Phenobarbital Disposition in Adult and Neonatal Rabbits

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INTRODUCTION

Breast fed infants are being exposed to a wider variety of drugs and environmental contaminants via milk (1, 2). To address the implications of this exposure, it is critical to know whether the amount of drug ingested poses a significant health problem to the newborn. As a first approach to determining the safety or hazard of drugs to the neonate, it is essential to be able to predict the amount of drug presented to the neonate following acute/chronic administration via milk. Even more critical is an understanding of the relationship between the amount of drug ingested (neonatal dose), the drug concentration at the site of action (usually a function of steady-state unbound concentration), and the pharmacological / toxicological effect in the neonate.

The excretion of phenobarbital into human breast milk has long been of clinical concern. Tyson, et al (3) in 1938 described possible drug-related sedative effects in two infants (out of 48 cases examined) which had been exposed to phenobarbital via breast milk. Kaneko, et al (4) reported a human milk to serum ratio (M/S) of 0.50 for phenobarbital and suggested that breast feeding should be supplemented with bottle feeding to reduce the phenobarbital intake. Research efforts in our laboratory have focused on the infant exposure to xenobiotics via milk using the rabbit as an animal model (5-7). Previously, we have reported the M/S ratio in the rabbit to be 0.59 (5).

Neonatal exposure must take into account not only the dose to which the infant is exposed, but also the ability of the infant to eliminate the xenobiotic and the differences in pharmacodynamic response in the neonate. The purpose of the present study is to characterize the pharmacokinetics (i.e., elimination characteristics and bioavailability) of PB in adult and neonatal rabbits.

MATERIALS AND METHODS

Chemicals. Phenobarbital sodium was obtained from Ransdell Co. (Louisville, KY). Hexobarbital was obtained from the Pharmacy Central Supply at the University of Kentucky (Lexington, KY).

Animals. Adult female nonpregnant (n = 12) and pregnant (n = 12) New Zealand White rabbits (3-6 kg, ~1 years old) were purchased from Myrtles Rabbitry (Thompson Station, TN) and were maintained under a 12/12-hr light/dark cycle. The twelve nonpregnant adult rabbits were used for PB pharmacokinetic studies. The pregnant rabbits (n = 12) were placed into welping cages containing a nesting box and were whelped from 30-32 days of gestation. The day of whelping was considered to be postnatal day 0. Rabbit pups of 17-22 days of age (250-350 g) were utilized for PB single dose pharmacokinetic studies (n = 24; 6 females; 18 males).

Study Design. A group of 6 nonpregnant adult female rabbits received PB as an i.v. bolus (30 mg/kg free base) *via* the left marginal ear vein. Serial venous blood samples (0.6 ml) were obtained by venipuncture from the right marginal ear vein at 0.5, 1, 3, 6, 12, 24, 36, 48, 72 and 96 hr after injection. A second group of 6 nonpregnant adult female rabbits received PB (10 mg/kg free base) as an oral dose *via* gavage and also as an i.v. bolus *via* the left marginal ear vein on separate occasions in a randomized fashion. Blood sampling protocol for 10 mg/kg i.v. dose was the same as described above. For oral administration, additional blood samples were collected at 0.25 and 2 hr after dosing. Two groups of rabbit pups (n = 6, each) were injected with either 10 or 30 mg/kg PB (free base) dissolved in saline as a bolus into the left marginal ear vein. Also, two additional groups of pups (n = 6, each) were administered p.o. 10 mg/kg PB (free base) dissolved in either saline or milk as a dosing vehicle (2 mg/ml) *via* gavage. Following i.v. injection, serial blood samples (0.4 ml) were collected at 1, 4, 10, 24, 48, 76, 120, and 168 hr. For oral administration, additional blood samples were taken at 0.25, 0.5 and 2 hr. Prior to dosing, an aliquot of blank serum was obtained from adult and neonatal rabbits by puncture of the central ear artery for protein binding measurement. Serum samples harvested were kept at −20°C until drug analysis. Serum protein binding was determined by equilibrium dialysis. Briefly, serum samples (0.3 ml) spiked with PB (15 and 60 μg/ml) were dialyzed in Plexiglass cells against pH 7.4, 0.133 M phosphate buffer at 37°C for 6 hr. The fluids in the dialysis cells were separated by Spectra/por dialysis membrane (molecular weight cutoff 12,000-14,000). At the end of dialysis, 100 μl aliquots of buffer and serum were harvested and frozen at -20°C until analysis. Serum free fraction was calculated as the ratio of buffer to serum concentrations.

PB Analysis. Serum PB concentrations were determined by a modified HPLC method used previously in our laboratory (8). To 100 μl of serum in a glass tube were added 100 μl of 0.2 M H_2SO_4 and 25 μl of internal standard (hexobarbital, 40 μg/ml H_2O). Samples were extracted with 2 ml methylene chloride by vortexing for 30 sec and centrifuged (3,400 × *g* for 5 min). The organic layer was transferred into a fresh tube containing 200 μl of 0.1 M NaOH and the mixture was then vortexed for 30 sec. Following centrifugation, an aliquot of 50 μl of aqueous layer was injected onto the HPLC system consisting of a Shimadzu Model SCL-6B controller, SIL-6B auto-injector, C-R6A integrator, SPD-6AV detector and a LC-6A pump (Shimadzu Scientific Instru-

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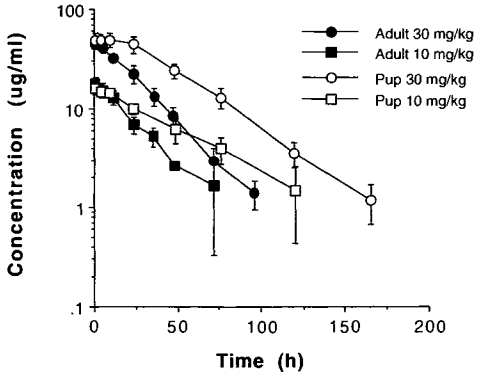

Figure 1 Mean (± S.D.) serum concentration-time curves of PB in adult rabbits and rabbit pups (n=6 each; 19-21 days old).

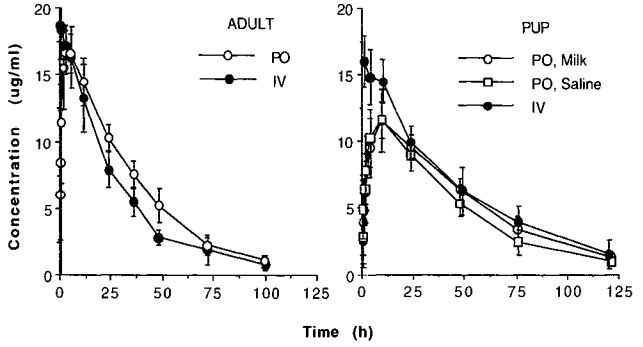

Figure 2 Mean (± S.D.) serum concentration-time curves of PB in adult rabbits and rabbit pups (n=6, 19-21 days old). Left panel; i.v. injection and oral administration of 10 mg/kg PB in adult rabbits. Right panel; i.v. injection and oral administration of 10 mg/kg PB dissolved in rabbit milk and saline in rabbit pups.

ments Inc., Columbia, MD). Separations were carried out on a C_{18} (10 μm particle size) 150 mm × 4.6 mm column (Jones Chromatography, Littleton, CO). The mobile phase (pH 2.9) consisted of acetonitrile:0.005 M phosphoric acid (3:7). The effluent was monitored at 225 nm, with a flow rate of 1 ml/min.

Data Analysis. Serum drug concentration-time data were analyzed by fitting mono-exponential (intravenous data) or biexponential (oral data) equations using nonlinear regression analysis (RSTRIP, MicroMath, Salt Lake City, UT). The area under the drug concentration-time curve (AUC) and area under the first moment curve (AUMC) were determined from the coefficients and exponents of the fitted relationship (9). Cls was determined from the equation: Cls = Dose/AUC. Clu was calculated by dividing Cls by unbound fraction (fu). Vss was determined from the equation: $Vss = Dose \cdot AUMC/AUC^2$. The bioavailability (F) was determined from the relationship: $F = (Dose_{iv} \cdot AUC_{oral})/(Dose_{oral} \cdot AUC_{iv})$.

Statistics. Changes in pharmacokinetic parameters were tested for statistical significance by two-way ANOVA followed by Tukey's multiple comparison test. Age and dose were the independent variables tested. An unpaired student t-test was used to test the difference in the mean oral pharmacokinetic parameters between the adult and the suckling pups, and the dosing vehicle (saline vs milk) in the suckling pups. The significance level was set at $p<0.05$.

RESULTS AND DISCUSSION

The average serum PB concentration-time curves obtained after 10 or 30 mg/kg i.v. bolus to the adult rabbits and rabbit pups (19-21 days of age) are shown in Figure 1. In the adult rabbits, there was no significant difference in the mean pharmacokinetic parameters between 10 and 30 mg/kg doses (Table 1). Compared with the adult rabbits, rabbit pups exhibited a lower Cls and Clu and a longer elimination $t_{1/2}$, with no significant change in Vss (Table 1). Phenobarbital did not bind extensively to pup serum protein, with a mean free fraction being 0.72 ± 0.17 and 0.68 ± 0.10 at 15 and 60 μg/ml concentrations, respectively. Phenobarbital binding was more extensive in the adult rabbit than in the pup ($p<0.05$), with a mean free fraction being 0.40 ± 0.02 and 0.46 ± 0.04 at 15 and 60 μg/ml concentrations, respectively.

Figure 2 shows average serum PB concentration-time profiles following i.v. injection and oral administration of the drug (10 mg/kg) to the adult rabbits (left panel) and rabbit pups (right panel). Cmax was lower in the pups whereas, the Tmax, and elimination half-life were significantly longer as compared to the adult (Table 2). In these suckling rabbits, the presence of milk did not have any influence on the rate and extent of PB absorption (Table 2).

Overall, the systemic clearance of total and unbound drug and the serum protein binding were lower in rabbit pups. These observations are similar to PB kinetics in the human neonate in which the elimination $t_{1/2}$ is prolonged and the absolute bioavailability of serum protein binding are reduced compared to the adult (10–13). The lower systemic clearance in the neonatal rabbit will result in a 3-fold greater

Table I. Pharmacokinetic Parameters (mean ± S.D.) Obtained from i.v. Bolus Injection of 10 and 30 mg/kg Doses of Phenobarbital to Adult Rabbits and Their Offspring

	Adult rabbits		Rabbit pups	
Parameter	10 mg (n = 6)	30 mg (n = 6)	10 mg (n = 6)	30 mg (n = 6)
Body weight (kg)	4.69 ± 0.40	4.33 ± 0.15[a]	0.27 ± 0.06[b]	0.28 ± 0.15[b]
Cls (ml/min/kg)	0.35 ± 0.06	0.39 ± 0.05	0.20 ± 0.06[b]	0.19 ± 0.02[b]
Clu (ml/min/kg)	0.88 ± 0.14	0.85 ± 0.13	0.30 ± 0.12[b]	0.23 ± 0.04[b]
Vss (l/kg)	0.55 ± 0.06	0.63 ± 0.03	0.62 ± 0.06	0.55 ± 0.07
$T_{1/2}$ (hr)	18.3 ± 1.6	18.9 ± 2.3	37.6 ± 11.6[b]	33.6 ± 4.2[b]

[a] Different from low dose adult, $p < 0.05$.
[b] Different from adult doses, $p < 0.05$.

Table II. Pharmacokinetic Parameters (mean ± S.D.) Obtained from Oral Administration of 10 mg/kg Phenobarbital to Adult Rabbits and Their Offspring

Parameter	Adult rabbits	Rabbit pups	
	Saline (n = 6)	Saline (n = 6)	Milk (n = 6)
Body weight (kg)	4.69 ± 0.40	0.31 ± 0.06[a]	0.29 ± 0.04
Cmax (μg/ml)	16.77 ± 2.84	12.20 ± 1.23[a]	11.76 ± 1.72
Tmax (hr)	3.53 ± 2.77	8.22 ± 3.88[a]	9.63 ± 4.67
$t_{1/2}$, absorption (hr)	0.85 ± 0.68	2.04 ± 1.23	2.55 ± 1.68
$t_{1/2}$, elimination (hr)	19.8 ± 3.4	31.4 ± 6.3[a]	34.9 ± 7.6
Bioavailability (%)	118.5 ± 46.3	63.3 ± 5.3	73.4 ± 5.3

[a] Different from adult saline dose using an unpaired t-test ($p < 0.05$).

steady-state serum concentrations of unbound PB than in the adult given comparable mg/kg doses.

In adult humans, PB is rapidly and completely absorbed, but its bioavailability shows a high degree of variability (13). The present findings also indicated a highly variable oral PB bioavailability in adult rabbits. The extent and the rate of PB absorption in the rabbit pups was less than the adults. Milk appeared to have no statistically significant impact on the rate or the extent of PB absorption in the pup. The lower bioavailability would not compensate for the overall lower systemic clearance of PB if dosed orally using milk as a vehicle; i.e., steady-state serum concentrations of unbound PB will be 2-fold higher in the pup compared with the adult given comparable mg/kg doses.

In summary, exposure to PB via lactation should take into account the diminished ability of the pup to eliminate PB as well as the bioavailable dose presented via nursing. Risk assessment of drug exposure via nursing should take into account not only estimates of the daily dose presented in the milk, but the ability of the neonate to eliminate drug and possible differences in the nature and characteristics of the pharmacokinetic response in the neonate.

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