperm in the vagina were considered to be evidence of successful mating. When copulation was confirmed, the median day of the mating period was regarded as GD 0. Pregnancy was confirmed between GD 20 and GD 25 by ultrasonography (SA-9900, Medison Co. Ltd., Korea). Pregnant females, weighing 2.51~4.50 kg on GD 0, were allocated randomly to four groups, and housed individually (Fig. 1).

**Administration of VPA.** The pregnant monkeys were administered once daily except for parturition day with valproic acid sodium salt (lot no. 036K0731, Sigma-Aldrich Co. Ltd., USA) at 0, 30, 90 and 270 mg/kg by nasogastric intubation using a volume of 0.5 ml/kg body weight from GD 100 to LD 31. The dosing solution was prepared by dissolving a weighted portion of VPA in saline.

**In-life observation of pregnant monkeys.** Clinical signs for each pregnant monkey were observed twice a day during the administration period and once a day during the non-administration period. Body weight and food consumption were observed as the schedule. Fetal growth and heart beat were examined on GDs 25, 40, 60, 90, 100, 110, 120, 130 and 140 using ultrasound. In addition, external abnormalities and body weights of F1 offspring were observed. When embryonic cardiac arrest was confirmed using ultrasonography, dosing for dams was terminated and these dams were excluded from the study.

**Sampling of maternal blood.** Blood samples (approximately 0.6 ml) were obtained from cephalic vein or femoral vein of dams at 0 (before treatment), 0.5, 1, 2, 4, 8 and 24 hr after administration of VPA on GD 100 (dosing initiation day), GD 140 and LD 30 (dosing termination day). Blood samples (approximately 0.5 ml) were taken from the pup at 1 hr after final dose of dams (LD 30). In addition,



**Fig. 2.** Chemical structure of valproic acid (VPA) and 4-ene valproic acid (4-ene-VPA).

milk (approximately 0.5 ml) was collected 1 hr after final dose on LD 31.

Blood taken from dams and pups was collected into a heparinized tube (6 IU/tube), centrifuged at 12000 rpm for 3 min, and plasma was stored at  $-80^{\circ}\text{C}$  until analysis. Milk was collected by aspirating a small volume of milk (0.5 ml) from dam, stored at  $-80^{\circ}\text{C}$  until analysis.

#### Bioanalysis of VPA and 4-ene-VPA in plasma and milk.

VPA and 4-ene-VPA (Fig. 2) in all prepared biological samples were analyzed by liquid chromatography/mass spectroscopy/mass spectroscopy (LC/MS/MS) using Agilent 1100 HPLC system (Agilent, USA) and API 3200 Qtrap mass spectrometer (Applied Biosystems, USA) equipped with a turbo ion spray interface in negative ionization mode. After acidifying the plasma and milk with the addition of 10% HCl, samples were extracted by solid-phase extraction using OASIS HLB cartridge (Waters, USA).

The spray needle voltage was  $-4500\text{ V}$ , and the source temperature was  $450^{\circ}\text{C}$ . The declustering potential energy was  $-30\text{ V}$ . Benzoic acid was used as an internal standard. Selected ion was simultaneously monitored at  $m/z$  143.3 for VPA,  $m/z$  141.3 for 4-ene valproic acid and benzoic acid, an internal standard, for  $m/z$  120.7. Chromatographic separation was achieved on  $\text{C}_{18}$  column ( $2.1\text{ mm} \times 100\text{ mm}$ ,  $3.5\text{ }\mu\text{m}$ ; Phenomenex, Inc, Torrance, CA). The mobile phase was composed of 10 mM ammonium formate-Methanol (25 : 75, v/v) and eluted at a flow rate of 0.2 ml/minute. Injection volume was set at  $3\text{ }\mu\text{l}$  and the column oven was maintained at  $30^{\circ}\text{C}$ . Data acquisition and processing were performed with Analyst software (version 1.4.2; Applied Biosystems, USA). Lower limit of quantification (LLOQ) of VPA in plasma and milk was  $1\text{ }\mu\text{g/ml}$  and LLOQ of 4-ene-VPA in plasma and milk was  $0.5\text{ }\mu\text{g/ml}$ .

#### Toxicokinetic analysis of plasma concentration data.

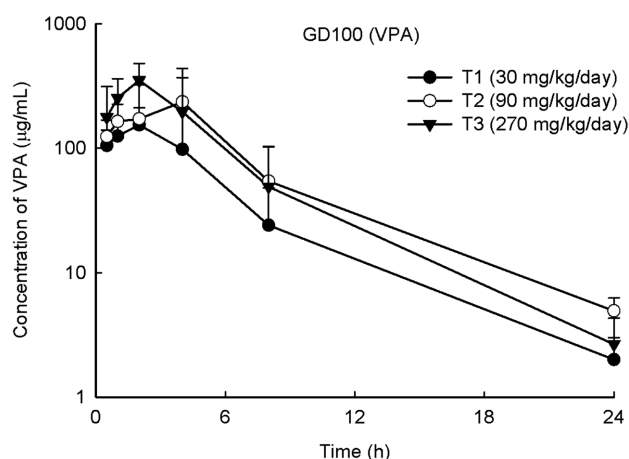
Non-compartmental toxicokinetic analysis was performed on individual plasma concentration data using a WinNonlin<sup>®</sup> program (version 5.2.1, Pharsight Corp., USA). The apparent terminal half life ( $t_{1/2,z}$ ) associated with the apparent terminal elimination phase was calculated from the equation,  $t_{1/2,z} = 0.693/\text{elimination rate constant}$ . The maximum observed peak plasma concentration ( $C_{\text{max}}$ ) and the time at which it is observed ( $T_{\text{max}}$ ) were obtained directly from the concentration-time data. The area under the plasma concen-

tration-time curve from the start of dosing to the last quantifiable sampling time point ( $\text{AUC}_{\text{last}}$ ) was calculated using linear-logarithmic trapezoidal rule. The mean residence time (MRT) from the start of dosing to the last quantifiable sampling time point ( $\text{MRT}_{\text{last}}$ ) was calculated from  $\text{MRT}_{\text{last}} = \text{AUMC}_{\text{last}}/\text{AUC}_{\text{last}}$ ; where  $\text{AUMC}_{\text{last}}$  is the area under the first moment curve from time 0 to the time of last sampling. Milk to maternal plasma ratio and pup plasma to maternal ratio were calculated using following equation.

$$\begin{aligned} \text{Milk to maternal plasma ratio} &= \text{concentration in milk } (\mu\text{g/ml}) \\ &\quad / \text{concentration in corresponding maternal plasma } (\mu\text{g/ml}) \\ \text{Pup plasma to maternal plasma ratio} \\ &= \text{concentration in pup plasma } (\mu\text{g/ml}) \\ &\quad / \text{concentration in corresponding maternal plasma } (\mu\text{g/ml}) \end{aligned}$$

## RESULTS

**Clinical signs, body weight and food consumption of pregnant monkeys and F1 pups.** No treatment related abnormal clinical signs were observed in dams treated with VPA at doses of 30, 90 and  $270\text{ mg/kg/day}$ . Although tem-

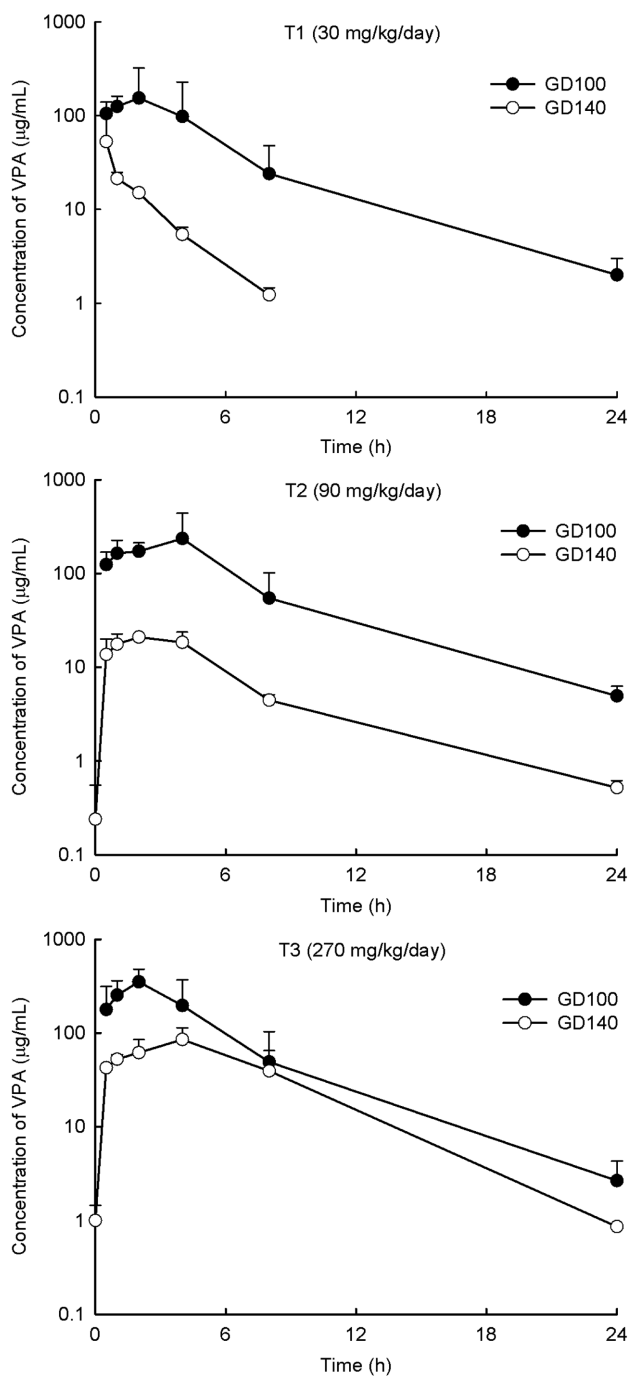


**Fig. 3.** Plasma concentrations of valproic acid (VPA) after oral administration of valproic acid at doses of 30, 90 and  $270\text{ mg/kg}$  in pregnant cynomolgus monkeys on day 100 of gestation (GD 100).

**Table 1.** Toxicokinetic parameters of valproic acid (VPA) after single oral administration of VPA in pregnant cynomolgus monkeys on the day 100 of gestation ( $n = 2$ )

Dose (mg/kg/kg)		$T_{\text{max}}$ (hr)	$C_{\text{max}}$ ( $\mu\text{g/ml}$ )	$\text{AUC}_{\text{last}}$ ( $\mu\text{g} \cdot \text{hr/ml}$ )	$T_{1/2,z}$ (hr)	$\text{MRT}_{\text{last}}$ (hr)
30	Mean	1.3	185.5	919	2.2	2.9
	SD	0.6	152.8	1165	1.1	1.6
90	Mean	3.0	262.0	1733	4.0	4.9
	SD	1.4	166.2	1224	0.7	0.4
270	Mean	3.0	380.5	1905	3.6	3.9
	SD	1.4	87.7	735	0.5	1.8

poral vaginal hemorrhage was found in all dams of the treatment groups during the gestation period, the animals were recovered. There was no significant difference in body weights and food consumption between the vehicle control and the treatment groups during the study period. In addition, treatment related changes in clinical signs and body



**Fig. 4.** Plasma concentrations of valproic acid (VPA) in pregnant cynomolgus monkeys on days 100 and 140 of gestation (GD 100 and GD 140).

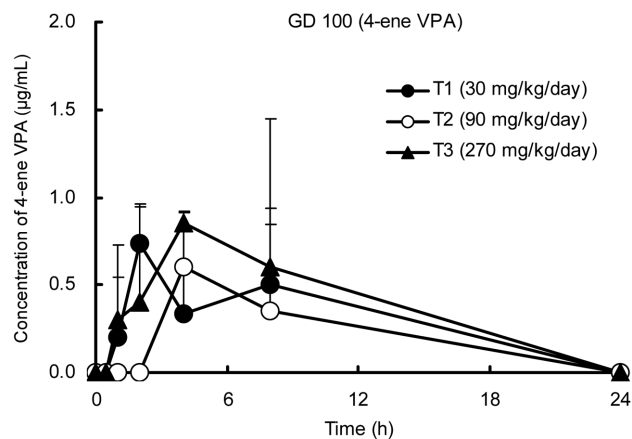
weights were not observed in F1 pups from dams treated with VPA at doses of 30, 90 and 270 mg/kg/day.

**Exposure of VPA in the plasma of pregnant cynomolgus monkeys.** Following single administration of VPA at doses of 30, 90 and 270 mg/kg/day to pregnant monkeys on GD 100, concentrations of VPA were generally quantifiable in the maternal plasma from all treatment groups up to 24 hr post-dose, demonstrating that the pregnant monkeys were systemically exposed to VPA.  $T_{max}$  was 1~4 hr after dosing at 30, 90 and 270 mg/kg/day and not showed a tendency with the increasing dose (Fig. 3).

There was no remarkable difference in the maternal systemic exposure (determined by  $AUC_{last}$  and  $C_{max}$ ) between the monkeys dosed at 90 and 270 mg/kg/day, but systemic exposure to VPA was increased with the dose in a less-than proportional manner in animals dosed with 30 and 90 mg/kg/day. The  $T_{1/2,z}$  was 2.2, 4.0 and 3.6 hr, and  $MRT_{last}$  was 2.9, 4.9 and 3.9 hr at 30, 90 and 270 mg/kg/day, respectively (Table 1).

Following repeated administration of VPA at doses of 30, 90 and 270 mg/kg/day to pregnant monkeys from GD 100 to GD 140, concentrations of VPA on GD 140 were generally quantifiable ( $> 1 \mu\text{g/ml}$ ) in the maternal plasma from all treatment groups up to 8 hr post-dose, demonstrating that the pregnant monkeys were systemically exposed to VPA. When comparing  $AUC_{last}$  of GD 140 with that of GD 100,  $AUC_{last}$  was 9, 7 and 26% and  $C_{max}$  was 30, 8 and 22% at 30, 90 and 270 mg/kg/day, respectively.  $MRT_{last}$  was 1.9, 3.2 and 3.8 hr at 30, 90 and 270 mg/kg/day, respectively (Fig. 4 and Table 2).

**Exposure of 4-ene-VPA in the plasma of pregnant cynomolgus monkeys.** Following single administration of VPA at doses of 30, 90 and 270 mg/kg/day to pregnant



**Fig. 5.** Plasma concentrations of 4-ene valproic acid (4-ene-VPA) after oral administration of valproic acid at doses of 30, 90 and 270 mg/kg in pregnant cynomolgus monkeys on day 100 of gestation (GD 100).

**Table 2.** Toxicokinetic parameters of valproic acid (VPA) after 40 day's repeated oral administration of VPA in pregnant cynomolgus monkeys on the day 140 of gestation (n = 2)

Dose (mg/kg/kg)		T <sub>max</sub> (hr)	C <sub>max</sub> (µg/ml)	AUC <sub>last</sub> (µg · hr/ml)	T <sub>1/2,z</sub> (hr)	MRT <sub>last</sub> (hr)
30	Mean	0.8	56.5	84	1.7	1.9
	SD	0.4	46.2	27	0.2	0.3
90	Mean	3.0	21.8	115	2.4	3.2
	SD	1.4	0.5	9	NA	0.3
270	Mean	4.0	85.1	488	NA	3.8
	SD	0.0	28.1	175	NA	0.3

NA = Not available due to the limited plasma concentrations.

**Table 3.** Toxicokinetic parameters of 4-ene valproic acid (4-ene-VPA) after single oral administration of VPA in pregnant cynomolgus monkeys on the day 100 of gestation (n = 2)

Dose (mg/kg/kg)		T <sub>max</sub> (hr)	C <sub>max</sub> (µg/ml)	AUC <sub>last</sub> (µg · hr/ml)	T <sub>1/2,z</sub> (hr)	MRT <sub>last</sub> (hr)
30	Mean	2.7	0.8	3.1	NA	3.8
	SD	1.2	0.3	3.0	NA	1.6
90	Mean	6.0	0.7	1.9	NA	4.9
	SD	2.8	0.1	1.8	NA	1.2
270	Mean	6.0	1.0	3.8	NA	4.4
	SD	2.8	0.3	4.2	NA	0.6

NA = Not available due to the limited plasma concentrations.

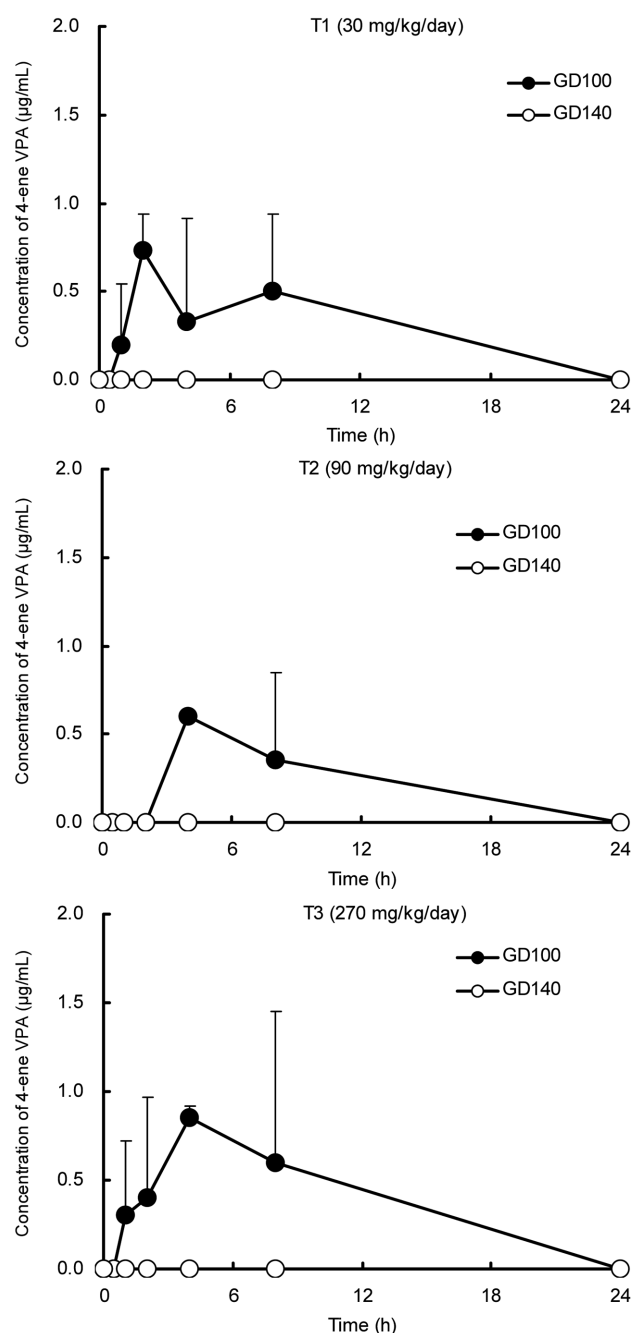
monkeys on GD 100, concentrations of 4-ene-VPA were extremely low compared to those of VPA and generally quantifiable in the maternal plasma from all treatment groups up to 8 hours post-dose (Fig. 5). Mean resident time (MRT<sub>last</sub>) was 3.8, 4.9 and 4.4 hr at 30, 90 and 270 mg/kg/day, respectively, which was similar as the corresponding values of VPA (Table 3).

Following repeated administration of VPA at doses of 30, 90 and 270 mg/kg/day to pregnant monkeys from GD 100 to GD 140, concentrations of 4-ene-VPA on GD 140 were not detected (< 0.5 µg/ml) in the maternal plasma from all treatment groups (Fig. 6).

#### **Milk transfer of VPA after repeated oral administration during gestation and lactation periods.**

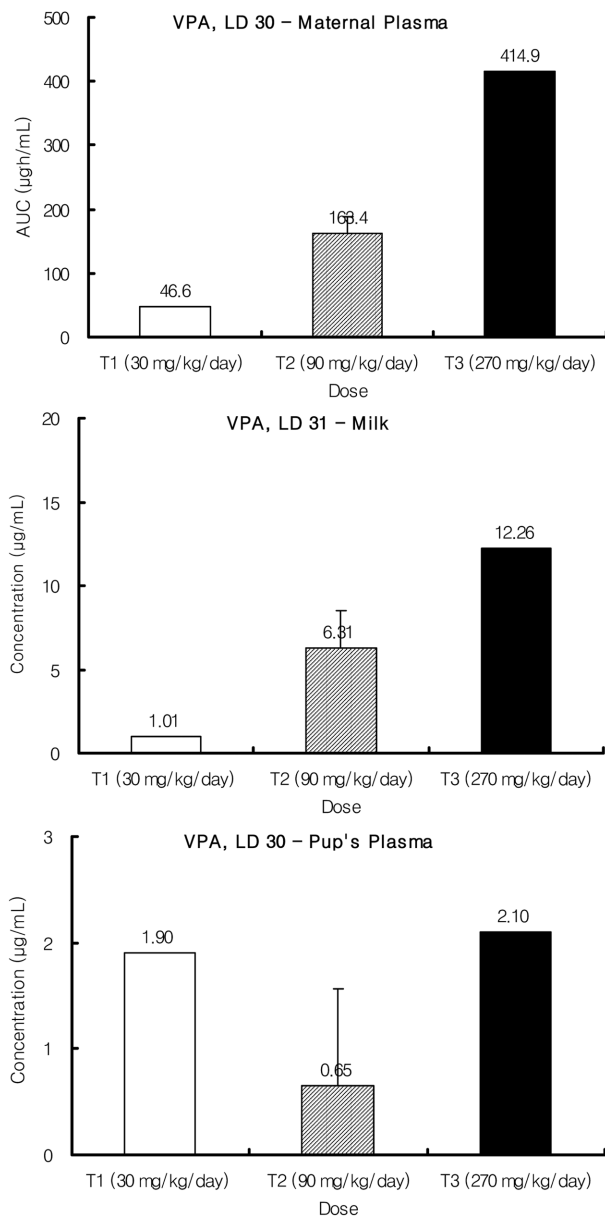
After repeated administration of VPA at doses of 30, 90 and 270 mg/kg/day from GD 100 to LD 30, concentrations of VPA were generally quantifiable (> 1 µg/ml) in the maternal plasma from all treatment groups up to 4~8 hr post-dose, indicating that the monkeys were systemically exposed to VPA. When comparing AUC<sub>last</sub> of LD 30 with that of GD 100, AUC<sub>last</sub> was 5, 9 and 22% and C<sub>max</sub> was 17, 23 and 27% at 30, 90 and 270 mg/kg/day, respectively. Mean resident time (MRT<sub>last</sub>) was 1.9, 3.2 and 3.8 hr at 30, 90 and 270 mg/kg/day, respectively.

VPA concentrations in pup's plasma and milk taken on LD 30 and LD 31, respectively, were generally quantifiable

**Fig. 6.** Plasma concentrations of 4-ene valproic acid (4-ene-VPA) in pregnant cynomolgus monkeys on days 100 and 140 of gestation (GD 100 and GD 140).

(> 1 µg/ml) in all treatment groups, demonstrating the potential of VPA from lactating dams to the nursing pups. The concentration of VPA in the milk at 1 hr post-dose to those of VPA in maternal plasma ratio was 4, 12 and 19% and the concentration of VPA in pup plasma at 1 hr post-dose to those of VPA in the maternal plasma was 8, 3 and 3% at 30, 90 and 270 mg/kg/day, respectively (Fig. 7).

The major metabolite, 4-ene-VPA, was not detected



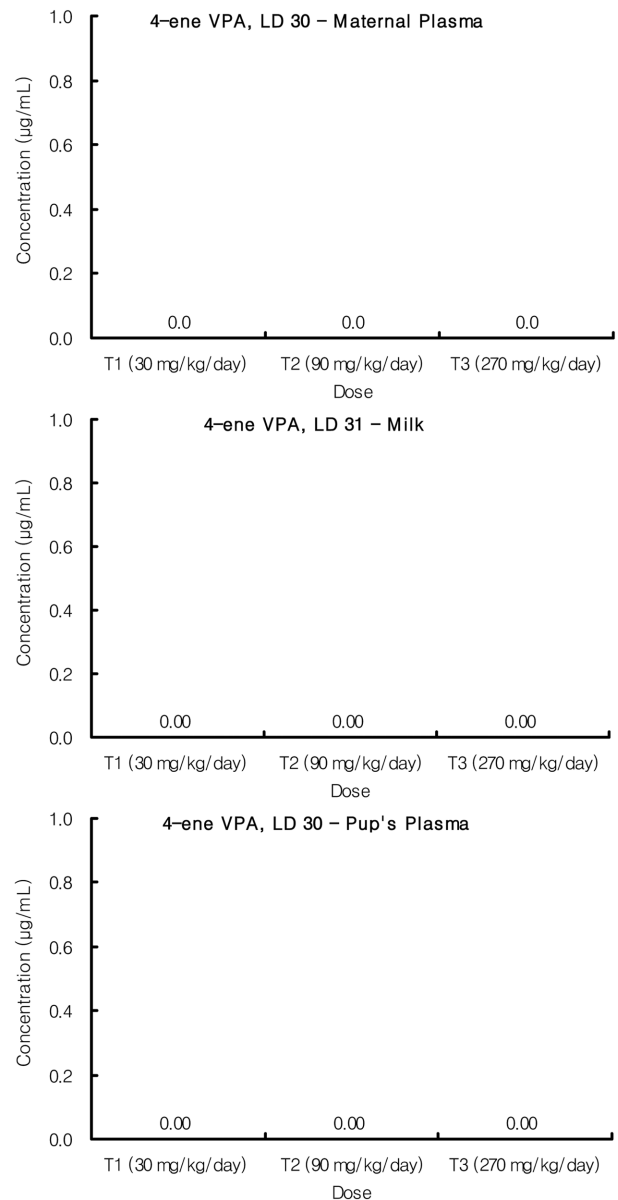
**Fig. 7.** Concentrations of valproic acid (VPA) in maternal plasma, milk and pup's plasma.

(< 0.5  $\mu\text{g}/\text{mL}$ ) in maternal plasma from all treatment groups.

Following repeated administration of VPA at doses of 30, 90 and 270 mg/kg/day to pregnant monkeys from GD 100 to LD 30, the major metabolite, 4-ene-VPA, on LD 30 was not detected (< 0.5  $\mu\text{g}/\text{mL}$ ) in the maternal plasma from all treatment groups. In addition, 4-ene-VPA, in the pup plasma and milk taken on LD 30 and LD 31, respectively, was not detected (< 0.5  $\mu\text{g}/\text{mL}$ ) in all treatment groups (Fig. 8).

## DISCUSSION

There are close relation between the species differences



**Fig. 8.** Concentrations of 4-ene valproic acid (4-ene-VPA) in maternal plasma, milk and pup's plasma.

in pharmacokinetics and drug teratogenesis (10), and a lot of studies have been performed in this area (11-14). There appears to be two major reasons for the observed species differences in response to teratogens: (a) intrinsic sensitivities of the developing tissues, e.g., extreme rarity of neural tube defects in nonhuman primates, but prominence in most other species including man; (b) differences in exposure of the embryo during the sensitive stages of gestation (10).

Although the species differences exist in pharmacokinetics and drug teratogenesis NHP than other species is usually used in toxicity and pharmacokinetic study because of the similarity between humans and NHP. The primary object of the present study was to establish the methods for milk

transfer study in cynomolgus monkeys as a one of the most commonly used NHP. VPA was selected as a model drug because firstly, VPA is a well known teratogen in humans and experimental animals but still is used in humans including pregnant population and application of VPA is getting expanded (4,15), and secondly peri- and postnatal toxicity and toxicokinetics of VPA have been conducted using various species, such as mice, rats, sheep and rhesus monkeys but not in cynomolgus monkeys (8).

In maternal toxicities, no treatment-related changes in general findings, such as clinical signs, body weight and food consumption, were noted after repeated administration of VPA at doses of 30, 90 and 270 mg/kg/day from GD 100 to LD 30, which is generally comparable to those previously reported in rhesus monkeys (9).

In pharmacokinetic analysis of VPA in maternal plasma after single oral administration on GD 100, pregnant monkeys were exposed to VPA and 4-ene-VPA at 30~270 mg/kg/day. The biphasic plasma elimination profile of VPA was showed in maternal plasma, which was similar as the results reported following oral administration of 20~180 mg/kg/day during gestational period (GD 20) in cynomolgus monkey (16) and following intravenous administration of 50 mg/kg/day during last pregnancy in rhesus monkey (17). The enterohepatic recirculation of VPA does not contribute to the biphasic elimination profile in primates (18). Although there was a trend toward longer apparent elimination half life with dose, the plasma elimination kinetics did not exhibit obvious dose-dependency at high dose.

Systemic exposure (determined by  $AUC_{last}$  and  $C_{max}$ ) increased with increasing dose in a less-than dose proportional manner between 30 and 270 mg/kg/day. The increases in  $C_{max}$  and  $AUC_{last}$  observed at 30~90 mg/kg/day were not born out at 270 mg/kg/day. These results suggest non-linear pharmacokinetics of VPA at dose range between 30 and 270 mg/kg/day although the large variation in individual  $AUC_{last}$  values at these doses. Lack of dose proportionality (implying nonlinear pharmacokinetics) may be due to many mechanisms but is typically due to the saturation of some component in the system, such as metabolizing enzymes or transporters. Common causes of dose non-proportionality due to nonlinear absorption include saturation of carrier-mediated uptake, poor aqueous solubility or slow release from the formulation, and saturation of pre-systemic metabolism (19). Lack of dose proportionality may have implications with regard to safety and efficacy. For a drug that shows dose-dependent absorption like VPA, typically higher doses lead to less absorption and sub-proportional drug concentrations.

After repeated administration of VPA to the pregnant monkeys, the systemic exposure of VPA on GD 140 was decreased to 7~26% of those after single administration on GD 100, suggesting that metabolic enzymes might have been induced or absorption might have been saturated after

repeated dosing. This result contrasts with our previous repeated dose study in pregnant cynomolgus monkeys from GD 20 to GD 50 at doses of 20, 60 and 180 mg/kg/day. In the present study, to elucidate the exposure differences caused by the repeated dose was out of scope. Further study may be needed in the future.

Among the metabolites of VPA, 4-ene-VPA is known as a hepatotoxic metabolite of VPA which generates a chemically reactive intermediate(s) that alkylate(s) cellular macromolecules (20,21). 4-ene-VPA was detected in the plasma with lower concentrations than those of VPA with less than 0.5% of VPA in  $C_{max}$  at all dose levels studied (30~270 mg/kg/day), which was similar with the result in rhesus monkeys, where  $C_{max}$  of 4-ene-VPA did not exceed 1  $\mu\text{g}/\text{ml}$  at 200 mg/kg/day (9). In addition, the monkeys exposed to 4-ene-VPA with 1.9~3.8  $\mu\text{g}\cdot\text{h}/\text{ml}$ , which is 0.5% of VPA in  $AUC_{last}$  at all dose levels, indicated extremely low amount of VPA was converted to 4-ene-VPA.

Following repeated administration of VPA at doses of 30, 90 and 270 mg/kg/day from GD 100 to LD 31, exposure of VPA in the milk and pups was increased with the increasing dose, although not linearly, to the dose level. Milk level would provide a relatively constant dose to the pup until weaning. Pup daily dose during lactation is a reflection of milk concentration, and milk concentrations were relatively consistent across the lactational period. Milk consumption increases as the pup grows, allowing for a relatively constant dose rate until milk consumption decreases by weaning. However, concentrations of 4-ene-VPA were under the detection limit ( $< 0.5 \mu\text{g}/\text{ml}$ ) in the maternal and pup's plasma from all treatment groups. No treatment-related changes to be related VPA or 4-ene-VPA in the postnatal development of pups were observed. This is associated with the fact that concentrations of 4-ene-VPA, a major metabolite, which causes liver toxicity and teratogenicity, were very low ( $< 0.5 \mu\text{g}/\text{ml}$ ) in the milk and pup plasma from all treatment groups.

VPA is excreted into human milk in low concentrations (22,23). Available reports suggest that a suckling infant may ingest less than 5% of the weight-adjusted maternal daily dose (24) and the ratio of VPA in the breast milk to maternal blood was about 2~3% (25). Hence, lactation by VPA treated mothers is permissible.

Taken together, following repeated administration of VPA at doses of 30, 90 and 270 mg/kg/day via oral route to pregnant monkeys from GD 100 to GD 140, dams were exposed to VPA and 4-ene-VPA which is one of the toxic metabolite of VPA. The detection of VPA in milk suggest the possibility of transfer of VPA through breast-feeding from dams to pup dose level of 30~270 mg/kg/day, and the VPA concentrations in pups was 3~8% of those of maternal plasma. However, 4-ene-VPA was detected under the detection limit ( $< 0.5 \mu\text{g}/\text{ml}$ ) in the milk and pup plasma from all treatment groups, demonstrating that 4-ene-VPA was not

transferred via milk to the pups.

The present study is the first one to report the toxicokinetics and milk transfer of VPA in cynomolgus monkeys. Considering the present situation that only limited numbers of milk transfer studies in NHP are available, the method and results reported in the present study provides primer information on the toxicokinetics and milk transfer of VPA in cynomolgus monkeys.

In conclusion, we established the method to study milk transfer in cynomolgus monkeys using VPA as a model drug, and demonstrated that VPA was transferred to milk, and the pups were exposed to VPA after repeated oral administration of VPA at doses of 30, 90 and 270 mg/kg/day in monkeys during the gestation and lactation periods.

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