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Short Communication

Difference in the concentration of tryptophan metabolites between maternal and umbilical foetal blood

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ABSTRACT

Maternal and umbilical foetal blood at delivery were analysed for tryptophan metabolites by using fully automated high-performance liquid chromatography. The metabolites detected in 100 μl of maternal plasma were kynurenine, serotonin, 5-hydroxyindoleacetic acid, indolelactic acid, indoleacetic acid and indolepropionic acid. These metabolites were present in various amounts in the protein-bound form. Except for indolepropionic acid, the concentrations of tryptophan metabolites were significantly higher in umbilical foetal plasma than in maternal plasma. In addition, 3-hydroxyanthranilic acid was present in umbilical foetal blood, but not in maternal blood. Furthermore, kynurenic acid was also detected in amniotic fluids.

INTRODUCTION

Tryptophan is essential for optimal growth in infants and for nitrogen equilibrium in human adults. In foetuses, nutrients such as amino acids are supplied from the mother and metabolized under autonomic regulation of foetuses. Foetal blood or amniotic fluids have been used for prenatal diagnosis of hereditary metabolic diseases and monitoring of foetal development [1–3].

Tryptophan is metabolized by two major pathways in humans, either through kynurenine or via a series of indoles (Fig. 1), and some of its metabolites are known to be biologically active. The levels of tryptophan and its metabolites in body fluids have been determined in nutritional or neurochemical investigations, as well as in studies of disease states, by using various analytical methodologies [4–7].

We have developed a new high-performance liquid chromatographic (HPLC) method with an on-line precolumn clean-up system that obviates sample pretreatment such as protein precipitation [8–10]. The fully automated HPLC system

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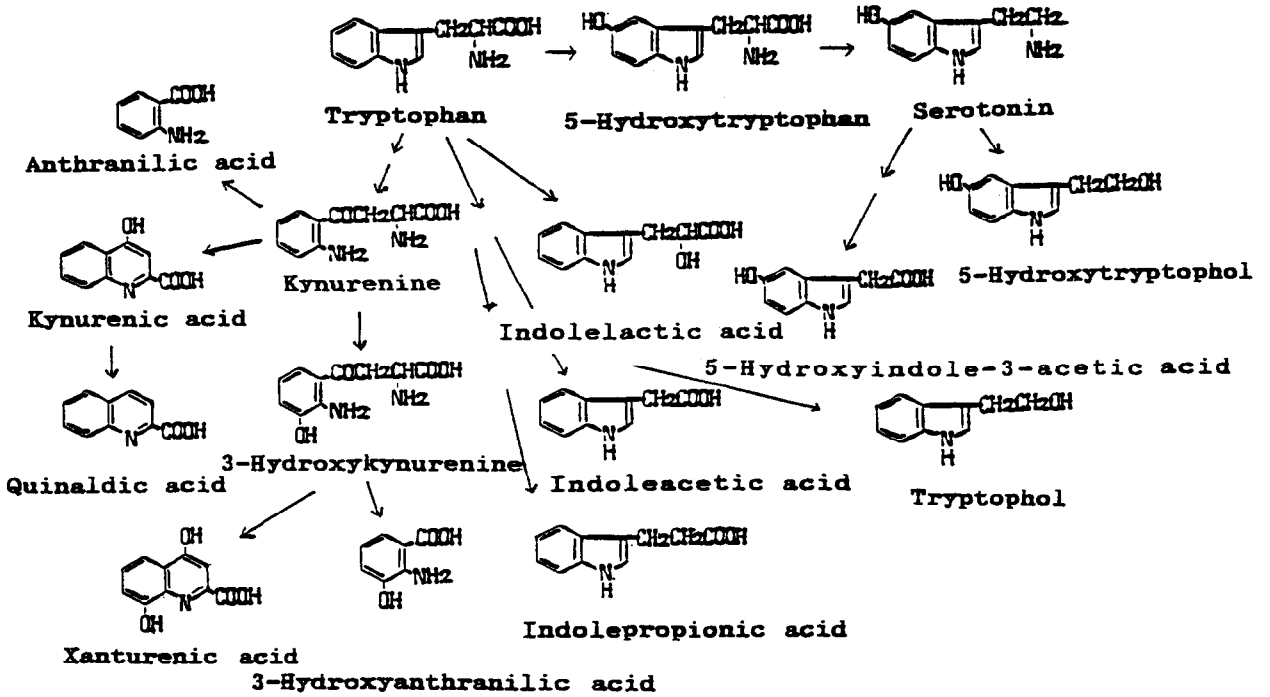

Fig. 1. Metabolism of tryptophan.

was described for the simultaneous determination of tryptophan (Trp) and its metabolites [10]. The HPLC method allowing direct injection of blood fluids into a column was preferable for routine use in a clinical laboratory to avoid time-consuming, less reproducible and laborious pretreatment steps. We applied this method to determine the concentrations of Trp metabolites in maternal and umbilical foetal blood at delivery, as a possible indicator of gestational maturity.

EXPERIMENTAL

Reagents

Standard Trp metabolites were obtained from Sigma (St. Louis, MO, USA). All other reagents, including the solvent, were of guaranteed grade and used as received.

Samples

Maternal blood was withdrawn from the cubital vein with a heparinized syringe immediately after the birth of a baby from a normal pregnancy. Umbilical foetal blood was collected separately from the umbilical artery and vein. Plasma was obtained by centrifugation at 1500 *g* for 15 min and kept at −20°C until analysis.

Free Trp metabolites in plasma samples were obtained by centrifugation of 0.5 ml of plasma by ultrafiltration with Ultracent (Tosoh, Tokyo, Japan).

Analysis of Trp metabolites

The HPLC system with a column-switching technique using two columns was analogous to that described in our previous paper [10]. The first column was a short precolumn of protein-coated ODS, which was made from an ODS column (40 × 4 mm I.D., 20–32 μm ODS, TSK-LS 120A; Tosoh), for deproteinization and also for trapping Trp metabolites. The characteristics of this protein-coated ODS column were discussed in ref. 11. The analytical column was octadecyl-bonded silica (TSK gel ODS-80TM, 5 μm, 150 × 4.6 mm I.D.; Tosoh).

A 100-μl voume of sample was injected into the

precolumn, which was equilibrated with purge solvent. After washing for 6 min with the purge solvent, the precolumn was connected to the analytical 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 content.

Fluorimetric detection (excitation 287 nm, emission 340 nm) was used to detect a series of indole metabolites, and UV spectrophotometric detection (at 350 nm) was used to detect the metabolites formed via kynurenine. Electrochemical detection (ED, applied voltage 600 mV on glassy carbon) was necessary for monitoring 5-hydroxyindoleacetic acid or 3-hydroxyanthranilic acid, which are not fluorimetrically or spectrophotometrically detectable at their endogenous concentrations. Thus, on-line use of the fluorimetric, UV absorbance and electrochemical detectors was essential to determine Trp metabolites simultaneously. The value obtained by direct plasma injection was estimated as the total (free plus bound to plasma proteins) according to our previous reports [8,9], and that of filtered plasma obtained with Ultracent was estimated as free.

Analysis of amino acids

Plasma amino acids were quantified using a laboratory-made amino acid analyser. The analytical conditions were analogous to our previous paper [12]. After deproteinization of the plasma sample with trichloroacetic acid, plasma amino acids were separated on a strong cation-exchange column (sulphonated porous polystyrene polymers) by programmed stepwise elution with citrate buffers, and by changing the salt concentration and pH. Post-column derivatization with *o*-phthaldialdehyde was carried out, followed by fluorimetric detection.

RESULTS AND DISCUSSION

Typical chromatograms showing the pattern of total Trp metabolites in maternal and umbilical foetal plasma are shown in Fig. 2. The following metabolites were detected in a 100-μl sample of maternal plasma: kynurenine, serotonin, 5-hydroxyindoleacetic acid, indolelactic acid, indoleacetic acid and indolepropionic acid. In umbilical foetal plasma, the peak of 3-hydroxyanthranilic acid was recognized, though it has not been detected in the analysis of 100 μl of maternal plasma or normal control plasma before.

The results of HPLC analyses of samples from 18 subjects are shown in Table I. Except for indolepropionic acid, the concentrations of all Trp metabolites found in umbilical foetal venous plasma (UV) and umbilical foetal arterial plasma (UA) were higher than in maternal plasma (MV). However, there were no significant differences between UV and UA.

The level of amino acids was also determined (data not shown). The tendency was similar to that reported previously [13], though there was less difference between the concentration of amino acids in maternal and umbilical foetal plasma compared with the concentration of Trp metabolites. In particular, the levels of kynurenine and 3-hydroxyanthranilic acid were significantly higher in umbilical foetal blood, and this might be correlated with higher enzymic activity of indolylamine 2,3-dioxygenase (IDO) in placentas [14]. As IDO is the first enzyme in the kynurenine pathway, this might lead to accelerated foetal synthesis of NAD, which is essential as a coenzyme in many enzyme reactions.

Free Trp metabolites in both maternal and umbilical foetal plasma were also analysed. As shown in Table II, the difference in the concentration of free TRP metabolites between maternal and foetal umbilical blood was not so large as that of total Trp metabolites. Therefore, the percentage of free metabolites was lower in umbilical foetal plasma than in maternal plasma (*e.g.* that of tryptophan was 19% in maternal plasma and 9% in foetal umbilical plasma). It was noteworthy that the level of free tryptophan was almost the same in maternal and foetal umbilical blood, considering that only the free form is effective in target cells.

The HPLC method was applicable to amniotic fluid, as well as blood. In the analysis of amniotic fluid, kynurenic acid was detected in addition to the Trp metabolites found in umbilical foetal

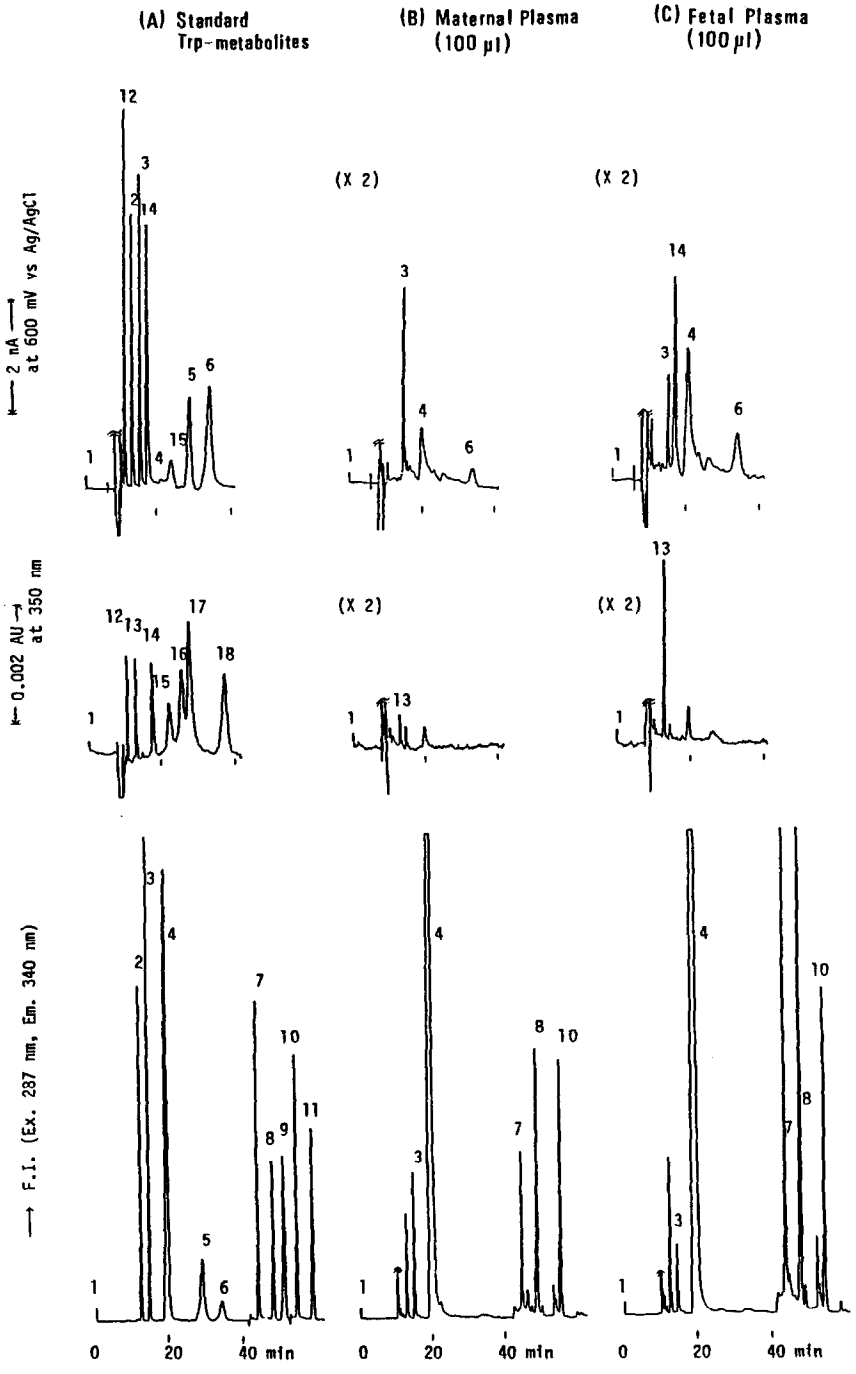

Fig. 2. Typical chromatograms of Trp metabolites in human maternal and umbilical foetal plasma. Samples: (A) standard Trp metabolites; (B) human maternal plasma (100 µl); (C) human umbilical foetal plasma (100 µl). Peaks (amounts in standard): 1 = injection marker; 2 = 5-hydroxytryptophan (50 pmol); 3 = serotonin (50 pmol), 4 = tryptophan (500 pmol); 5 = 5-hydroxytryptophol (50 pmol); 6 = 5-hydroxyindole-3-acetic acid (50 pmol); 7 = indolelactic acid (50 pmol); 8 = indoleacetic acid (50 pmol); 9 = tryptophol (50 pmol); 10 = indolepropionic acid (50 pmol); 11 = indolebutyric acid (50 pmol); 12 = 3-hydroxykynurenine (500 pmol for UV detection, 100 pmol for ED); 13 = kynurenine (500 pmol); 14 = 3-hydroxyanthranilic acid (2 nmol for UV detection, 100 pmol for ED); 15 = xanturenic acid (500 pmol); 16 = kynurenic acid (500 pmol); 17 = quinaldic acid (2 nmol); 18 = anthranilic acid (2 nmol). Columns: protein-coated ODS (40 × 4 mm I.D.) for precolumn; analytical ODS column (150 × 4.6 mm I.D.) (TSK gel ODS-80 TM, 5 µm, Tosoh). Purge solvent, 0.1 *M* phosphate buffer (pH 2.0) containing 1.0% TCA, 6 min, 0.9 ml/min. Analytical solvents: (1) 0.1 *M* phosphate solution (pH 3.3) containing 4% acetonitrile, 32 min; (2) 0.1 *M* phosphate solution (pH 4.5) containing 20% acetonitrile, 12 min; (3) 0.1 *M* phosphate solution (pH 4.5) containing 30% acetonitrile, 10 min; flow-rate, 1.3 ml/min; analytical column temperature, 35°C. Detection: top, electrochemical detection (600 mV); middle, UV absorption (350 nm); bottom, fluorimetric detection (F.I.) (excitation 287 nm, emission 340 nm).

TABLE I

TRYPTOPHAN METABOLITE LEVELS IN UMBILICAL FOETAL PLASMA (UV, UA) AND MATERNAL PLASMA (MV)

Samples were obtained from 18 subjects. N.D. = Below detection limit.

	UV, mean ± S.D. (pmol/ml)	UA, mean ± S.D. (pmol/ml)	MV, mean ± S.D. (pmol/ml)
Tryptophan	75 900 ± 17 000	68 900 ± 16 500	35 100 ± 10 000
Kynurenine	4960 ± 1100	4590 ± 1300	910 ± 260
3-Hydroxyanthranilic acid	270 ± 80	260 ± 80	N.D.
Indolelactic acid	1360 ± 300	1310 ± 310	350 ± 120
Indoleacetic acid	1730 ± 630	1730 ± 560	620 ± 235
Indolepropionic acid	840 ± 735	800 ± 650	730 ± 700
5-Hydroxyindoleacetic acid	103 ± 30	100 ± 30	40 ± 20

plasma. Attempts to correlate the state of foetal maturity with the level of a variety of constituents of amniotic fluid or serum have been made for lecithin and sphingomyelin [15], catecholamine [16] and polyamine [17]. However, as far as we know, there have been no reports concerning the investigation of tryptophan and its metabolites in clinical condition in foetal growth.

CONCLUSION

Trp and its metabolites in maternal and umbilical foetal plasma at delivery have been determined by automated HPLC, and Trp metabolites were found to be bound to plasma proteins to various extents. The concentrations of Trp metabolites, except for indolepropionic acid, were significantly higher in umbilical foetal plasma than in maternal plasma, though there was less difference in the concentrations of free Trp metabolites. This method was applicable to amniotic fluid, and kynurenic acid was detected in addition to the metabolites found in foetal plasma.

In addition to individual metabolites, the assay of a complete spectrum of Trp metabolites might be interesting for predicting the existence of abnormal states. The determination of Trp metabolites by this method could be used to monitor foetal maturity.

TABLE II

FREE TRYPTOPHAN METABOLITES IN FOETUSES AND GRAVIDA

Samples were taken from 23 subjects, N.D. = Below detection limit.

	Foetuses		Gravida	
	Mean ± S.D. (pmol/ml)	Free/total (%)	Mean ± S.D. (pmol/ml)	Free/total (%)
Tryptophan	7000 ± 4400	9	6300 ± 3700	19
Kynurenine	510 ± 350	11	220 ± 100	20
3-Hydroxyanthranilic acid	125 ± 55	46	N.D.	–
Indolelactic acid	N.D.	–	110 ± 80	18
Indoleacetic acid	140 ± 72	10	110 ± 80	18
Indolepropionic acid	N.D.	–	N.D.	–
5-Hydroxyindoleacetic acid	59 ± 32	58	44 ± 18	77

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