Liver Fibrosis in an Extremely Small Infant for Gestational Age

Hirokazu Arai,^{1,2} Atsuko Noguchi,¹ Ryoji Goto,² Takefumi Matsuda,² Hatsushi Nakajima² and Tsutomu Takahashi¹

¹Department of Pediatrics, Akita University Graduate School of Medicine, Akita, Japan

²Department of Pediatrics, Akita Red Cross Hospital, Akita, Japan

Premature infants with intrauterine growth restriction (IUGR) are at greater risk for an adverse perinatal outcome. IUGR affects hepatocyte function, but the histopathological changes in the postnatal liver are not well known. We report a male infant with severe IUGR. His mother was transferred to our hospital at 26 weeks of gestation because of preterm labor and severe IUGR. An emergency cesarean section was carried out because of a non-reassuring fetal status. The birth weight of the infant was 332 g. He showed congestive heart failure and marked hepatomegaly from birth. After 1 week, blood examinations showed hyperbilirubinemia with high direct bilirubin. Because of liver dysfunction, he received the minimal total parenteral nutrition for 7 days. After 1 month, he progressively developed ascites and coagulopathy, and died 3 months after birth. Liver autopsy showed diffuse perisinusoidal fibrosis. Fibrosis was also prominent around the central vein. Immunohistochemical study revealed many α -smooth muscle actin-positive cells, which represent activated hepatic stellate cells, and a few transforming growth factor- β 1-positive cells in the perisinusoidal fibrotic area. These results indicate that the infant developed chronic (end stage) liver failure by 3 months of age. We excluded congenital infection, metabolic syndrome and citrin deficiency. It is therefore conceivable that intrauterine cardiac failure may be responsible for liver fibrosis. Early detection of liver dysfunction soon after birth is a key to predict the prognosis of severe IUGR in preterm infants.

Keywords: liver failure; IUGR; preterm infant; liver fibrosis; extremely low birth weight
Tohoku J. Exp. Med., 2010, **221** (3), 181-185. © 2010 Tohoku University Medical Press

Premature infants with intrauterine growth restriction (IUGR) are at greater risk for postnatal growth and development as well as acute and chronic morbidities (Rosenberg 2008). IUGR influences hepatocyte function (Alonso et al. 2007). Compared to appropriate for gestational age (AGA) infants of similar birth weight, small for gestational age (SGA) infants show a higher rate of neonatal cholestasis (Boehm et al. 1990); however, only one report has shown postnatal liver histopathological changes for SGA infants that received prolonged total parenteral nutrition (TPN) (Baserga and Sola 2004).

Herein, we report the unusual case of an extremely low birth weight (ELBW) infant with severe IUGR. At birth, the infant showed congestive heart failure. Severe coagulopathy, thrombocytopenia, and jaundice persisted. The infant received the minimal TPN for only 7 days, and subsequently developed severe perisinusoidal liver fibrosis.

Patient Report

A 36-year-old woman, gravida 2, para 1, was referred to another hospital at 25 weeks of gestation with suspected IUGR. IUGR was first suspected at 23 weeks of gestation at another clinic. She was transferred to our hospital at 26

weeks of gestation because of preterm labor and severe IUGR. Ultrasound revealed severe growth restriction of the fetal biparietal diameter and abdominal circumference (each below -2 standard deviation). A first-trimester ultrasound had confirmed gestational age. She had reliable date parameters confirmed by the crown-rump length at 8 weeks' gestation. During her first pregnancy at 26 years old, she had undergone an artificial abortion. There had been no complications in her second pregnancy; a healthy child was born spontaneously at term. There was no family history of hereditary disorders. She had not taken medicine during the pregnancy. On admission, she did not have premature rupture of the membranes. Fetal ultrasound showed reverse end-diastolic flow in the umbilical artery and umbilical venous pulsations. Middle cerebral artery flow pattern showed redistribution (resistance index = 0.66). We did not examine the fetal karyotype because her family refused.

At 26 weeks of gestation, an emergency cesarean section was carried out because of a non-reassuring fetal status (minimal fetal heart rate variability). The birth weight of the male infant was 332 g. The infant had asphyxia, with Apgar scores of 3 at one minute and 5 at five minutes, and required resuscitation with tracheal intubation. On admis-

Received January 12, 2010; revision accepted for publication May 7, 2010. doi:10.1620/tjem.221.181

Correspondence: Hirokazu Arai, M.D., Ph.D., Department of Pediatrics, Akita University Graduate School of Medicine, Hondo 1-1-1, Akita, Akita 010-8543, Japan.

e-mail: arahiro@med.akita-u.ac.jp

Fig. 1. Infant echocardiography. Echocardiography showed dilated inferior vena cava (left) and tricuspid regurgitation (right) at day 0.

Table 1. Laboratory findings on admission (day 0).

pH	7.202	AST	52 U/L	Na	140 mmol/L
pCO ₂	35.7 mmHg	ALT	3 U/L	K	3.6 mmol/L
BE	-13.3 mEq/L	LDH	851 U/L	Cl	99 mmol/L
BS	127 mg/dL	GGT	204 U/L	Ca	9.4 mg/dL
		T.Bil	2.4 mg/dL	iP	5.2 mg/dL
WBC	3,600/ μ L	D.Bil	0.5 mg/dL	Fe	79 μ g/dL
Hb	13.6 g/dL	ALP	199 U/L	IgG	353 mg/dL
Plt	0.2×10^4 / μ L	CK	309 U/L	IgM	1 mg/dL
		TP	3.2 g/dL	CRP	0.0 mg/dL
APTT	69.9 sec	Alb	2.2 g/dL		
PT	34%	T.Chol	21 mg/dL		
Fib	< 50 mg/dL	TG	27 mg/dL		
D-dimer	2.0 mg/dL	BUN	9.7 mg/dL		
AT-III	< 20%	Cr	0.66 mg/dL		
HPT	24%				

sion, he had respiratory distress syndrome, and received artificial surfactant replacement therapy. A chest radiograph showed cardiomegaly. Echocardiography showed left ventricular dysfunction with ejection fraction of 46% (Murae et al. 2002), a dilated inferior vena cava, and tricuspid and mitral regurgitation (Fig. 1). Soon after birth, the patient showed congestive heart failure. Congenital cardiac malformation was absent. The patient was treated with inotropic and vasodilatory agents, and his cardiopulmonary condition improved gradually. No renal anomaly could be found on ultrasonography. Ophthalmological examination yielded normal findings. We could not examine his karyotype as the parents did not consent, but his face, appearance, and physical examination did not suggest trisomy 21 or 18.

Initial laboratory studies (Table 1) indicated a platelet count of 0.2×10^4 / μ L. Blood examinations showed metabolic acidosis (pH 7.202, BE -13.3 mmol/L), and coagulopathy was identified: fibrinogen (< 50 mg/dL) and prothrombin time (PT) % (34%) were low. Blood ammonia and blood glucose levels were within the normal ranges.

He showed marked hepatomegaly from birth. After 1 week, blood examinations showed hyperbilirubinemia with high direct bilirubin (total and direct bilirubin of 17.7 and

13.3 mg/dL at 17 days of life, respectively) (Fig. 2). Because of liver dysfunction, TPN (amino acids 0.3-1 g/kg/day; glucose infusion rate, max 6 mg/kg/minute; and vitamins) was used for only 7 days and we did not use intravenous lipid infusion; however, cholestasis did not improve, and we started ursodeoxycholic acid (UDC) (15 mg/kg/day).

After 1 month, he progressively presented with severe liver dysfunction with cholestasis, which led to the development of ascites, coagulopathy, and hypoalbuminemia. We administered fresh frozen plasma and vitamin K infusion frequently to improve coagulopathy, but these therapies were unsuccessful; his condition became unstable and he progressed to grade IV intraventricular hemorrhage. Plasma amino acid examination on day 91 showed hypertyrosinemia (1,113 nmol/mL). We suspected tyrosinemia type I, but no accumulation of upstream metabolites and succinylacetone was detected. Alpha-fetoprotein was 8,264.6 ng/mL (normal range) on the same day. Total bile acids were 119 μ mol/L (day 91). We used special milk formulas (medium chain triglyceride (MCT)-enriched formulas or tyrosine-free milk), but his condition did not improve. He died 3 months after birth because of sepsis.

Fig. 2. Clinical course. After 1 week, blood examinations showed hyperbilirubinemia with high direct bilirubin.

Fig. 3. Histology of the liver by Elastica-Masson staining. Liver specimens were fixed in formalin, embedded in paraffin, sectioned thinly, and stained with Elastica-Masson. Increased collagen fibers (arrows) were present along the sinusoids. Fibrosis was prominent around the central vein (arrowhead), and the hepatocytes showed frequent disruption (asterisk). Cellular infiltration of the parenchyma was absent. Fatty liver change was absent. Bar = 50 μ m

After death, congenital infection (varicella zoster, herpes, cytomegalovirus, toxoplasmosis, syphilis, and rubella), extrahepatic obstruction, citrin deficiency (neonatal intrahepatic cholestasis), and inborn errors of bile acid metabolism (e.g., primary 3-oxo-delta 4-steroid 5 beta-reductase deficiency) were excluded. The results of neonatal screening for metabolic diseases (galactosemia, homocystiuria, phenylketouria, and maple syrup urine disease) and endocrine diseases (hypothyroidism and adrenal hyperplasia) were negative. A liver sample was the only specimen obtained after death with informed consent from the parents. No extrahepatic biliary atresia was noted. Microscopically, diffuse perisinusoidal liver fibrosis with increased collagen fibers was observed (Fig. 3). Fibrosis was prominent around the central vein, and the hepatocytes showed frequent disruption. Iron deposits were mildly present. Cellular infiltration in the parenchyma was absent. Fatty liver change was also absent (Fig. 3).

Immunohistochemically, many α -smooth muscle actin-positive cells, which represent activated hepatic stellate cells (HSCs), were detected in the perisinusoidal fibrotic area (Fig. 4a and c); moreover, a few transforming growth factor (TGF)- β 1-positive cells were present in the same area (Fig. 4b and d).

Discussion

This patient received TPN for only 1 week because of cholestasis. In spite of minimal TPN, he showed severe liver fibrosis. Baserga et al. (2004) reported that SGA-ELBW infants have a risk factor for TPN (for >7 days)-associated cholestasis. Some of these infants showed portal/sinusoidal fibrosis; however, compared with our patient, they used higher doses of amino acids, glucose and intravenous lipids.

Moreover, infection, metabolic disease, and heart failure contribute to liver failure (cholestasis in neonates and infants) (Alonso et al. 2007); however, we excluded con-

Fig. 4. Immunohistochemical staining of the liver. Immunohistochemical staining was performed using frozen sections. Antibodies against α -smooth muscle actin (a) (Boehringer Mannheim, Mannheim, Germany, dilution: 1:10) and TGF- β 1 (b) (Santa Cruz Biotechnology, Inc., CA, USA, dilution: 1:200) were obtained commercially. (a) Immunoreactivity of spindle-like cells (arrows) was found along sinusoids. (b) Immunoreactivity was detected in round cells (arrows) in sinusoidal areas. (c) Negative control section for (a). (d) Negative control section for (b). Bar = 50 μ m

genital infection, metabolic syndrome, and neonatal intrahepatic cholestasis.

Our patient showed hypertyrosinemia, but we excluded tyrosinemia type I because of no accumulation of upstream metabolites and succinylacetone. SGA infants are more sensitive to metabolic imbalances than AGA infants (Baserga et al. 2004). Also, Robles et al. (1984) reported hypertyrosinemia in small-for-date (SFD) infants. Hypertyrosinemia results from immature function of the enzyme 4-hydroxyphenylpyruvate dioxygenase, most frequently in premature infants (Russo et al. 2001).

A cause of IUGR is uteroplacental insufficiency secondary to maternal morbidities such as hypertension (Baserga et al. 2004). IUGR fetuses demonstrate progressive hemodynamic changes, and develop earlier and more pronounced right than left ventricular function deterioration (Bahtiyar and Copel 2008). Soon after birth, this patient showed congestive heart failure, which is associated with the development of liver fibrosis (Naschitz et al. 2000). An animal-based study of right ventricular pressure overload showed liver fibrosis and α -smooth muscle actin expression (Gieling et al. 2004). Although it could not be excluded that liver failure had been caused by another etiology, we suggested that intrauterine cardiac failure contributed to liver fibrosis.

An immunohistochemical study showed many α -smooth muscle actin-positive cells (myofibroblasts) and a few TGF- β 1-positive cells in the perisinusoidal fibrotic area. Liver fibrosis contributes to the differentiation of HSCs into myofibroblasts (Hinz et al. 2007). TGF- β 1 is the

most potent profibrogenic cytokine that activates HSC, but the present patient showed only a few TGF- β 1-positive cells, despite that fibrotic changes developed in the liver at 3 months of age.

Early detection of liver dysfunction (including the examination of markers of liver fibrosis) soon after birth is a key to predict the prognosis of severe IUGR in preterm infants.

References

- Alonso, E.A., Squires, R.H. & Whittington, P.F. (2007) Acute liver failure in children. In *Liver disease in children*, edited by F.J. Suchy. Cambridge University Press, NY, pp. 71-96.
- Bahtiyar, M.O. & Copel, J.A. (2008) Cardiac changes in the intrauterine growth-restricted fetus. *Semin. Perinatol.*, **32**, 190-193.
- Baserga, M.C. & Sola, A. (2004) Intrauterine growth restriction impacts tolerance to total parenteral nutrition in extremely low birth weight infants. *J. Perinatol.*, **24**, 476-481.
- Boehm, G., Müller, D.M., Teichmann, B. & Krumbiegel, P. (1990) Influence of intrauterine growth retardation on parameters of liver function in low birth weight infants. *Eur. J. Pediatr.*, **149**, 396-398.
- Gieling, R.G., Ruijter, J.M., Maas, A.A., Van Den Bergh Weerman, M.A., Dingemans, K.P., ten Kate, F.J., Lekanne dit Deprez, R.H., Moorman, A.F. & Lamers, W.H. (2004) Hepatic response to right ventricular pressure overload. *Gastroenterology*, **127**, 1210-1221.
- Hinz, B., Phan, S.H., Thannickal, V.J., Galli, A., Bochaton-Piallat, M.L. & Gabbiani, G. (2007) The myofibroblast: one function, multiple origins. *Am. J. Pathol.*, **170**, 1807-1816.
- Murase, M., Ishida, A. & Momota, T. (2002) Serial pulsed Doppler assessment of early left ventricular output in critically ill very low-birth-weight infants. *Pediatr. Cardiol.*, **23**, 442-448.

- Naschitz, J.E., Slobodin, G., Lewis, R.J., Zuckerman, E. & Yeshurun, D. (2000) Heart diseases affecting the liver and liver diseases affecting the heart. *Am. Heart J.*, **140**, 111-120.
- Robles, R., Gil, A., Faus, M.J., Periago, J.L., Sánchez-Pozo, A., Pita, M.L. & Sánchez-Medina, F. (1984) Serum and urine amino acid patterns during the first month of life in small-for-date infants. *Biol. Neonate*, **45**, 209-217.
- Rosenberg, A. (2008) The IUGR newborn. *Semin. Perinatol.*, **32**, 219-224.
- Russo, P.A., Mitchell, G.A. & Tanguay, R.M. (2001) Tyrosinemia: a review. *Pediatr. Dev. Pathol.*, **4**, 212-221.
-