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Pharmacokinetics of *p*-Phenylbenzoic Acid in Near-Term Fetus of the Rat

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The pharmacokinetics of *p*-phenylbenzoic acid (PPBA) in near-term fetus of the rat was investigated after intramuscular injection of ^{14}C-PPBA into the fetus. The elimination rate of plasma PPBA in the isolated fetus was 15.8% of that in the non-isolated fetus. The area under the blood concentration curve in the isolated fetus was 5.2-fold higher than that in the non-isolated fetus. The transplacental clearance and the fetal tissue clearance were 0.127 ml/min and 0.0299 ml/min, respectively. The urinary and biliary excretion was less than 1.0% of the injected amount. No significant difference in various laboratory parameters or in uridine diphosphate–glucuronyltransferase activity was observed between the isolated and non-isolated fetuses.

Keywords——*p*-phenylbenzoic acid; isolated fetus; transplacental clearance; total body clearance; fetal tissue clearance

Drug clearance in the fetus consists of transplacental clearance (Cl_p) and fetal tissue clearance (Cl_f). The clearance of the fetus was demonstrated in ewes.[1,2] However, little is known in the case of rats. Previous reports[3,4] showed that *p*-phenylbenzoic acid (PPBA) was excreted into the bile as the unchanged compound and its glucuronide in the fetus after the injection of PPBA into a maternal vein. This work was undertaken to investigate the pharmacokinetics of the clearance of PPBA in near-term fetus of the rat.

Experimental

Material and Animal——^{14}C-PPBA was injected into pregnant rats on the 21st day of gestation in the manner described previously.[3]

Administration to Non-isolated Fetus——Animals were anesthetized lightly with ether. The uterus was exposed by midline incision and ^{14}C-PPBA was injected into the femoral muscle of one fetus at the dose of 135 nmol with a microsyringe.

Administration to Isolated Fetus——A fetus was removed by caesarian section and ^{14}C-PPBA was injected into the femoral muscle of the fetus at a dose of 135 nmol with a microsyringe. Urinary excretion was prevented by applying an adhesive (Aron Alpha, Toa Gosei Kagaku).

The fetus was then kept in an incubator at 38 °C.

Determination of Plasma Level, Urinary Excretion and Intestinal Contents——Plasma PPBA level was determined in the way described previously.[3] The radioactivity in urine of the isolated fetus was determined by assay of the urinary bladder containing urine. The intestine of the isolated fetus was minced and then shaked in 2.0 ml of ice-cold isotonic saline solution to wash out the intestinal contents. After centrifugation of the wash solution, an aliquot of the supernatant was assayed for radioactivity.

Pharmacokinetic Analysis——The plasma PPBA concentrations of both isolated and non-isolated fetuses were analyzed by using a one-compartment open model with the non-linear least-squares method.

Transplacental clearance (Cl_p) was obtained from the plasma PPBA concentrations of mother and non-isolated fetus. The relationship between fetal and maternal compartments is described by the following equation according to the model in Fig. 1:

$$V_m \frac{dM(t)}{dt} = V_f \cdot F(t) \cdot k_f - V_m \cdot M(t) \cdot k_m \quad (1)$$

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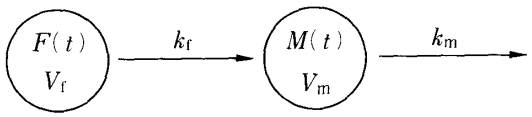

Fig. 1. Model for Transplacental Clearance

where V_m is the distribution volume of the maternal compartment, V_f the distribution volume of the fetal compartment, $M(t)$ the maternal plasma PPBA concentration, $F(t)$ the fetal plasma PPBA concentration, k_m the first-order elimination constant from the maternal compartment and k_f the first-order rate constant from the fetal compartment to the maternal compartment *via* the placenta. The Laplace transform and the inverse transform of Eq. (1) give:

$$M(t)=\int_0^t F(t-\theta)\cdot\frac{Cl_p}{V_m}\cdot\exp(-k_m\cdot\theta)\,d\theta \qquad (2)$$

where Cl_p is $V_f\cdot k_f$. Cl_p/V_m was obtained according to the method of Umemura *et al.*[5,6] Cl_p was obtained by using a value of 34.8 ml for V_m from the previous report.[2]

Laboratory Investigation——Using blood samples from isolated and non-isolated fetuses, hematological examination was carried out with a Coulter counter® S_{sr}. Biochemical examination were performed with an autoanalyzer (Hitachi 716) using the serum from isolated and non-isolated fetuses.

Activity of Uridine Diphosphate Glucuronyltransferase (UDPGT)——Hepatic UDPGT activity in isolated and non-isolated fetuses was determined in the manner described previously.[3]

Results and Discussion

The present study used isolated fetuses to determine the pharmacokinetic parameters of PPBA. It was first necessary to check that the isolation of the fetus did not lead to differences in pharmacokinetic character from non-isolated fetus. To examine the physiological affect of the isolation, various laboratory parameters were checked and the assay of UDPGT activity was carried out. As shown in Table I, no significant difference was observed between the isolated and non-isolated fetuses. This finding indicates that the data from the isolated fetus can be used for pharmacokinetic analysis.

Figure 2 shows the plasma PPBA concentrations of the fetus and mother after the intramuscular injection of ^{14}C-PPBA into isolated and non-isolated fetuses. The plasma PPBA concentration of the non-isolated fetus reached the maximum at 10 min after the injection, whereas that of the maternal plasma increased until 40 min. The plasma PPBA concentration of the isolated fetus reached the maximum at 20 min and then decreased slowly, remaining at a higher level than that of the non-isolated fetus. The PPBA concentration of the non-injected fetus was below 10% of the maternal PPBA concentration at the corresponding

Table I. Laboratory Parameters of the Fetus

	Non-isolated	Isolated
GOT, K-unit	128±31	171±45
GPT, K-unit	26± 7	32± 7
Alk. phosphatase, KA-unit	32± 8	42±11
Red blood cells, $10^4/\mu l$	224±41	294±59
White blood cells, $10^3/\mu l$	13± 2	14± 2
Hemoglobin, g/dl	12± 2	14± 3
Hematocrit, %	33.0± 5.9	38.6± 7.1
Total protein, g/dl	2.3± 0.4	2.2± 0.4
Albumin, %	55±11	56±12
UDPGT picomol/mg protein/min	116±18	104±19

The data represent the mean±S.E. of five fetuses.

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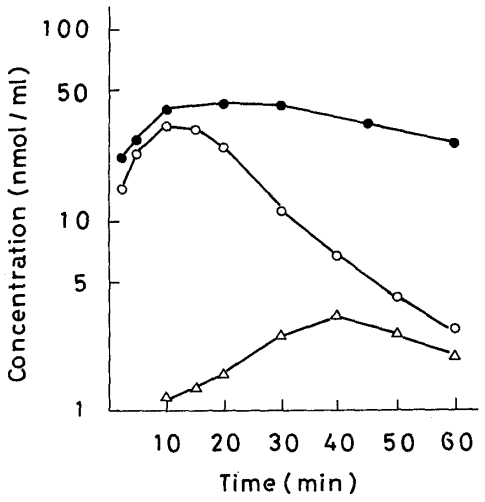


Fig. 2. Concentration of *p*-Phenylbenzoic Acid in Fetal and Maternal Plasma after Intramuscular Injection into Intact and Isolated Fetuses

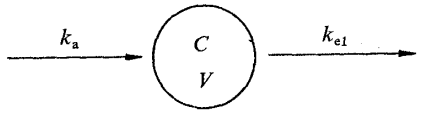
The values are the means of five litters or rats. ○, intact fetus; ●, isolated fetus; △, mother.

TABLE II. Pharmacokinetic Parameters for *p*-Phenylbenzoic Acid from Fetal Plasma[a)]

	Non-isolated	Isolated
C_{max}, nmol/ml	31.0±3.7	41.9±5.0
T_{max}, min	10.1±1.4	17.6±2.6[c)]
$T_{1/2}$, min	9.67±1.14	61.3±7.2[c)]
V, ml	2.19±0.26	2.65±0.32
k_a, min^{-1}	0.133±0.016	0.165±0.018
k_{el}, min^{-1}	0.0717±0.0086	0.0113±0.0013[c)]
Cl_{tot}, ml/min[b)]	0.157±0.018	0.0299±0.0034[c)]
AUC, min μmol/ml	0.859±0.102	4.50±0.51[c)]

The data are the means±S.E. of five litters.

a) Parameters for the model.



c; fetal plasma PPBA concentration.
V; distribution volume of fetus.
k_a; first-order absorption rate from injection site.
k_{el}; first-order elimination rate from fetus.

b) Cl_{tot}; total body clearance obtained from $V \cdot K_{el}$.

c) Significantly different from the values for non-isolated fetus ($p<0.05$).

time, probably due to the large distribution volume of the maternal compartment compared with that of the fetal compartment. This finding suggests that the transfer of ^{14}C-PPBA from the maternal compartment to the fetal compartment had no significant affect on the pharmacokinetic analysis in the fetal–maternal unit. The PPBA level in the amniotic fluid was also negligible compared with that in the fetus. This result suggests that the transfer of ^{14}C-PPBA between amniotic fluid and the fetal body played no significant role.

In view of these findings, the transfers of ^{14}C-PPBA from the maternal compartment to the fetal compartment and between the fetal body and amniotic fluid were neglected for the pharmacokinetic analysis in the present study.

Table II shows the pharmacokinetic parameters obtained from the plasma PPBA concentration of the fetus based on a one-compartment open model. No remarkable difference in C_{max} between the isolated and non-isolated fetuses was observed, whereas k_{el} and area under the blood concentration curve (AUC) in the isolated fetus were 15.8% lower and

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5.2 times higher than the corresponding values in the non-isolated fetus. The total body clearance (Cl_{tot}) in the non-isolated fetus was 5.3-fold higher than that in the isolated fetus. The clearance in the non-isolated fetus is given as follows,

$$Cl_p = Cl_{tot} - Cl_f$$

where Cl_p is the transplacental clearance from the fetus to the mother, Cl_{tot} the total body clearance from the fetal compartment, and Cl_f the fetal tissue clearance. Cl_{tot} in the isolated fetus is equal to Cl_f in the non-isolated fetus. By using the Cl_{tot} of the isolated and non-isolated fetuses presented in Table II, the Cl_p of 0.127 ml/min was obtained. On the other hand, Cl_p obtained from the fetal and maternal PPBA levels in the fetal-maternal unit was 0.142 ml/min. The similarity in the Cl_p obtained by both methods suggests that the pharmacokinetic model used in the present study is applicable.

The finding that both values of Cl_p were over 80% of Cl_{tot} indicates that PPBA in the fetal compartment is mainly eliminated *via* the placenta. The contents in the urinary bladder and the intestine corresponded to less than 1% of the injected radioactivity during the experimental period of 1 h. This result is presumably due to the immaturity in the functions of both urinary and biliary excretion, producing the low value of Cl_f. In conclusion, the clearance of the fetal compartment depends on the transplacental clearance.

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