CHANGE IN PHARMACOKINETIC CHARACTER OF *p*-PHENYL BENZOIC ACID IN DEVELOPING FETUS OF RAT

TOSHIO NANBO

Tokai Laboratories, Daiichi Pure Chemicals Co., Ltd., 2117, Tokaimura, Nakagun, Ibaraki

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The change in the pharmacokinetic character of *p*-phenyl benzoic acid (PPBA) in developing fetus of rat was investigated by a constant infusion of ^{14}C -PPBA. PPBA level in tissues increased with the day of gestation. The hepatic clearance for PPBA showed the increase with the stage of development. The excretion of the glucuronide from liver into bile increased with the stage, remarkably between the 19th and 21st day of gestation. The developmental profile of UDP-glucuronyltransferase activity in hepatic microsomes of fetus resembled that of the hepatic excretory function for the glucuronide. The glucuronide level in the amniotic fluid increased with the day of gestation, remarkably between the 19th and 21st day of gestation.

The elimination rate of the glucuronide from amniotic fluid determined by intra-amniotic injection of the glucuronide decreased linearly with the day of gestation.

Keywords— ^{14}C -*p*-phenyl benzoic acid; glucuronide; developing fetus; constant infusion; hepatic excretory function; storage capacity; UDP-glucuronyltransferase; elimination rate from amniotic fluid

Previous report¹⁾ has demonstrated that the pharmacokinetic character of *p*-phenyl benzoic acid (PPBA) in fetal compartment was different from that in maternal compartment and changed with the day of gestation. The change in the pharmacokinetic character of PPBA with the stage can be associated with the change in the hepatic excretory function, UDP-glucuronyltransferase activity and the elimination of the glucuronide in amniotic fluid.

The immaturity of hepatic excretory function in new born animals was demonstrated by comparing with adult animals^{2–6)} and the development of UDP-glucuronyltransferase activity in fetal liver was investigated using drug forming ether type glucuronide.⁷⁾

The clarification of the change in the pharmacokinetic character with the day of gestation is considered to be important from the viewpoint of the change in the detoxication ability for xenobiotics in fetus.

The present study was designed to clarify the

mechanism related to the change of the pharmacokinetic character in fetal compartment with the stage of gestation observed in previous report.¹⁾

MATERIALS AND METHODS

Animal Treatment—Pregnant Wister rats from the 16th to the 21st day of gestation were used. The stage of gestation was determined by the method in a previous report.¹⁾ The bile duct of animal was cannulated as previously reported.¹⁾ ^{14}C -PPBA synthesized as described previously¹⁾ was infused at a constant rate of 1.25 mg/kg/h into the femoral vein of mother for 8 h with an infusion pump (Harvard Apparatus Model 975). Animals were anesthetized with pentobarbital during infusion period and placed in Bollman's cage after the end of infusion. Samples were taken in the same manner as that described in a previous report.¹⁾

Assay of UDP-glucuronyltransferase Activity—Fetal liver were collected as described in a

previous report.¹⁾ Microsomes from fetal liver were prepared as described by Lucier *et al.*⁸⁾ and resuspended in 3 volumes of isotonic 0.02 M Tris-HCl buffer (pH 7.6). The resultant microsomes (0.5ml) were incubated with 1 μ mol of 14 C-PPBA, 40 μ mol of uridine diphosphate glucuronic acid and 4 μ mol of MgCl_2 . The incubation mixture was adjusted to a final volume of one ml with 0.02 M Tris-HCl buffer (pH 7.6) and was incubated for 20 min at 37°C. The reaction rate was found to be linear under these conditions. The reaction was stopped by adding 0.5 ml of ethanol and then centrifuged. The supernatant was used to assay the glucuronide. The microsomal protein was determined according to the method of Lowry *et al.*⁹⁾

Elimination Rate of the Glucuronide in Amniotic Fluid — The bile from adult was injected into the amniotic fluid as described in a previous report.¹⁾ The volume of amniotic fluid and body weight of fetus on each stage were assayed using another rat and then the initial glucuronide concentration in amniotic fluid was determined to be 2 n mol/ml per g fetal body weight. The elimination rate was estimated from the glucuronide remaining in amniotic fluid at 0, 5 and 10 min.

Measurement and Analysis — Analytical method of radioactive substances in tissue and assay of radioactivity were as described in the a previous report.¹⁾

RESULTS

Fig. 1 shows PPBA and its glucuronide level in plasma during and after infusion of 14 C-PPBA on the 18th and 20th day of gestation. On the 20th day of gestation, PPBA levels in mother and fetus attained approximately to a steady state at 8 h after the start of infusion. PPBA level in fetus was 63% of that of mother at 8 h. After the end of infusion, PPBA level in mother decreased with a half life of 3.9 h. PPBA in fetus, however, maintained nearly a constant level for 4 h after the end of infusion, then, decreased slowly. The glucuronide in mother showed the same pattern as PPBA with lower concentration. The fetal glucuronide level was 10% of fetal PPBA level at

the end of infusion, then appeared to increase slightly.

On the 18th day of gestation, PPBA and its glucuronide level in mother appeared to be approximately similar to the corresponding levels on the 20th day of gestation. Fetal PPBA level showed the same pattern as that on the 20th day of gestation with lower concentration. Fetal glucuronide level, however showed a constant level after the end of infusion. PPBA and its glucuronide levels on the 18th day of gestation were about 60% of the corresponding levels on the 20th day of gestation at 24 h.

Liver and Intestine Level of Fetus

Fig. 2 shows PPBA and its glucuronide levels in liver and intestine of fetus on the 20th day of gestation during and after the infusion of 14 C-PPBA. PPBA levels in liver and intestine attained approximately to a steady state at 8 h after the

FIG. 1. Plasma Level of PPBA and Its Glucuronide during and after Continuous Intravenous Infusion of 14 C-PPBA to Rat

Each point indicates the mean of five rats or litters.

- — ■ maternal PPBA (18-d pregnant),
- — □ maternal PPBA (20-d pregnant),
- --- ■ maternal glucuronide (18-d pregnant),
- --- □ maternal glucuronide (20-d pregnant),
- △ — △ fetal PPBA (18-d pregnant),
- — ○ fetal PPBA (20-d pregnant),
- ▲ — ▲ fetal glucuronide (18-d pregnant),
- — ○ fetal glucuronide (20-d pregnant).

start of infusion and then decreased with the same elimination rate as that in fetal plasma.

The glucuronide in liver showed nearly the same pattern as PPBA in liver. Whereas, the glucuronide in intestine increased during and after the infusion of ^{14}C -PPBA.

Blood and Plasma Level of Developing Fetus

Fig. 3 shows the concentration of radioactivity in blood, and PPBA and its glucuronide in plasma of fetus at the end of infusion. PPBA level in plasma increased with the day of gestation. The rate of increase tended to be lower during last two days of gestation. The level of PPBA on the 21st day of gestation was 2.3 fold that on the 17th day of gestation. The radioactivity level in blood increased with the rate similar to PPBA level, and the ratio of the blood radioactivity level to the plasma PPBA level showed no significant change with the day of gestation. The radioactivity level in blood on the 21st day of gestation was 4.5 fold

that on the 16th day of gestation. The glucuronide level in plasma increased slightly with the day of gestation.

Fig. 4 shows the fetus/mother blood concentration ratio of the radioactivity at the end of infusion. The value of the ratio increased approximately linearly with the day of gestation. The value of the ratio on the 21st day of gestation was about 6 fold that on the 16th day of gestation.

Liver and Intestine Level of Developing Fetus

Fig. 5 shows PPBA and its glucuronide levels in fetal liver and intestine at the end of infusion. PPBA level in intestine increased nearly linearly with the day of gestation. The glucuronide level increased with lower rate than PPBA level from 17th to 19th day of gestation and with higher rate

FIG. 2. PPBA Levels in Liver and Intestine of Fetus on the 20th Day of Gestation during and after Continuous Intravenous Infusion of ^{14}C -PPBA to Rat. Each point indicates the mean $\pm$ S.E. of five litters.

- — ○ PPBA in liver,
- — ● glucuronide in liver,
- △ — △ PPBA in intestine,
- ▲ — ▲ glucuronide in intestine.

FIG. 3. Blood and Plasma Level of PPBA and Its Glucuronide in Developing Fetus

Each point indicates the mean $\pm$ S.E. of five litters.

- — total radioactivity in blood (equivalent to PPBA),
- — PPBA in plasma,
- — glucuronide in plasma.

during last two days of gestation. The glucuronide level appeared to be higher than PPBA level on the 21st day of gestation. The level on the 21st day of gestation was 3.8 fold higher in PPBA and 63.0 fold higher in the glucuronide compared with that on the 17th day of gestation, respectively.

PPBA and its glucuronide levels in liver showed lower rate of increase compared with the corresponding levels of intestine. Furthermore, the glucuronide level was lower than PPBA level throughout the gestation days. The levels on the 21st day of gestation were 2.4 fold and 11.5 fold higher in the glucuronide compared with the corresponding levels on the 17th day of gestation, respectively.

Concentration Ratio between Tissues of PPBA and Its Glucuronide in Developing Fetus

Table I shows the ratio of the concentration between tissues at the end of infusion. The

FIG. 4. Ratio of the Radioactivity Level in Fetal Blood to That in Maternal Blood
Each point indicates the mean $\pm$ S.E. of five rats and litters.

liver/plasma and intestine/liver PPBA concentration ratios showed no significant increase. Whereas, the intestine/plasma PPBA concentration ratio increased gradually with the day of gestation. The value of the ratio on the 21st day of gestation was about 2 fold that on the 17th day of gestation. PPBA level in the liver was lower than that in plasma throughout the days of gestation.

The intestine/liver glucuronide concentration ratio showed low rate of increase from the 17th to 18th day of gestation and remarkable increase between the 19th and 21st day of gestation. The value of the ratio on the 21st day of gestation was 6.6 fold higher than that on the 17th day of gestation.

PPBA Glucuronide in Amniotic Fluid and Its Elimination Rate

Fig. 6 shows the glucuronide level in amniotic

FIG. 5. Tissue Level of PPBA and Its Glucuronide in Developing Fetus

Each point indicates the mean of five litters.

- △ PPBA in liver,
- ▲ glucuronide in liver,
- PPBA in intestine,
- glucuronide in intestine.

fluid at the end of infusion and the elimination rate of the glucuronide in the amniotic fluid. The glucuronide level at the end of infusion increased with the day of gestation, remarkably between the 19th and 21st day of gestation. The elimina-

tion rate of the glucuronide in amniotic fluid decreased linearly with the day of gestation. The elimination rate on the 17th day of gestation was 4 fold that on the 21st day of gestation.

UDP-glucuronyltransferase Activity in Hepatic

TABLE I. *Concentration Ratio between Tissues of PPBA and Its Glucuronide in Developing Fetus*

		Day of gestation				
		17	18	19	20	21
Liver/ plasma	PPBA	0.15±0.02	0.16±0.02	0.17±0.03	0.19±0.04	0.21±0.05
Intestine/ Liver	PPBA	1.10±0.17	1.47±0.21	1.64±0.24	1.69±0.29	1.73±0.28
	Glucuronide	1.31±0.21	1.53±0.22	2.42±0.24 ^{a)}	3.35±0.33 ^{a)}	8.61±1.32 ^{b)}
Intestine/ Plasma	PPBA	0.17±0.03	0.24±0.04	0.28±0.05	0.32±0.05	0.36±0.05

The data indicates the mean ± S.E. of five litters.

a) Significantly different from the former day of gestation ($p < 0.05$).

b) Significantly different from the former day of gestation ($p < 0.01$).

FIG. 6. *Accumulation and Elimination Rate of the PPBA Glucuronide in Amniotic Fluid*
Each point indicates the mean of five litters.
● glucuronide concentration,
○ elimination rate.

FIG. 7. *Development of UDP-glucuronyltransferase Activity in Hepatic Microsomes of Fetus*
Each point indicates the mean ± S.E. of five litters.

Microsome of Fetus

Fig. 7 shows the change of UDP-glucuronyltransferase activity in fetal liver microsome with the day of gestation. The activity increased with the day of gestation, remarkably between the 19th and 21st day of gestation. The activity on the 21st day gestation was 4 fold that on the 17th day of gestation.

DISCUSSION

Previous report¹⁾ has demonstrated that the pharmacokinetic character of PPBA changes with the stage in fetal compartment after intravenous injection. The mechanism of the change of pharmacokinetic character in developing fetus was investigated in this study.

PPBA levels in both maternal and fetal compartments attained approximately to a steady state at the end of infusion. This result suggests that the transfer of PPBA reaches an equilibrium state in both compartments and in the fetal tissues at 8 h after the start of infusion.

The uptake of fetal blood cell for radioactive substance was low throughout the gestation days, since the plasma/blood radioactivity concentration ratios were over 1.5. PPBA was mainly recognized in plasma of developing fetus. The fetus/mother blood concentration ratio and PPBA level in the fetal plasma showed increase with the day of gestation. These results suggest that the placental transfer of PPBA or the storage capacity of fetal plasma, probably, associating with the change in the concentration of fetal plasma protein increased with the day of gestation.

The change in hepatic clearance of fetus with the stage was suggested in a previous report¹⁾. The elimination of PPBA from plasma into bile occurs in a stepwise manner — uptake of PPBA from plasma into liver, intrahepatic metabolism and storage and excretion from liver into bile. The developmental change of the PPBA concentration ratio between tissues suggests that the uptake of PPBA from plasma into liver and its excretion from liver into bile didn't increase significantly with the day of gestation. Whereas, the intestine/liver glucuronide concentration ratio

increased with the day of gestation. However, the developmental profile of the intestine/liver glucuronide concentration ratio resembled the change of the glucuronyltransferase activity with the day of gestation. This finding suggests that the increase of the hepatic excretory function for the glucuronide reflected mainly that of the glucuronide formation.

In a previous report,¹⁾ it was assumed that the glucuronide in fetal intestine was reabsorbed *via* hydrolysis from intestinal wall as well as PPBA. Therefore, the finding that the glucuronide in fetal intestine showed high level in later stage suggests that the enterohepatic circulation of the glucuronide *via* hydrolysis may play an important role in the long duration of plasma PPBA level in later stage.

The glucuronide level in amniotic fluid at the end of the infusion increased with the day of gestation, whereas the elimination rate of the glucuronide from amniotic fluid decreased with the day of gestation. Based on the present data, it is possible to assume that the increase in the formation of the glucuronide and the decrease in its elimination rate from amniotic fluid with the day of gestation are related to the increase in the accumulation of the glucuronide in amniotic fluid with the day of gestation.

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