ORIGINAL ARTICLE

Significant correlations between the flow volume of patent ductus venosus and early neonatal liver function: possible involvement of patent ductus venosus in postnatal liver function

K Murayama, H Nagasaka, K Tate, Y Ohsone, M Kanazawa, K Kobayashi, Y Kohno, M Takayanagi

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See end of article for authors' affiliations

Correspondence to: Dr Murayama, Division of Metabolism, Chiba Children's Hospital, 579-1, Heta-cho, Midori-ku, Chiba, 266-0007 Japan; kmuraya@mri.biglobe.ne.jp

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Background: The biochemical features of portosystemic venous shunt with high flow volume are hypergalactosaemia, hyperammonaemia, prolonged blood coagulation time, and raised serum bile acid concentration. The ductus venosus remains open with shunt flow in most neonates for a certain period after birth. However, the effects of blood flow through the ductus venosus on neonatal liver function remain unclear.

Objective: To elucidate the effect of patency of the ductus venosus on liver function in early neonates.

Methods: Subjects were divided into three groups by gestational age (group I, 29–32 weeks; group II, 33–36 weeks; group III, 37–41 weeks). The shunt flow volume through the ductus venosus was examined serially using ultrasonography, and correlations between flow volume and liver function in the respective groups were calculated during the first week after birth.

Results: Group I had a higher flow volume and later functional closure than the other two groups. Plasma ammonia and serum total bile acid concentrations correlated with flow volume in groups I and II, and blood galactose and galactose 1-phosphate concentrations correlated significantly with flow volume in group III. Percentage hepaplastin also correlated significantly with flow volume in all groups, but plasma vitamin K concentration did not in any group.

Conclusions: Patent ductus venosus has a considerable effect on crucial liver functions such as ammonia detoxification, blood coagulation, and regulation of serum total bile acid concentration in early neonates.

The clinical manifestations of congenital portosystemic venous shunt (PSVS) with a higher shunt ratio are well documented. They are characterised by hyperammonaemia, hypergalactosaemia, prolonged coagulation time, and raised serum total bile acid (TBA) concentration.[1–7] Furthermore, Uchino and colleagues[1 7] reported that patients with an extremely high shunt ratio develop severe hepatic steatosis, which eventually progresses to hepatic failure. On the other hand, the effect of congenital PSVS with a low shunt ratio on liver function has not been well studied.

The ductus venosus is a bypass between the umbilical vein and the inferior vena cava in the fetus.[8] The blood flow through this bypass decreases immediately after birth. Functional closure, which is followed by anatomic closure, is virtually complete within a few weeks of birth, whereas most of the other forms of congenital PSVS are permanent.[8–11] According to earlier reports, [1 3 7 12–14] patent ductus venosus (PDV) with extremely high blood flow accounts for some of the manifestations of congenital PSVS.

There are only a few available reports describing changes in blood flow through the ductus venosus before functional closure in early life.[10] Further, under the assumption that most cases of PDV in neonates have little pathophysiological significance, its effect on neonatal liver function has not been extensively studied. There is only one study in the literature, by Fugelseth and colleagues,[15] who reported that PDV was not related to alimentary galactosaemia in preterm neonates.

In this study, we investigated changes in PDV flow in the early neonatal period and associations between PDV flow and liver function, using analysis of blood ammonia, TBA, galactose and galactose 1-phosphate concentrations and the hepaplastin test (HPT). The possible effects of PDV on crucial liver functions of neonates are discussed.

MATERIALS AND METHODS

Study design and subjects

A total of 167 neonates born at the perinatal centre of Kimitsu Chuo Hospital between December 2000 and February 2002, with birth weight appropriate for date and gestational ages ranging from 29 to 41 weeks, were enrolled in the study. To eliminate potential confounding variables, neonates with congenital heart disease, vascular anomalies, bowel disease, severe respiratory distress, or who had been given corticosteroids (which have been shown to promote the closure of PDV) were excluded.[9] Subjects were divided into three groups by gestational age: group I, 29–32 weeks (n = 36); group II, 33–36 weeks (n = 77); group III, 37–41 weeks (n = 54). All were fed with breast milk eight times a day, and each feed was completed within 15 minutes. The milk intake volume was recorded for each subject, and the mean (SE) value (ml/kg/day) for each group was calculated at 3 and 7 days of age. Some neonates in groups I and II were given glucose intravenously to avoid hypoglycaemia, and the blood sugar levels of all of the subjects were kept in an appropriate range (43–109 mg/dl). No other parenteral nutrition or chemical agents were administered during the study. Table 1 gives basic information on the three groups of subjects.

Abbreviations: PSVS, portosystemic venous shunt; PDV, patent ductus venosus; TBA, total bile acids; HPT, hepaplastin test

Table 1 Background information on subjects in each group

	Group I	Group II	Group III
Number (male/female)	36 (20/16)	77 (37/40)	54 (32/22)
Gestational age range (weeks)	29–32	33–36	37–41
Birth weight (g)*	1583 (36)	2133 (35)	3056 (66)
Birth weight range (g)	1322–2318	1426–2992	2300–4400
Milk intake	Breast milk	Breast milk	Breast milk
Day 3 (ml/kg/day)*	48.5 (5.2)	65.5 (8.1)	81.5 (7.7)
Day 7 (ml/kg/day)*	91.0 (5.1)	112.2 (8.8)	137.4 (11.1)

*Values are mean (SE).

The flow volume through the ductus venosus was determined serially in each subject until functional closure (defined as the disappearance of measurable blood flow on a colour Doppler image) was confirmed. The same observer performed all ultrasound examinations throughout the study. From the beginning of feeding, the flow volume at about 30 minutes after a feed was determined. Concurrently, a blood sample was collected, and concentrations of ammonia and serum TBA were determined. Galactose and galactose 1-phosphate concentrations in a dried blood spot were determined. Blood sugar concentration was also determined and compared with that before a feed to confirm adequate absorption of nutrients in milk from the intestine. Further, to evaluate blood coagulating ability, percentage hepaplastin (% HPT) and concurrent plasma vitamin K concentration were determined. For each group, the correlations between flow volume and these concentrations were determined to evaluate the effect of PDV on liver function.

This study was approved by an institutional review board, and parents of all neonates provided written informed consent before the start.

Determination of the flow volume of the ductus venosus

The patency of the ductus venosus is shown by downward blood flow from the portal sinus to the inferior vena cava. In this section, the flow volume in the ductus venosus was measured by pulsed Doppler sonography (Sonos 4500; Hewlett-Packard Company, Tokyo, Japan) with a 12 MHz transducer, as described previously.[5 9]

Determination of plasma ammonia and serum TBA concentrations

Plasma ammonia and serum TBA concentrations were determined at 3 and 7 days of age by the respective enzymatic method.[16–19]

HPT and determination of plasma vitamin K concentration

To prevent vitamin K deficiency, neonates in groups I and II were given 1 mg vitamin K intravenously within 24 hours of birth, whereas those in group III were given 2 mg vitamin K. To evaluate the ability of the blood to coagulate, the HPT was performed at 3 and 7 days of age using a commercial kit (HP Tests; Sankyo Co Ltd, Tokyo, Japan). Also at 3 days of age, plasma vitamin K concentration was determined fluorimetrically as described by Haroon and coworkers.[20]

Determination of galactose and galactose 1-phosphate in dried blood spots

Galactose and galactose 1-phosphate concentrations in dried blood spots were determined using a fluorimetric microplate reader as described by Yamaguchi and colleagues.[21 22]

Statistical analysis

Flow volumes in infants at 1, 3, and 7 days of age and biochemical data at 3 and 7 days of age were compared between groups using the Kruskal-Wallis test. Differences between the groups in age at closure of the PDV were also evaluated using the Kruskal-Wallis test. Relations between flow volume and biochemical data were evaluated by Spearman's correlation test. $p<0.05$ was considered significant.

RESULTS

The flow volume of the ductus venosus differed by group and age (table 2). The age at functional closure was also significantly different among the three groups. At birth, the flow volume was similar in the three groups, but thereafter the flow volume in group I was significantly higher than in groups II and III. The flow volume in group I reached a maximum at 3 days of age (mean (SE) 29.1 (4.8) ml/min/kg). At 7 days of age, a considerable flow volume (11.0 (3.1) ml/min/kg) was sustained. On the other hand, the flow volumes of groups II and III were highest at birth (12.2 (1.1) ml/min/kg for group II, 7.3 (1.3) ml/min/kg for group III). At 3 days of age, the flow volume of group II decreased to half of that at birth, whereas the flow volume of group III remained the same as at birth. At 7 days of age, both groups II and III exhibited very low flow volumes of 2.5 (0.6) and 0.5 (0.3) ml/min/kg respectively. Functional closure of the ductus venosus occurred latest in group I and earliest in group III (mean (SE) 10.2 (1.2) days of age for group I, 7.1 (0.8) days for group II, and 4.6 (0.4) days for group III).

Table 2 Changes in flow volume in the ductus venosus after birth and age at functional closure

	Group I (n=36)	Group II (n=77)	Group III (n=54)	p Value			
				I *v* II *v* III	I *v* II	II *v* III	I *v* III
Day 0	17.8 (3.3)	12.2 (1.1)	7.3 (1.3)	0.0860	0.1645	0.1052	0.0665
Day 1	15.5 (1.7)	9.0 (1.2)	6.7 (1.3)	<0.001***	0.0039**	0.3110	<0.001***
Day 3	29.1 (4.8)	5.3 (0.8)	6.6 (3.0)	<0.001***	<0.001***	0.5159	<0.001***
Day 7	11.0 (3.1)	2.5 (0.6)	0.5 (0.3)	<0.001***	<0.001***	0.0049**	<0.001***
Age (days)	10.2 (1.2)	7.1 (0.8)	4.6 (0.4)	<0.001***	0.0146*	0.0071**	<0.001***
Range	5–17	1–16	1–16				

Data for flow volume (ml/kg/min) and age at functional closure are mean (SE).
Differences between groups were evaluated with the Kruskal-Wallis test: *p<0.05, **p<0.01, ***p<0.001.

Table 3 Changes in biochemical data after birth

				p Value			
	Group I	Group II	Group III	I *v* II *v* III	I *v* II	II *v* III	GI *v* GIII
Ammonia (μg/dl)							
Day 3	97.0 (4.1) (n=29)	73.5 (3.1) (n=71)	63.3 (4.5) (n=30)	<0.001***	<0.001***	<0.001***	<0.001***
Day 7	85.3 (3.5) (n=29)	68.2 (3.1) (n=71)	56.8 (2.6) (n=40)	<0.001***	<0.001***	<0.001***	<0.001***
Total bile acid (μmol/l)							
Day 3	28.7 (2.9) (n=29)	18.8 (1.1) (n=71)	21.9 (2.5) (n=28)	0.0030**	0.001**	0.5291	0.0117*
Day 7	23.5 (2.0) (n=29)	19.3 (1.0) (n=71)	21.4 (1.4) (n=38)	0.2057	0.0842	0.5499	0.2262
Hepaplastin test (%)							
Day 3	57.4 (2.9) (n=29)	66.9 (1.7) (n=71)	69.3 (3.7) (n=34)	<0.001***	<0.001***	0.0182*	<0.001***
Day 7	59.9 (4.7) (n=29)	68.1 (5.6) (n=71)	71.2 (6.9) (n=40)	<0.001***	<0.001***	0.0144*	<0.001***
Plasma vitamin K (ng/ml)							
Day 3	69 (6) (n=29)	71 (4) (n=71)	67 (6) (n=34)	0.3900	0.3933	0.2139	0.5859
Galactose (mg/dl)							
Day 3	0.32 (0.02) (n=26)	0.43 (0.08) (n=45)	0.33 (0.04) (n=32)	0.7856	0.8493	0.6317	0.4758
Day 7	0.55 (0.06) (n=29)	0.52 (0.16) (n=47)	0.47 (0.06) (n=32)	0.4999	0.8045	0.2542	0.4038
Galactose 1-phosphate (mg/dl)							
Day 3	0.71 (0.11) (n=26)	1.11 (0.18) (n=44)	1.11 (0.22) (n=32)	0.0185*	0.1139	0.1265	0.005**
Day 7	1.20 (0.22) (n=29)	1.35 (0.22) (n=47)	1.82 (0.23) (n=32)	0.0017**	0.5964	0.0013**	0.0013**

Data are mean (SE). Differences between group were evaluated by the Kruskal-Wallis test: *p<0.05, **p<0.01, ***p<0.001.

For all groups, there was a significant (p<0.0001) increase in blood sugar after a feed (3 days of age: group I, 53 (5) mg/dl before milk, 68 (7) mg/dl 30 minutes after; group II, 55 (6) mg/dl, 73 (8) mg/dl; group III, 59 (6) mg/dl, 86 (8) mg/dl; 7 days of age: group I, 58 (7) mg/dl, 79 (6) mg/dl; group II, 58 (6) mg/dl, 82 (7) mg/dl; group III, 61 (5) mg/dl, 86 (7) mg/dl), confirming that nutrients in milk were adequately absorbed from the intestine.

Table 3 shows the biochemical data for all groups. At both 3 and 7 days of age, plasma ammonia concentration was significantly (p<0.001) higher in group I than in groups II and III, and significantly (p<0.001) higher in group II than in group III. Serum TBA concentration was significantly higher in group I than in groups II and III at 3 days of age (28.7 (2.9), 18.8 (1.1), and 21.9 (2.5) μmol/l respectively). However, at 7 days of age, no significant difference in TBA concentration was observed among the three groups. The galactose concentration in the dried blood spot was no different among the three groups on either day, whereas galactose 1-phosphate was significantly higher in group III than in group I at both ages and higher than in group II at 7 days of age. For the HPT, the percentage value was significantly (p<0.001) lower for group I than for groups II and III at both 3 and 7 days of age. However, no significant difference in plasma vitamin K concentration was observed among the three groups.

Table 4 summarises the correlation coefficients between flow volume and biochemical data. In group I, a strong positive correlation of the plasma ammonia concentration and a strong inverse correlation of % HPT with the concurrent flow volume were observed at 3 days of age (p<0.001). At 7 days of age, plasma ammonia concentration still correlated with flow volume but % HPT no longer correlated. In addition, a significant positive correlation of serum TBA concentration with flow volume was observed (p = 0.0307). In group II, ammonia and TBA concentrations and % HPT strongly correlated with flow volume at 3 days of age (ammonia, p<0.001; TBA, p = 0.0039; % HPT p<0.001) but not at 7 days of age. In group III, % HPT correlated with flow volume at 3 days of age (p = 0.0057). Furthermore,

Table 4 Correlation coefficients between biochemical data and flow volume

	Group I	Group II	Group III
3 days of age			
Ammonia	*r*=0.7014; **p<0.001**; n=29	*r*=0.4653; **p<0.001**; n=71	*r*=0.3346; p=0.0816; n=29
Total bile acid	*r*=0.3430; p=0.0727; n=29	*r*=0.3819; **p=0.0039**; n=71	*r*=0.2480; p=0.2174; n=29
Hepaplastin test	*r*=−0.6292; **p<0.001**; n=29	*r*=−0.5797; **p<0.001**; n=71	*r*=−0.4675; **p=0.0057**; n=32
Vitamin K	*r*=−0.0973; p=0.5838; n=29	*r*=0.1311; p=0.3152; n=71	*r*=0.2100; p=0.2763; n=32
Galactose	*r*=0.0759; p=0.8449; n=26	*r*=−0.2365; p=0.1758; n=44	*r*=06556; **p=0.0094**; n=18
Galalactose 1-phosphate	*r*=0.2089; p=0.2969; n=26	*r*=−0.0331; p=0.7374; n=44	*r*=0.5460; **p=0.0304**; n=18
7 days of age			
Ammonia	*r*=0.5337; **p=0.0052**; n=29	*r*=−0.0362; p=0.1093; n=71	*r*=0.4777; p=0.2735; n=38
Total bile acid	*r*=0.4172; **p=0.0307**; n=29	*r*=0.1980; p=0.5161; n=71	*r*=0.2794; p=01087; n=38
Hepaplastin test	*r*=−0.1167; p=0.4790; n=29	*r*=−0.0149; p=0.1560; n=71	*r*=0.4438; p=0.5176; n=38
Galactose	*r*=0.2674; p=0.1860; n=29	*r*=0.0835; p=0.6362; n=47	*r*=0.4314; p=0.3783; n=23
Galactose 1-phosphate	*r*=0.2727; p=0.1609; n=29	*r*=0.1938; p=0.5842; n=47	*r*=0.2282; p=0.5681; n=23

Data are p values for Spearman's correlation coefficient by rank test. p Values in bold are significant.

only in this group did galactose and galactose 1-phosphate concentrations significantly correlate with flow volume (galactose, p = 0094; galactose 1-phosphate, p = 0.0304). However, at 7 days of age, no significant correlations between biochemical data and flow volume were observed in this group.

In contrast with the other biochemical data, plasma vitamin K concentration did not correlate with flow volume at any stage in any group.

DISCUSSION

Recently, case reports of congenital PSVS, including PDV, have been increasing in accordance with advances in imaging techniques, and, in turn, the clinical manifestations and the long term prognosis have been clarified.[1–7] However, the effects of PDV with varying shunt flows on neonatal liver function remain unclear, although patients with hypergalactosaemia due to PDV accompanied by high shunt flow have been found in neonatal mass screening.[6]

The ductus venosus of almost all neonates remains open with blood flow, the volume of which differs in each individual, for a certain period after birth.[8–11] However, changes in blood flow through the ductus venosus during the neonatal period has never been studied in detail, although there are several reports of the age at functional closure.[8–11]

This study reveals that the volume of blood flowing through the ductus venosus was higher in group I than in groups II and III, and that functional closure was more delayed in neonates born at earlier gestational ages, showing the difference in the nature of PDV according to the gestational age of the neonate. However, we could not determine the corrective shunt ratio because determination of blood flow volume through the truncus of the portal vein was often disrupted by gas in the gut and a mixture of signals derived from blood flow through the hepatic artery.[8]

PDV in the neonatal period has been considered to be of little pathophysiological significance until now, and therefore the effects of PDV on important liver functions of neonates involving ammonia detoxification and coagulation have never been investigated. It is well known that the neonatal liver is cholestatic and metabolically immature, and that liver function in early life is readily affected by a number of factors such as infection and administration of chemical agents.[23–28] Therefore it is reasonable to assume that PDV also influences neonatal liver function.

This study also shows that urea cycle function and metabolism of galactose and bile acid during the first seven postnatal days differs according to gestational and postnatal age. During this period, plasma ammonia concentration was significantly higher in group I than in groups II and III. Further, serum TBA concentration was also significantly higher in this group than in the other two groups at 3 days of age. In contrast, blood galactose 1-phosphate concentrations in group III at 3 and 7 days of age were high compared with the other two groups, although blood galactose concentration did not differ among the three groups.

According to previous reports, the maturity levels of both the urea cycle and bile acid metabolism in the fetus, including key enzyme activities and protein concentrations, are strongly dependent on gestational age, and concentrations at birth are still considerably lower than adult concentrations.[23–25] Accordingly, the differences in plasma ammonia and serum TBA concentrations by gestational age are possibly explained by differences in maturation of these metabolic pathways. However, the distinct difference in PDV flow volume between group I and the other two groups suggests that the differences in ammonia and TBA concentrations are, at least in part, attributable to the differences in PDV flow.

On the other hand, for galactose metabolism, it has been shown that the maturation level in preterm neonates is similar to that in term neonates.[15 26] As shown in table 1, milk intake in group III was significantly higher than in the other two groups. Therefore we speculate that the high galactose 1-phosphate concentration in group III is, at least in part, attributable to high milk intake, as a major component of milk is lactose which comprises galactose and glucose.

Significant correlations between liver metabolic function and PDV flow volume in each group were found in this study. In group I, a strong correlation of plasma ammonia concentration with flow volume was observed at 3 and 7 days of age. In group II, plasma ammonia and serum TBA concentrations correlated strongly with flow volume at 3 days of age. In group III, blood galactose concentration correlated strongly with flow volume at 3 days of age. These results strongly suggest that PDV influences neonatal liver functions involving ammonia detoxification, bile acid regulation, and galactose metabolism, and that the magnitude of the effect differs with gestational and postnatal age.

In older patients with high PSVS shunt flow, blood coagulation time is often delayed, with concomitant increases in ammonia and TBA concentrations.[1–7 12–14] Intracranial haemorrhage and intestinal haemorrhage are often life threatening problems in neonates.[29 30] Our study shows that there is a significant inverse correlation between flow volume and % HPT, suggesting high susceptibility of neonates with high PDV flow to the development of life threatening haemorrhages. HPT is a representative examination to evaluate the ability of the blood to coagulate, reflecting vitamin K and production of the vitamin K dependent coagulation factors II, VII, IX, and X by liver cells.[31 32] As PDV flow did not have a significant effect on plasma vitamin K concentration, a decrease in % HPT in accordance with an increase in PDV flow must be due to decreased production of coagulation factors. These results suggest that, besides the direct effects of the shunt itself as a PSVS on ammonia and TBA concentrations, PDV in neonates has an effect on protein synthesis in the neonatal liver.

In this experiment, we were unable to investigate whether PDV is relevant to the severity of neonatal hyperbilirubinaemia, because many of the neonates, especially in group I, received phototherapy to avoid hyperbilirubinaemia. However, considering that a significant positive correlation existed between PDV flow and TBA concentration (a marker of cholestasis), it is possible that a high PDV flow may also be associated with neonatal hyperbilirubinaemia.

This study shows that PDV is closely related to crucial liver functions, especially ammonia detoxification and blood

What is already known on this topic

- The ductus venosus remains open with shunt flow in most neonates for a certain period after birth
- The association between patency of ductus venosus and neonatal liver function remains unclear

What this study adds

- Patent ductus venosus is closely related to crucial liver functions such as ammonia detoxification, blood coagulation, and regulation of serum total bile acid concentration in early neonates

coagulation, in the early neonatal period. The pathophysiological significance of PDV should be highlighted in clinical practices dealing with neonates.

Authors' affiliations

K Murayama, M Takayanagi, Division of Metabolism, Chiba Children's Hospital, Chiba, Japan
H Nagasaka, K Kobayashi, Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan
K Tate, Y Ohsone, Department of Neonatology, Kimitsu Chuo Hospital, Kisarazu, Japan
K Murayama, M Kanazawa, Y Kohno, Department of Pediatrics, Chiba University Graduate School of Medicine, Chiba

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