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Abstract

Concentrations of tryptophan (free and protein bound) and its metabolites in plasma of maternal vein at delivery, umbilical vein, umbilical artery, neonatal vein and breast milk were determined by high performance liquid chromatography. The plasma levels of tryptophan and most of its metabolites in umbilical vein and artery were significantly higher than those in maternal vein. The concentration of total tryptophan in plasma of neonatal vein showed marked decrease at 24 h after birth in comparison with that at birth, but the total kynurenine concentration was not decreased in plasma of neonatal vein. The colostrum contained a high level of total tryptophan. There were high ratios of free to total tryptophan in colostrum, transitional and mature milk. In the blood, ratios of free to total of tryptophan and kynurenine were kept at constant level throughout the perinatal period.

KEYWORDS: tryptophan, kynurenine, perinatal period, human breast milk

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Tryptophan and its Metabolite Concentrations in Human Plasma and Breast Milk During the Perinatal Period

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Concentrations of tryptophan (free and protein bound) and its metabolites in plasma of maternal vein at delivery, umbilical vein, umbilical artery, neonatal vein and breast milk were determined by high performance liquid chromatography. The plasma levels of tryptophan and most of its metabolites in umbilical vein and artery were significantly higher than those in maternal vein. The concentration of total tryptophan in plasma of neonatal vein showed marked decrease at 24 h after birth in comparison with that at birth, but the total kynurenine concentration was not decreased in plasma of neonatal vein. The colostrum contained a high level of total tryptophan. There were high ratios of free to total tryptophan in colostrum, transitional and mature milk. In the blood, ratios of free to total of tryptophan and kynurenine were kept at constant level throughout the perinatal period.

Key words : **tryptophan, kynurenine, perinatal period, human breast milk**

Tryptophan is the only essential amino acids, which is mostly (80–90 %) bound to the circulating serum albumin in human blood (1, 4). However, it is free tryptophan that works functionally in the living body since, for example, only free form is able to enter the brain to control cerebral serotonin synthesis (1, 4, 5, 8).

Tryptophan metabolites in the human blood have not been studied extensively because of their low levels. Therefore, physiological roles of tryptophan in the perinatal period has not well been investigated.

In the present study, we determined concentrations of total and free tryptophan and its metabolites by high performance liquid chromatography (HPLC) (14, 15) in maternal, umbilical and neonatal blood at delivery and of breast milk, and investigated tryptophan metabolism in fetuses and neonates.

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Materials and Methods

Materials. Forty-eight women with normal delivery at from 37 to 41 weeks of gestation were studied. The maternal blood samples were withdrawn by antecubital venipuncture with a heparinized syringe immediately before delivery. The umbilical blood samples were taken directly from the umbilical vein and artery at birth, separately. The blood samples of 20 full-term healthy neonates were obtained by heel puncture.

Breast milk samples were obtained from 10 healthy mothers of full-term neonates on the 3rd, 5th, and 7th

day postpartum. All the mothers breast-fed their babies.

HPLC system. Tryptophan and its metabolites were analyzed by direct-injection HPLC system with the column switching method, using two columns. The first was a short precolumn of two-layer gel ODS for deproteinization, and also for trapping tryptophan and its metabolites. The second was an analytical ODS column (16, 17). A 200-μl volume of plasma sample was injected onto the precolumn, which was equilibrated with the purge solvent. After washing for 6 min with the purge solvent, the precolumn was connected with an analytical ODS column in the flow-through mode. The metabolites were separated on the analytical column by stepwise elution with 0.1 M phosphate solution with increasing acetonitrile concentration. The analytical column was monitored with two detectors : fluorescence (UV-8 ; Tosoh, Tokyo, Japan), and spectrophotomeric (RF535 ; Shimazu, Tokyo Japan) detectors (19).

Preparation of samples. The blood samples were mixed with heparine (approximately 0.3 mg/ml blood) and stored at 4°C in sealed containers for less than 24 h before centrifugation at 3000 × g for 10 min at room temperature. Tryptophan (total and free) and Kynurenine (total and free) in plasma were separated by ultrafiltration (5).

Tryptophan (total), kynurenine (total) and other metabolites in plasma and in the plasma ultrafiltrate (free tryptophan and kynurenine) were analyzed by HPLC system described above. This system showed the reproducibility with C. V. (%) of less than 3% and approximately 90% of recovery rate. A typical authentic chromatogram of tryptophan and its metabolites is shown in Fig 1.

The breast milk samples were analyzed with the same technique.

The statistical analysis was performed using the Student's t test.

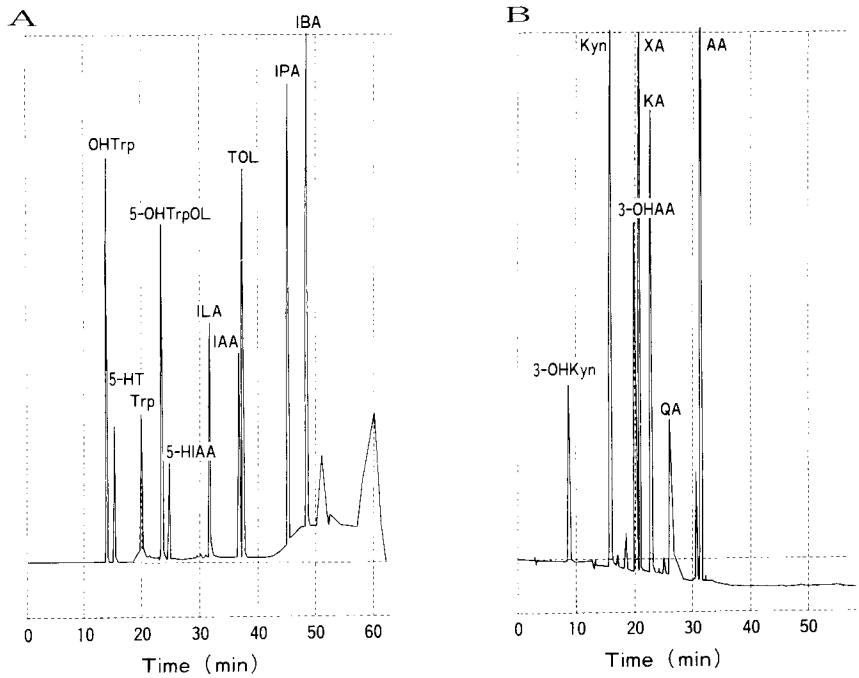

Fig. 1 Chromatogram of tryptophan and its metabolites using column switching method (Pure standard): (A) Tryptophan metabolites in series of indole were determined by spectrophotmeric detector (EX 287, EM 340) ; (B) Metabolites through kynurenine were determined by ultra violet spectrophotometry (350nm) (Trp, 1nmol ; other indole metabolites, 100pmol ; 3-OHKyn, Kyn, XA and AA, 500pmol ; 3-OHAA, QA and AA, 2nmol). Peaks: Trp, tryptophan ; OHTrp, 5hydroxytryptophan ; 5HT, serotonin ; 5-OHTrpOL, 5hydroxytryptophol ; 5-HIAA, 5-hydroxy indoleacetic acid ; ILA, indolelactic acid ; IAA, indoleacetic acid ; TOL, tryptophol ; IPA, indolepropionic acid ; IBA, indolebutyric acid ; 3-OHKyn, 3-hydroxykynurenine ; Kyn, kynurenine ; 3-OHAA, 3-hydroxyanthranilic acid ; XA, xanthurenic acid ; KA, kynurenic acid ; QA, quinaldic acid ; AA, anthranilic acid.

Results

Concentrations of tryptophan and its metabolites in blood plasma of maternal vein and umbilical vein and artery. Table 1 presents concentrations of tryptophan and its metabolites in the plasma of maternal vein and umbilical blood of 48 women with normal pregnancy. Concentrations of tryptophan, indolelactic acid (ILA) and indoleacetic acid (IAA) were significantly higher in the plasma of umbilical vein and artery blood than those in maternal vein plasme ($p < 0.01$).

Concentrations of kynurenine and its metabolites in the plasma of umbilical vein and umbilical artery were higher than those in the plasma of maternal vein, except for anthranilic acid (AA). Plasma levels of kynurenine in the umbilical vein and umbilical artery were about 3 times higher than that in the maternal vein, and plasma levels of 3 OH-anthranilic acid (3 OHAA) in umbilical vein and umbilical artery were about 40 times higher than that in the maternal vein.

Plasma levels of tryptophan and its metabolites showed no significant difference between the umbilical vein and umbilical artery, as shown in Table 1.

The ratio of tryptophan and kynurenine in the plasma of maternal vein was different from that in umbilical vein.

Free tryptophan and kynurenine concentrations in plasma of maternal vein, umbilical vein and umbilical artery and neonatal vein. Table 2 shows the plasma levels of total and free tryptophan in maternal vein, umbilical vein and umbilical artery. Both of plasma levels of free tryptophan in plasma of umbilical vein and artery were higher than those in maternal vein, however there is no difference between ratios of free to total of tryptophan in the plasma of umbilical vein and artery. Ratios of free to total of tryptophan were about the same in maternal vein. umbilical vein, and umbilical artery.

Plasma levels of free kynurenine in umbilical vein and artery were higher than those in maternal vein. In ratios of free to total kynurenine, free kynurenine was around 5 percent of the total in the plasma of maternal vein, umbilical vein and umbilical artery, showing no difference among them.

Plasma levels of free tryptophan and kynur-

Table 1 Concentration of tryptophan and its metabolites in the blood plasma of maternal vein, umbilical vein and umbilical artery

	Plasma of maternal vein (pmol/ml)	Plasma of umbilical vein (pmol/ml)	Plasma of umbilical artery (pmol/ml)
Trp	39700±19500	83500±24500**	83200±12100**
OHTrp	41.9±14.9	10.3±1.9**	11.2±1.9**
ILA	352±195	831±379**	902±405**
IAA	592±324	1856±322**	1752±268**
IPA	322±132	570±185*	602±112*
Kyn	1700±800	4200±1800**	4300±1500**
3OHAA	12.0±23.0	527.0±233.0***	702.0±312.0***
XA	188±84	295±28**	258±27**
KA	20.5±11.3	38.1±7.7*	39.8±9.3*
AA	413±15	387±15	432±24

Values are expressed as mean ± SD (n = 48)
Abbreviations: Trp, tryptophan ; OHTrp, hydroxy tryptophan ; ILA, indolelactic acid ;
IAA, indoleacetic acid ; IPA, indolepropionic acid ; Kyn, kynurenine ;
3OHAA, 3hydroxy anthranilic acid ; XA, xanturenic acid ; KA, kynurenic acid ; AA, anthranilic acid.
Significantly different from maternal vein blood: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 2 Total and free tryptophan and kynurenine concentrations in maternal vein, umbilical vein, umbilical artery and neonatal vein

	Tryptophan			Kynurenine		
	Total (nmol/ml)	Free (nmol/ml)	Free (%)	Total (nmol/ml)	Free (nmol/ml)	Free (%)
Maternal vein	39.6±21.4	7.5±0.7	19.0±2.0	1.17±1.91	0.09±0.02	5.33±0.94
Umbilical vein	82.6±37.3*	16.4±2.3*	19.9±2.5	4.12±1.64*	0.23±0.06*	5.63±1.32
Umbilical artery	83.4±14.2*	17.5±3.5*	21.0±2.7	4.25±1.62*	0.22±0.14*	5.18±1.67
Neonatal vein Days after birth						
1	53.5±18.2	9.4±3.5	17.5±0.25	4.47±4.10	0.21±0.05	3.85±0.30
5	69.5±3.2	12.7±1.8	18.3±0.42	4.90±2.90	0.29±0.09	4.12±0.18

Values are expressed as mean ± SD (n = 20).
Significantly different from values in maternal vein and neonatal vein on the 1st day after birth: *, $p < 0.01$.

enine also showed no difference between those in maternal vein and umbilical vein.

Concentration of total tryptophan in the plasma of neonatal vein decreased significantly ($p < 0.01$) at 24 h after birth compared with those in the plasma of neonatal vein at delivery and tended to increase slightly on the 5th day. These values, however, were lower than those at delivery.

On the other hand, concentrations of kynurenine in the plasma of neonatal vein did not decrease after delivery. The concentration of free tryptophan in plasma of neonatal vein decreased significantly ($p < 0.01$) at 24 h after birth compared with that at delivery. As to ratios of free to total of tryptophan, there was not a difference between plasma of umbilical vein and neonatal vein. The free kynurenine did not decrease, and ratios of free to total of kynurenine did not show any particularly significant change in the neonatal period either.

Concentrations of total and free tryptophan in human milk. Total and free tryptophan contents in the colostrum, on the 3rd puerperal day, were significantly higher ($p < 0.01$) than those of the 5th-puerperal-day-transitional milk, and of the 30th-day-mature milk. Free tryptophan was about 80% (ratio of free tryptophan to total tryptophan) in colostrum and, about 70% in transitional and mature milk, as shown in Table 3.

Table 3 Concentration of total and free tryptophan in breast milk

Day of lactation	Tryptophan		
	Total (nmol/ml)	Free (nmol/ml)	Free (%)
3	39.4±3.1	31.9±2.9	81.0±2.1
5	6.3±1.1*	5.0±0.7*	81.4±3.5
30	3.5±0.8	2.5±0.3	71.8±8.5

Values are expressed as mean ± SD (n = 20)
Significantly different from values of 3days of lactation: *, $p < 0.01$.

Discussion

Essential amino acids are supplied by active transport through the placenta in the intrauterine life (1, 2). In our present study, the concentration of tryptophan was higher in plasma of umbilical vein than in plasma of maternal vein like other amino acids. Furthermore, the plasma levels of kynurenine and its metabolites except for AA, the plasma levels in umbilical vein and artery were higher than those in maternal vein as well.

The kynurenine metabolism starts after tryptophan is metabolized into kynurenine by the

reactions including tryptophan pyrrolase (E. C. 1. 1. 3. 12) in mammal tissues (7, 17). There is a paper reporting that the activity of this enzyme is very low in the fetal liver and placenta, and, after delivery, the activity increased in neonatal liver (18).

On the other hand, the activity of indoleamine 2, 3-dioxygenase (EC 1. 13. 11. 11) (13, 17) that splits the indole ring of tryptophan is reported to be very high in the placenta (17). The activity is highest in the placenta followed by the lung and small intestine in human adults (17). Therefore, the metabolism of tryptophan to kynurenine may be catalyzed mainly by intraplacental indoleamine 2, 3-dioxygenase during fetal period.

Furthermore, the concentration of tryptophan in the plasma of umbilical vein was about twice the concentration in the plasma of maternal vein, and concentration of kynurenine in the plasma of umbilical vein was approximately 3 times higher than that in plasma of maternal vein. This tendency was more conspicuous of kynurenine than tryptophan. For this reason, it is supposed that tryptophan may be metabolized into kynurenine by indoleamine 2, 3-dioxygenase of the placenta in the fetal life (17). Concentrations of total and free tryptophan and kynurenine in plasma of umbilical vein and in umbilical artery were significantly higher than in maternal vein. In addition, concentrations of both tryptophan and kynurenine in plasma of umbilical vein and umbilical artery of the neonates with intrauterine growth retardation were significantly low compared with those of neonates born appropriate for date (20). Therefore, kynurenine and its metabolites may play an important role during fetal development.

Both of plasma levels of total and free tryptophan in neonatal vein at 24 h after birth were significantly lower than those at birth. These low levels may be due to the removal of the placenta (1). But the plasma levels of total and free kynurenine did not decrease, indicating the importance of kynurenine metabolism even in the neonatal period (1, 5).

Furthermore, since human milk is thought to be the possible supply source of tryptophan for neonates, we determined total and free tryptophan in human milk as well. The total tryptophan content was higher in colostrum than in transitional and mature milk. The ratios of free to total tryptophan of colostrum was also high, about 80 percent. The free tryptophan levels compared with the total were high even in transitional and mature milk than those in human plasma (free form was approximately 20 percent of the total). This high percentage of free tryptophan in human milk (about 80 %) may result from albumin level which is less than about one half of the level in human blood. Kynurenine, however, could not be detected in human milk.

As we mentioned above, tryptophan, which was supplied through the placenta in the fetal life, temporarily decreased at 24 h after birth due to removal of the placenta at birth (9, 18). However, tryptophan was supplied by human milk containing high concentration of tryptophan during neonatal period. On the other hand, kynurenine did not decrease in the neonatal period. Therefore, it may be suggested that tryptophan pyrrolase could be activated in the neonatal liver shortly after delivery.

Generally, the main role of the kynurenine metabolism *in vivo* is said to be nicotinamide adenine dinucleotide (NAD) synthesis via 3 OHAA, and energy production via the TCA cycle (7, 15, 17). And there is an experimental report that free kynurenine controls the uptake of free tryptophan and the synthesis of serotonin in the brain tissue (6, 7).

In this study, it is of great interest that neither ratios of free to total of tryptophan nor kynurenine showed any change in plasma of maternal vein, umbilical vein, umbilical artery and neonatal vein, in spite of the fact that binding of tryptophan to albumin is susceptible to disturbance by many factors (for example ; intake of food, stressful manipulation, administration of certain pharmacological agents) which have been reported (1).

Plasma levels of ILA and IAA of indole metabolites in umbilical vein and artery were

higher than those in maternal vein. The physiological action of indole metabolism among others is obscure at present, but there is an interesting report that the blood levels of these metabolites increase in patients with colon cancer (14). So, these metabolites might be concerned with cell proliferation. The work of indole metabolites during the perinatal period remains to be investigated furthermore.

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