PHARMACOKINETICS IN MATERNAL-FETAL UNIT AFTER INTRAVENOUS ADMINISTRATION OF \( p \)-PHENYL BENZOIC ACID TO RAT

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The pharmacokinetics in maternal-fetal unit of \(^{14}\text{C}-\text{p}\)-phenyl benzoic acid was studied in intact and bile duct-cannulated pregnant rats following the intravenous administration. The half life of the maternal blood level was shortened by bile duct-cannulation. However, the effect of bile duct-cannulation on the decay curve of fetal blood level was not recognized. There was no difference in the distribution pattern from whole body autoradiogram and tissue binding between mother and fetus.

PPBA level in fetal plasma was different from that in maternal central and peripheral compartment based on pharmacokinetic analysis. The glucuronide levels in fetal intestine and amniotic fluid increased with time.

The levels of PPBA and its glucuronide in fetal tissues on the 18th day of gestation were lower than the corresponding levels on the 20th day of gestation. The elimination rate of the glucuronide in amniotic fluid was higher on the 18th day of gestation.

Keywords—\(^{14}\text{C}-\text{p}\)-phenyl benzoic acid; pregnant rat; intravenous administration; distribution; glucuronide; fetus; autoradiography; intra-amniotic injection; tissue binding

It has been known that nonsteroidal anti-inflammatory drugs with biphenyl and carboxyl groups show a longer duration in fetus than in mother.\(^{1,2}\) The metabolic fate of the nonsteroidal anti-inflammatory drug is characterized by the enterohepatic circulation through glucuronide formation in adult rat.\(^{3-5}\) A likely mechanism in fetus can be estimated to contribute partly to the long duration of anti-inflammatory drug in fetus.

Shah and McLachlan\(^{4}\) reported the long duration of diethylstilbestrol glucuronide in fetus. However, relatively little attention has been directed to the pharmacokinetics in maternal-fetal unit of anti-inflammatory drug associated with a long duration in fetus. Additionally, the pharmacokinetic change with the stage is poorly understood.

The present work shows the pharmacokinetics in the maternal-fetal unit and the change of pharmacokinetic character with the stage in rat by using \( p \)-phenyl benzoic acid (PPBA) as a convenient “Model Substance” of nonsteroidal anti-inflammatory drug with biphenyl and carboxyl groups.

MATERIALS AND METHODS

Labelled Compound—\(^{14}\text{C}-\text{PPBA}\) was synthesized by reacting Grignard reagent of \( p \)-bromophenyl benzoic acid with \(^{14}\text{CO}_2\). The specific activity was 4.5 mCi/mmol. The radiochemical purity was ascertained to be over 98% by thin layer chromatography (TLC).

Animal Treatment—Normal male and female Wistar rats were bred and the day of finding vaginal spermatozoa was considered the 0 d of pregnancy. On the 18th and 20th day of gestation, pregnant female rats were selected and used. The bile duct of rats was cannulated with polyethylene tubing and the animals were placed in Bollman’s cage. Sodium salt of \(^{14}\text{C}-\text{PPBA}\) was administered intravenously at a dose of 2.5 mg/kg (free acid).
Whole Body Autoradiography — The animals receiving $^{14}$C-PPBA was anesthetized with ether, followed by immersion in dry ice-acetone mixture. Whole 30 μ sagittal sections of the frozen rat were taken with a microtome (PMV Type 400) according to Ullberg’s method$^{69}$ and freeze dried. The dried sections were exposed to X-ray film (Sakura Type H).

Analysis of Radioactive Substance in Tissues — Animals receiving $^{14}$C-PPBA were sacrificed, then fetuses were decapitated. Maternal and fetal blood were collected in heparin-treated tube. The organs from all fetuses and amniotic fluid of the same litter was pooled and kept frozen until analyzed. Half volume of ethanol was added to plasma and amniotic fluid sample and centrifuged at 4°C. Organs were homogenized in 1 volume of 50% methanol and then centrifuged at 4°C. The supernatant fraction of sample was subjected to TLC. The plate (Merck Silica gel F$_{254}$) was developed in n-butanol : acetic acid : water (4:1:2) at 4°C and contacted with X-ray film (Sakura Type H). The radioactive areas were then scraped off and mixed with 80% methanol in counting vials. The stability of the radioactive substances under development was confirmed by two dimensional TLC.

Identification of PPBA Glucuronide — Fetal intestines at 24 h after administration of $^{14}$C-PPBA to mother were homogenized with 1 vol. of methanol, then the supernatant was subjected to centrifugation. TLC of the supernatant showed two spots. After hydrolyzing the supernatant with β-glucuronidase or 0.5 N sodium hydroxide, TLC showed one spot with high Rf value corresponding to PPBA. In addition, the spot with low Rf value agreed with the spot of the glucuronide formed in liver preparations of adult rats according to Lucier's method.$^{69}$

Intra-amniotic Injection of Biliary Excretes — Fifty μl of bile obtained from an adult rat receiving $^{14}$C-PPBA was injected into amniotic fluid according to Chamberlain’s method.$^{70}$ Fifty μl of bile contained 2 nmol of the glucuronide. After killing mother, fetal organs and amniotic fluid were taken and analyzed.

Intravenous Injection of $^{14}$C-PPBA Glucuronide — The bile obtained from an adult rat administered with $^{14}$C-PPBA was extracted with ether to remove $^{14}$C-PPBA. The aqueous layer containing 0.5 μmol of $^{14}$C-PPBA glucuronide was injected intravenously to pregnant rats on the 20th day of gestation. The level in maternal and fetal plasma was assayed at 5 and 10 min after injection.

Tissue Binding — Maternal and fetal tissues of rat on the 20th day of gestation were homogenized in 2 vol. of isotonic 0.05 M phosphate buffer (pH 7.4). One ml of tissue homogenate or plasma was placed into cellophane tube (Visking 8/24) and sealed. The tubing was immersed in 5 ml of isotonic 0.05 M phosphate buffer (pH 7.4) and dialyzed for 1 h at 37°C with shaking. Radioactivities inside and outside the tubing were counted to determine the degree of tissue binding.

Determination of Radioactivity — Tissue samples were combusted by Sample Oxidizer (Packared Model 305). The supernatant of homogenates and the silica gel scraped off were mixed with 15 ml of toluene liquid scintillation medium. The radioactivity in each samples was measured in a liquid scintillation counter (ALOKA 305).

Pharmacokinetic Analysis — PPBA level in maternal plasma was analyzed using a two compartment open model. PPBA concentration in the peripheral compartment was calculated according to the method of Gibaldi and Perrier.$^{8}$

RESULTS

Blood Level

Fig. 1 shows the blood level of mother and fetus after the administration of $^{14}$C-PPBA to intact and bile duct-cannulated rats on the 20th day of gestation. The maternal blood of bile duct-cannulated rats showed the same level as that of intact rats during the first 3 h, then, decreased with a half life of 9.9 h in intact rats, 4.2 h in bile duct-cannulated rats, respectively. At 24 h, the maternal blood level in bile duct-cannulated rats was 35% of that in intact rats. Whereas, there was no significant difference in the blood level and its
half life of fetus between bile duct-cannulated and intact rats. The radioactivity in bile obtained from bile duct-cannulated rats contained 85% of the dose in 24 h.

Whole Body Autoradiogram

Fig. 2 is the autoradiogram of bile duct-cannulated female rats on the 20th day of gestation after the administration of $^{14}$C-PPBA. In mother at 5 min, the highest radioactivity was seen in blood, followed by placenta, liver, lung and kidney. The radioactivity in fetus was lower than that in maternal muscle. Fetal organs showed no specific localization. At 4 h, maternal liver showed higher concentration of radioactivity than maternal blood. In fetus, the highest radioactivity was shown in intestinal contents, followed by blood, liver and lung. Intestinal wall showed low radioactivity. The radioactivity in fetal blood was lower than that in maternal blood. At 24 h after dosing, the radioactivity in placenta and kidney was higher than that in maternal blood. The radioactivity in fetal organs decreased slowly compared with that in maternal organs, and fetal blood showed higher level than maternal blood. The pattern of distribution at 24 h was similar to that at 4 h.

Plasma Level

Fig. 3 shows the concentration of PPBA and its glucuronide in maternal and fetal plasma after the administration of $^{14}$C-PPBA to bile duct-cannulated rats on the 18th and 20th day of gestation. The maternal PPBA level on the 20th day of gestation decreased with biphasic curve having half lives of 0.15 h and 3.6 h. PPBA level of fetus reached a maximum at 4 h, then decreased with a half life of 13.1 h. The fetal PPBA level was higher than maternal PPBA level at 24 h after dosing. Maternal glucuronide level reached a maximum at 3 h after dosing, then decreased with a half life of 6.2 h. Whereas, fetal glucuronide level increased until 6 h after dosing, then maintained a constant level.

On the 18th day of gestation, the pattern of maternal PPBA and glucuronide levels were nearly similar to those on the 20th day of gestation. PPBA level of fetus reached a maximum at 4 h, then decreased with a half life of 12.5 h. The level was lower than maternal level throughout the experimental period. The glucuronide level reached a maximum at 8 h, then maintained nearly a constant level. The ratio of the 0 to 24 h AUC of the earlier stage to that of the latter stage was 35.8% in PPBA level of fetus and 36.7% in the glucuronide level of fetus, respectively.

PPBA level in maternal peripheral compartment obtained from maternal PPBA level on the 20th day of gestation is plotted in Fig. 4 as the broken line. The level in the maternal peripheral compartment appeared to peak in approximately 50 min, thereafter declined in parallel with that in central compartment. The level in maternal lung was nearly consistent with that in peripheral compartment. On the other hand, the fetal lung appeared to show the same pattern as the fetal plasma.

Fetal Liver

Fig. 5 shows the level of PPBA and its glucuronide in fetal liver after the administration
FIG. 2. Wholebody Autoradiograms after Intravenous Administration of $^{14}$C-PPBA to Bile Duct-Cannulated Rat

of $^{14}$C-PPBA to bile duct-cannulated rats on the 18th and 20th day of gestation. PPBA level on the 20th day of gestation reached a maximum at 4 h after dosing, thereafter, decreased with a half life of 9.2 h. The glucuronide level increased with the lapse of time and showed nearly the same level as PPBA level at 24 h after dosing.

On the 18th day of gestation, PPBA level showed a peak at 4 h then decreased with a half life of 9.3 h. The glucuronide level reached a maximum at 4 h, thereafter, maintained nearly a constant level. The levels of PPBA and its glucuronide were lower than the corresponding levels on the 20th day of gestation.

_Fetal Intestine_

Fig. 6 shows the level of PPBA and its glucuronide in fetal intestine after the administration of $^{14}$C-PPBA to bile duct-cannulated rats on the 18th and 20th day of gestation. PPBA level of the 20th day of gestation reached a maximum at 4 h after dosing, then decreased. The glucuronide level increased with the lapse of time. After 24 h, the level of glucuronide was 3 fold higher than that of PPBA. On the 18th day of gestation, PPBA level reached a maximum at 4 h after dosing, then decreased. The glucuronide level increased until 12 h, then maintained nearly a constant level. At 72 h, the glucuronide level tended to decrease. The PPBA and its glucuronide levels on the 18th day of gestation

**FIG. 3.** Plasma Level after Intravenous Administration of $^{14}$C-PPBA to Bile Duct-Cannulated Rat

Each point indicates the men 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),
- Δ fetal PPBA (18-d pregnant),
- ○ fetal PPBA (20-d pregnant),
- △ fetal glucuronide (18-d pregnant),
- ● fetal glucuronide (20-d pregnant).

**FIG. 4.** Time Course of Plasma and Lung Levels after Intravenous Administration of $^{14}$C-PPBA to Bile Duct-Cannulated Rat

Each point indicates the mean ± S.E. of five rats or litters.

- ▲ maternal plasma level,
- ○ maternal lung level,
- ● fetal plasma level,
- O fetal lung level,
- --- calculated central compartment level,
- --- calculated peripheral compartment level.

Pharmacokinetic parameters were obtained from two compartment model method.
FIG. 5. Liver Level of Fetus after Intravenous Administration of $^{14}$C-PPBA to Bile Duct-Cannulated Rat

Each point indicates the mean of five rats or litters. 
△ PPBA (18-d pregnant), ▲ glucuronide (18-d pregnant), ○ PPBA (20-d pregnant), ● glucuronide (20-d pregnant).

FIG. 6. Intestine Level of Fetus after Intravenous Administration of $^{14}$C-PPBA to Bile Duct-Cannulated Rat

Each point indicates the mean of five rats or litters. 
△ PPBA (18-d pregnant), ▲ glucuronide (18-d pregnant), ○ PPBA (20-d pregnant), ● glucuronide (20-d pregnant).

FIG. 7. Amniotic Fluid Level after Intravenous Administration of $^{14}$C-PPBA to Bile Duct-Cannulated Rat

Each point indicates the mean of five rats or litters. 
△ PPBA (18-d pregnant), ▲ glucuronide (18-d pregnant), ○ PPBA (20-d pregnant), ● glucuronide (20-d pregnant).

were lower than the corresponding levels on the 20th day of gestation.

Amniotic Fluid

Fig. 7 shows the level of PPBA and its glucuronide in amniotic fluid after the administration of $^{14}$C-PPBA to bile duct-cannulated rats on the 18th and 20th day of gestation. PPBA levels of both gestations reached a peak at 2 h, then decreased with a half life of 10 h.

The glucuronide level on the 20th day of gestation increased with the lapse of time and was higher than PPBA level at 24 h after dosing. While, the glucuronide level on the 18th day of gestation reached a maximum at 2 h and thereafter maintained nearly a constant level.

Placental Transfer of the Glucuronide

Five min and 10 min after the injection of the bile containing the glucuronide, maternal plasma showed 5 picomol/ml and 0.4 picomol/ml of radioactive substances, respectively. But, fetal plasma showed no significant radioactivity.

Elimination of the Glucuronide in Amniotic Fluid
The rate of elimination from amniotic fluid is shown in Fig. 8. The elimination ratio of the glucuronide was 42% in 10 min and 59% in 1 h on the 20th day of gestation, respectively. On the 18th day of gestation, 58% of the glucuronide was eliminated from amniotic fluid in 10 min and about 100% was eliminated in 30 min.

**Tissue Binding of \(^{14}\)C-PPBA**

Table I shows the binding ratio of \(^{14}\)C-PPBA to maternal and fetal tissue. There was no significant difference in the degree of tissue binding between mother and fetus.

**DISCUSSION**

PPBA was used as a convenient “Model Substance” of nonsteroidal antiinflammatory drug with biphenyl and carboxyl groups for following reasons. Its chemical structure is similar to the nonsteroidal antiinflammatory drugs such as Ketoprofen and the simple metabolism that a great part of metabolism of PPBA is the conjugation with glucuronic acid which made it convenient to investigate the pharmacokinetics maternal-fetal unit.

There was a significant difference in the effect of bile duct-cannulation on the pharmacokinetic character between mother and fetus. The radioactivity in maternal blood decreased in both the half life and the AUC by the bile duct-cannulation. This change is estimated to be due to the removal of the reabsorption of biliary excretes by the bile duct-cannulation. Whereas the pharmacokinetic character of the radioactivity in fetal blood showed no significant effect by the bile duct-cannulation. This result suggests that the pharmacokinetic character of PPBA in fetal compartment is different from that in maternal compartment. This speculation is supported by the pharmacokinetic analysis. The pattern of PPBA level in fetal plasma was different from that in both maternal central and peripheral compartments. PPBA level in maternal lung was nearly consistent with that in maternal peripheral compartment, whereas PPBA level in fetal lung showed the pattern paralleled to that in fetal plasma. Thus, it is speculated that the fetal compartment has the storage capacity for PPBA passed through placenta shortly after dosing.

**TABLE I. Binding of \(^{14}\)C-PPBA to Maternal and Fetal Tissues**

<table>
<thead>
<tr>
<th></th>
<th>Binding ratio (%)</th>
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<tbody>
<tr>
<td></td>
<td>Mother</td>
</tr>
<tr>
<td>Lung</td>
<td>17.5±2.5</td>
</tr>
<tr>
<td>Liver</td>
<td>24.5±3.2</td>
</tr>
<tr>
<td>Kidney</td>
<td>16.7±1.8</td>
</tr>
<tr>
<td>Muscle</td>
<td>19.4±2.3</td>
</tr>
<tr>
<td>Plasma</td>
<td>18.6±2.8</td>
</tr>
</tbody>
</table>

The value indicates the mean ± S.E. of five rats or litters.
The pharmacokinetics in maternal-fetal unit was investigated using bile duct-cannulated animals to avoid the affect of reabsorption of biliary excretes in maternal compartment. No difference in the distribution pattern between mother and fetus was observed in the whole body autoradiogram. This result agreed with the finding that there was no difference in the tissue binding between mother and fetus. Therefore, the pharmacokinetic difference between mother and fetus is not related to the qualitative nature in the tissue binding.

The glucuronide was observed in fetal tissue. It is well known that the glucuronide does not pass the placenta due to its hydrophilic property. It was confirmed by intravenous injection of \(^{14}\)C-PPBA glucuronide to mother that PPBA glucuronide does not pass the placenta. Therefore, the glucuronide in fetus was formed in fetus, probably, in fetal liver. The fetal intestine showed the high level of PPBA in a short time and the glucuronide in a later time after dosing of \(^{14}\)C-PPBA. The level in intestine reflects mainly the level in the intestinal contents from whole body autoradiogram. Therefore, PPBA and the glucuronide in intestinal contents are estimated to be derived from the biliary excretes. PPBA level in intestinal contents decreased with time and PPBA in amniotic fluid showed no accumulation. These findings suggest that PPBA in the intestinal contents on the 20th day of gestation was absorbed from intestinal wall of fetus. Whereas, the glucuronide in intestine on the 20th day of gestation increased with time. The glucuronide in the intestinal contents on the 18th day of gestation, however, tended to decrease at 72 h. This result suggests that the glucuronide in intestine is reabsorbed mainly from intestinal wall, probably after deconjugation, and the aglycon enters an enterohepatic circulation system. It is estimated that the enterohepatic circulation of PPBA in fetal compartment gave rise to the pharmacokinetic difference in fetal compartment from maternal compartment.

The finding that the glucuronide in amniotic fluid increased with nearly the same pattern as that in intestine and showed higher level than that in plasma suggests that the glucuronide in amniotic fluid was mainly derived from intestinal contents. The excretion of the glucuronide from intestinal contents into amniotic fluid should not be neglected since the total amount of the glucuronide in amniotic fluid on the 20th day of gestation was about 30% of the glucuronide in intestinal contents at 24 h.

It was well known that the substance in amniotic fluid is absorbed from digestive loop via swallowing of amniotic fluid. The glucuronide injected amniotically was well absorbed into fetal body. This result suggests that the glucuronide swallowed was reabsorbed after hydrolysis by intestinal \(\beta\)-glucuronidase. The glucuronide level in amniotic fluid on the 20th day of gestation increased with time and then reached nearly the same level as PPBA in fetal plasma. Thus, the glucuronide in amniotic fluid is estimated to contribute partly to the duration of fetal plasma level after reabsorption into fetal body on the 20th day of gestation.

Shah and McLachlan\(^{10}\) reported that the level of the glucuronide of diethylstilbestrol in fetal plasma was higher than those of diethylstilbestrol in fetal plasma and its glucuronide in maternal plasma. Whereas, diethylstilbestrol in fetal plasma showed the same level as that in maternal plasma. They demonstrated that the mechanism for these findings is that the glucuronide in fetus can not pass through the placenta. Lucier \etal\(^{9}\) demonstrated the same mechanism in the report using hydroxylated metabolites of polychlorinated biphenyls. The present study also showed the accumulation of the glucuronide in fetal plasma, probably, due to the mechanism described by Shah and McLachlan.\(^{40}\) PPBA level in fetal plasma, however, was higher than the glucuronide level in both stages of gestation. It is estimated that the difference from Shah and Lucier's results is related to the difference in the rate of conjugation and deconjugation between hydroxyl and carboxyl groups. Considering that aglycon has the biological effect and the glucuronide is detoxicated,\(^{10}\) it is noticeable that
substance forming ester type glucuronide shows the longer duration as aglycon in fetal tissues compared with substance forming ether type glucuronide.

The difference in the level of fetal tissue and amniotic fluid between the 18th and 20th day of gestation was observed. PPBA decay curves of plasma, liver and intestine on the 18th day of gestation were nearly parallel to the corresponding PPBA decay curves on the 20th day of gestation with lower level. This result suggests that the function of the storage capacity including an enterohepatic circulation for PPBA in fetal compartment changed with the day of gestation. While the deviation in the tissue glucuronide level of both stages increased with time, remarkably in intestinal contents. The deviation of the glucuronide level in both stages is estimated to be due to the difference in the formation of the glucuronide with the day of gestation. A striking difference between both stages was that the glucuronide in the amniotic fluid of the later stage increased with time, whereas that of the earlier stage tended to decrease with time. The difference between both stages is estimated to be due to the difference in the formation of the glucuronide and its elimination rate from amniotic fluid between the two stages. The elimination rate of the glucuronide injected intraamnionically was higher in the earlier stage. This result suggests that the immaturity of fetal tissue in earlier stage makes the passage of the glucuronide easier.

REFERENCES

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