CHANGE IN KINETIC PROPERTIES AND METABOLIC CLEARANCE OF UDP-GLUCURONYLTRANSFERASE FOR *p*-PHENYL BENZOIC ACID IN DEVELOPING FETUS OF RAT

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The hepatic microsomal UDP-glucuronyltransferase (UDPGT) for *p*-phenyl benzoic acid (PPBA) in the developing fetus of rat was investigated. The kinetic properties of UDPGT in microsome of fetus changed during developmental period. The value of the $V_{\max}$ for PPBA and uridine 5'-phosphoglucuronic acid (UDPGA) per mg protein increased with the day of gestation, remarkably between the 19th and 21st day of gestation. The developmental profile of the $V_{\max}$ per g liver was similar to the $V_{\max}$ per mg protein. Whereas the $V_{\max}$ in whole liver showed rapid increase during development comparing with the $V_{\max}$ per g liver. The K_m for PPBA and UDPGA showed slower increase with the day of gestation than the corresponding $V_{\max}$.

Keywords — UDP-glucuronyltransferase; kinetic properties ($V_{\max}$ and K_m); liver size; *p*-phenyl benzoic acid; developing fetus

Previous reports^{1,2)} showed that the glucuronidation of *p*-phenyl benzoic acid (PPBA) in fetus increased with the day of gestation, remarkably between the 19th and 21st day of gestation, after the administration of PPBA to maternal rat from the 17th to 21st day of gestation. The increase of the glucuronidation of PPBA in fetus with gestation day was related to the development of the specific activity of UDP-glucuronyltransferase (UDPGT) in hepatic microsomes of fetus.²⁾

The development in the capability for the glucuronidation in the fetal liver depends on the development in the specific enzyme activity, protein content of liver and size of liver.³⁾ The present study demonstrated the development in the capability for the glucuronidation of PPBA to clarify the change in the ability to conjugate PPBA in developing fetus of rat. In the present study, the microsomal fraction was chosen as the enzyme source because many other studies have used microsomal fraction as the enzyme source and UDPGT activity distributes predominantly in microsomal fraction.^{4,5)}

MATERIALS AND METHODS

Chemicals — ¹⁴C-PPBA with specific activity of 45 mCi/mmol was synthesized as described previously.¹⁾ Uridine 5'-phosphoglucuronic acid (UDPGA) was obtained from the Sigma Chemical Company.

Animal — Pregnant Wistar strain rats with the pregnant day determined by the method described in a previous report¹⁾ were used.

Preparation of Tissues — Fetuses were removed from mother killed by cervical dislocation and decapitated. Livers were immediately excised and homogenized with 3 ml of isotonic 0.02 M Tris-KCl buffer (pH 7.8) per g of liver. The preparation of microsomes and estimation of microsomal protein content were performed as described previously.²⁾ The microsomal fractions were resuspended in isotonic 0.02 M Tris-KCl buffer (pH 7.8).

Incubation System — The incubation conditions are described in the respective tables. The range of PPBA concentration used in the determination of the $V_{\max}$ for PPBA included the liver PPBA levels in the *in vivo* experiment in

the previous report.²⁾ The incubation mixture was preincubated for 10 s at 37°C in a water bath before starting the reactions by the addition of substrate and then further incubated for 20 min. At the end of the reaction, 50 μ l of 50% eth-

anol was added and then centrifuged. The supernatant was used to assay the glucuronide according to the previous report.¹⁾ Enzyme kinetic constants were obtained by a computerized least-squares method. The $V_{\max}$ of the liver was ob-

TABLE I. *Developmental Change in Body Weight, Liver Weight and Microsomal Protein*

Gestation day	Body weight g	Liver weight g	g/100g body weight	Microsomal protein mg/g liver
15	0.286 $\pm$ 0.043	0.0152 $\pm$ 0.0020	5.31 $\pm$ 1.02	10.5 $\pm$ 1.6
16	0.538 $\pm$ 0.092 ^{a)}	0.0308 $\pm$ 0.0043 ^{a)}	5.72 $\pm$ 0.95	11.8 $\pm$ 1.7
17	0.959 $\pm$ 0.128 ^{a)}	0.0617 $\pm$ 0.0080 ^{a)}	6.43 $\pm$ 0.96	13.3 $\pm$ 1.9
18	1.70 $\pm$ 0.22 ^{a)}	0.117 $\pm$ 0.015 ^{a)}	6.89 $\pm$ 1.08	14.5 $\pm$ 2.2
19	2.32 $\pm$ 0.31	0.171 $\pm$ 0.022	7.37 $\pm$ 1.25	16.4 $\pm$ 2.5
20	4.35 $\pm$ 0.64 ^{a)}	0.274 $\pm$ 0.035 ^{a)}	6.30 $\pm$ 0.95	18.2 $\pm$ 2.3
21	5.53 $\pm$ 1.02	0.344 $\pm$ 0.046	6.22 $\pm$ 0.92	20.8 $\pm$ 2.8

Data are expressed as the mean $\pm$ S.E. of five litters.

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

TABLE II. *Developmental Change in Kinetic Properties of Hepatic Microsomal UDPGT for PPBA*

	Gestation day			
	15	16	17	
K_m mM	0.330 ± 0.051	0.367 ± 0.060	0.485 ± 0.073	
V_{max} nmol/mg protein/min	0.0421 ± 0.0062	0.0472 ± 0.0061	0.0543 ± 0.0076	
nmol/g liver/min	0.442 ± 0.057	0.557 ± 0.062	0.722 ± 0.094	
nmol/whole liver/min	0.00672 ± 0.00090	0.0172 ± 0.0022 <i>a)</i>	0.0445 ± 0.0058 <i>a)</i>	
	Gestation day			
	18	19	20	21
	0.566 ± 0.085	0.583 ± 0.092	0.690 ± 0.110	0.714 ± 0.107
	0.0851 ± 0.0103 <i>a)</i>	0.141 ± 0.019 <i>a)</i>	0.236 ± 0.033 <i>a)</i>	0.451 ± 0.056 <i>a)</i>
	1.23 ± 0.13 <i>a)</i>	2.31 ± 0.24 <i>a)</i>	4.29 ± 0.51 <i>a)</i>	9.38 ± 1.22 <i>a)</i>
	0.144 ± 0.019 <i>a)</i>	0.393 ± 0.051 <i>a)</i>	1.18 ± 0.18 <i>a)</i>	3.22 ± 0.42 <i>a)</i>

The incubation mixture contained the following in a final volume of 0.1 ml:

UDPGA, 4 μ mol; $MgCl_2$, 0.4 μ mol; hepatic microsomal protein of fetus, 0.15 mg;

Data are expressed as the mean $\pm$ S.E. of five litters.

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

tained by multiplying the specific enzyme activity (nmol/mg protein/min) by the microsomal protein content in the liver with correction for microsomal yield. Microsomal yield was determined by recovery of glucose-6-phosphatase.⁶⁾

RESULTS

Body Weight, Liver Weight and Protein Content in Developing Fetus

Table I shows the body weight, liver weight and protein content in liver of developing fetus. The body weight and liver weight increased rapidly with the day of gestation. The liver/body weight ratio, however, showed no significant change with the day of gestation. Protein content showed low increase with the day of gestation comparing with the liver weight. Protein content on the 21th day of gestation was 2-fold that on the 15th day of gestation.

Kinetic Properties of UDPGT in Developing Fetus

Table II shows the development in the kinetic properties ($V_{\max}$ and K_m) of hepatic microsomal UDPGT for PPBA. The $V_{\max}$ expressed as nmol/mg protein tended to increase slowly by the 18th day of gestation. The rapid increase in the $V_{\max}$ was observed between the 19th and 21th day of gestation. The $V_{\max}$ on the 21th day of gestation was 10.7-fold and 5.3-fold higher comparing with those on the 15th and 18th day of gestation, respectively. The $V_{\max}$ expressed as

nmol/g liver showed nearly the same developmental profile as that expressed as nmol/mg protein. Whereas, the $V_{\max}$ of whole liver increased rapidly comparing with that expressed as nmol/g liver throughout all gestation days. The $V_{\max}$ of whole liver on the 21th day of gestation was 479 times and 22 times those on the 15th and 18th day of gestation, respectively. The apparent K_m increased throughout all gestation days. The rate of increase with the day of gestation in the K_m was lower comparing with that in the $V_{\max}$ between the 18th and 21th day of gestation. The apparent K_m on the 21th day of gestation was 2.2 times that on the 15th day of gestation.

Table III shows the development in the kinetic properties of UDPGT for UDPGA. The developmental patterns of the $V_{\max}$ and the apparent K_m resembled those of the corresponding kinetic properties for PPBA throughout all gestation days. The $V_{\max}$ for UDPGA on the 21th day of gestation was 12.2-fold and 7.3-fold higher comparing with those on the 15th and 18th day of gestation, respectively. The apparent K_m for UDPGA on the 21th day of gestation was 2.7 times that on the 15th day of gestation.

DISCUSSION

The $V_{\max}$ expressed as nmol/mg protein for PPBA increased with the day of gestation, re-

TABLE III. Developmental Change in Kinetic Properties of Hepatic Microsomal UDPGT for UDPGA

Gestation day	K_m mM	$V_{\max}$ picomol/mg protein/min
15	1.49 ± 0.20	19.3 ± 2.5
16	1.72 ± 0.18	22.4 ± 2.8
17	2.25 ± 0.26	25.6 ± 3.3
18	2.63 ± 0.34	32.5 ± 3.9
19	2.78 ± 0.29	59.5 ± 7.6 ^{a)}
20	3.02 ± 0.32	101 ± 13 ^{a)}
21	3.98 ± 0.31	236 ± 28 ^{a)}

The incubation mixture contained the following in a final volume of 0.1 ml: ¹⁴C-PPBA, 50 nmol; MgCl₂, 0.4 μmol; hepatic microsomal protein of fetus, 0.15 mg;

Data are expressed as the mean ± S.E. of five litters.

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

markably between the 19th and 21st day of gestation. UDPGT activity is known to show the different developmental pattern towards substrate.^{7,8)} Wishart⁹⁾ reported that UDPGT activity for 2-aminophenol and 1-naphthol increased remarkably comparing with that for bilirubin and testosterone during the late fetal period in rat. The developmental pattern of the $V_{\max}$ expressed as nmol/mg protein for PPBA was similar to that of the activity for 2-aminophenol and 1-naphthol in rat.

The rate of increase with the day of gestation in the content of microsomal protein per g liver was lower comparing with that in the $V_{\max}$ per g liver. Whereas, the developmental profile of the $V_{\max}$ per mg protein was similar to that of the $V_{\max}$ per g liver. This finding suggests that the synthesis of UDPGT was higher than that of the total protein in microsomal fraction during maturational development of fetal liver. The $V_{\max}$ of whole liver for PPBA showed dominant increase with the day of gestation comparing with the $V_{\max}$ per g liver. From these results, the change in the total capability for glucuronidation in developing fetus is assumed to mainly depend on the changes in the specific microsomal UDPGT activity and liver size. The developmental profile of the $V_{\max}$ of whole liver corresponded well with the change in the glucuronide level of fetal intestine with the day of gesta-

tion in the previous report.²⁾ This finding indicates that the total capability for the glucuronidation of PPBA from *in vitro* experiment reflects well the result from *in vivo* experiment.

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