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Abstract

Previously, we pointed out the importance of the kynurenine metabolism in fetuses and neonates. We examined localization and developmental change of indoleamine 2,3-dioxygenase activity in human placenta. The indoleamine 2,3-dioxygenase was found localized in syncytiotrophoblast in the placenta. The indoleamine 2,3-dioxygenase activity was not detected in placenta in the early stage of gestation. It was first detected at around 14 weeks of gestation, increased rapidly thereafter and was maintained at high levels till near term. The indoleamine 2,3-dioxygenase activity was significantly lower in placenta with retarded intrauterine development. These results suggest the importance of placental indoleamine 2,3-dioxygenase during fetal development.

KEYWORDS: indoleamine 2, 3-dioxygenase, human placenta, tryptophan, kynurenine

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Localization and Developmental Change of Indoleamine 2, 3-Dioxygenase Activity in the Human Placenta

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Previously, we pointed out the importance of the kynurenine metabolism in fetuses and neonates. We examined localization and developmental change of indoleamine 2, 3-dioxygenase activity in human placenta. The indoleamine 2, 3-dioxygenase was found localized in syncytiotrophoblast in the placenta. The indoleamine 2, 3-dioxygenase activity was not detected in placenta in the early stage of gestation. It was first detected at around 14 weeks of gestation, increased rapidly thereafter and was maintained at high levels till near term. The indoleamine 2, 3-dioxygenase activity was significantly lower in placenta with retarded intrauterine development. These results suggest the importance of placental indoleamine 2, 3-dioxygenase during fetal development.

Key words : indoleamine 2, 3-dioxygenase, human placenta, tryptophan, kynurenine

Tryptophan is essential amino acid which is metabolized by active substances such as serotonin, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) in the human body. The main metabolic route of tryptophan metabolism in the liver is the kynurenine pathway, NAD and NADP are also formed by this route (1-4).

The kynurenine pathway starts with L-tryptophan, which is converted into N- α -folmyl-L-kynurenine by tryptophan pyrrolase (EC 1.1.3.12) in the liver of human adult. However, the tryptophan pyrrolase activity is scarcely detected in the placenta and fetal liver (6, 9, 10).

It has also been shown that the levels of tryptophan and most of its metabolites are

significantly higher in plasma of umbilical blood than those in plasma of maternal blood (5, 8)

In the present study, we examined indoleamine 2, 3-dioxygenase (EC 1.13.11.11) activity in the fetuses and placenta in 12 cases with normal development and 9 cases with retarded intrauterine growth. This enzyme splits the indole ring of tryptophan (7, 11, 12) and exhibits the highest activity in the placenta, followed by the lung and small intestine in human adults (11).

Materials and Methods

Materials. The placenta obtained from 21 cases of appropriate for date neonates and 9 cases of retarded intrauterine development (type II) were used in this study.

Immunohistological staining for the identification of

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placental indoleamine 2, 3-dioxygenase. The placenta was taken almost simultaneously with delivery and cut into small blocks. After washing with water, the blocks were fixed in 10 % formaline. The tissue sample was embedded in paraffin and a 5 μ m section was prepared. After the usual procedure (11) for the endogenous peroxidase staining, a monoclonal antibody against indoleamine 2, 3-dioxygenase and biotinylated rabbit anti-mouse IgG were used as the primary and secondary antibodies, respectively. These were bound with peroxidase-bound streptavidin to form avidin-biotin-peroxidase complex, which was made luminous with 3-amino-9 ethylcarbazole.

Detection of placental indoleamine 2, 3-dioxygenase activity. The indoleamine 2, 3-dioxygenase activity was assayed according to the Takigawa's method (11). The placenta was washed thoroughly, minced to pices of about 0.1 to 0.2 g, and then homogenized with a Polytron homogenizer for 45 sec. The homogenate was centrifugated at $2,500 \times g$ for 15 min and the supernatant

obtained was used as the sample. The sample (0.2 ml) was added to the standard reaction mixture (total 1 ml) containing 50 mM potassium phosphate buffer, pH 6.5, 25 μ M methylene blue, 20 mM ascorbic acid, 50 μ g of catalase and 0.4 mM L-tryptophan. The reaction, at 37 °C, was started with the addition of L-tryptophan and terminated after 30 min by adding 0.2 ml of 10 % (W/V) trichloroacetic acid. The mixture was further incubated at 50 °C for 30 min to hydrolyze N-formyl kynurenine to kynurenine. After centrifugation at $2,500 \times g$ for 15 min, the resultant supernatant (0.8 ml) was added to 0.8 ml of 2 % acetic acid containing 16 mg of p-dimethylaminobenzalhyde. The absorbance at 480 nm was read.

Results

Localization of placental indoleamine 2, 3-

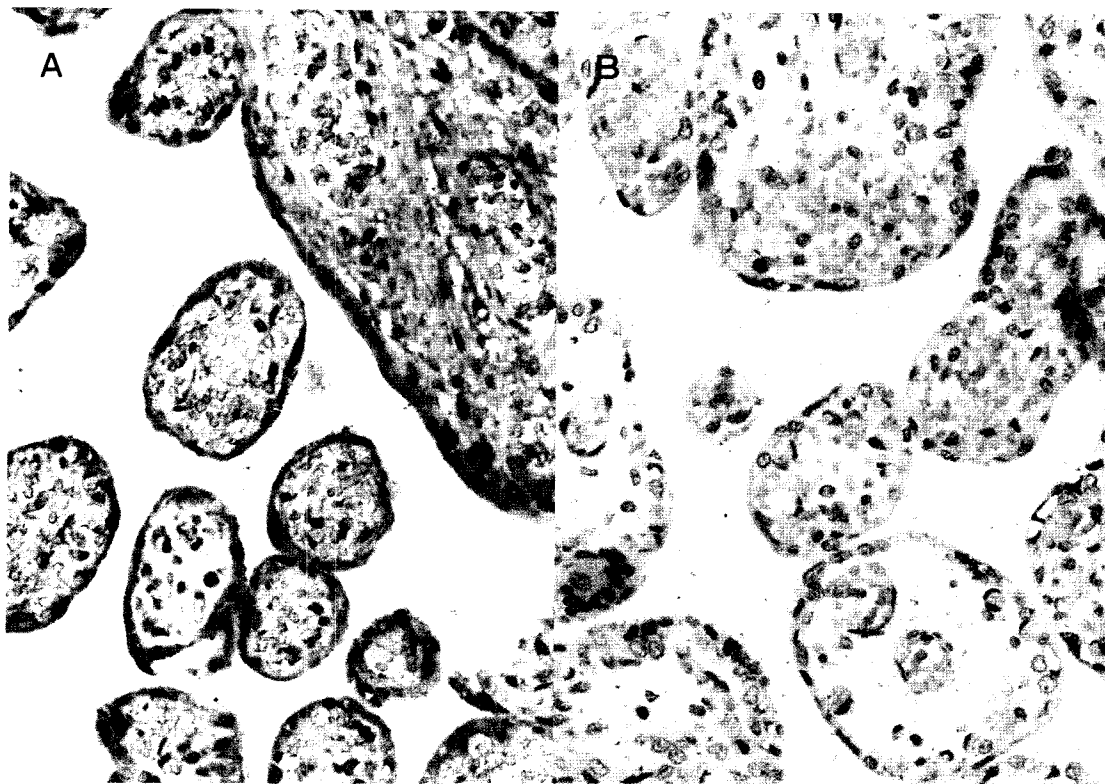


Fig. 1 Localization of indoleamine 2, 3-dioxygenase with immunohistological staining in human placental tissue at 18 weeks of gestation. Syncytiotrophoblast was stained brown (A) as compared with the control (B).

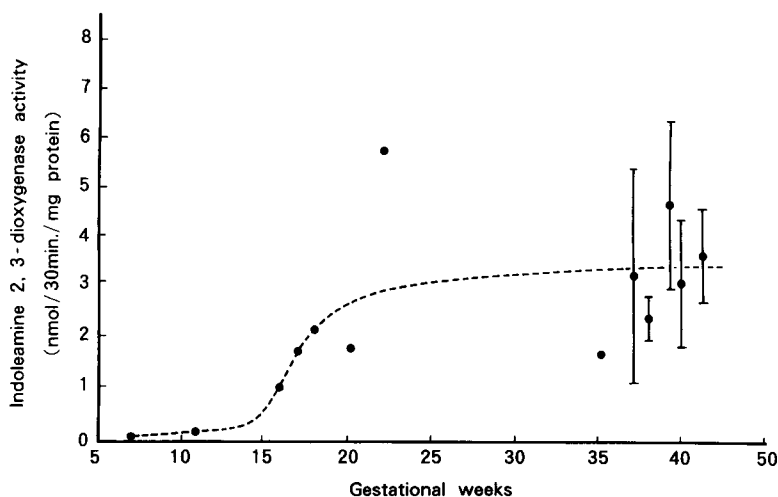


Fig. 2 Changes of activity of indoleamine 2, 3-dioxygenase in human placenta. When the activity of placental indoleamine 2, 3-dioxygenase was examined at each period of pregnancy, the indoleamine 2, 3-dioxygenase activity became noticed at around 14 weeks of gestation, increased rapidly thereafter, reached 3 nmol/30 min/mg protein at around 25 weeks of gestation and maintained high level till near term. The data are expressed as mean \pm SD.

dioxygenase. Immunological staining of indoleamine 2, 3-dioxygenase in the placenta at 18 weeks of gestation is presented in Fig. 1. Syncytiotrophoblasts stained brown (Fig. 1A) in contrast with the control (Fig. 1B). The decidua did not stain. These findings suggest that intraplacental indoleamine 2, 3-dioxygenase is localized in syncytiotrophoblast.

The indoleamine 2, 3-dioxygenase was first detected with the present staining method at around 14 weeks of gestation, at the time when placenta is matured, and it was detected thereafter until near term.

Activity of placental indoleamine 2, 3-dioxygenase. When the activity of placental indoleamine 2, 3-dioxygenase was examined at various periods of pregnancy, the activity became noticeable from around 14 weeks of gestation, and increased rapidly thereafter. A value of about 3 nmol/30 min/mg protein was reached at around 25 weeks of gestation, and this high level was maintained till near term (Fig. 2).

The indoleamine 2, 3-dioxygenase activity (1.8 ± 0.7 nmol/30 min/mg protein) of the placenta of neonates with intrauterine growth

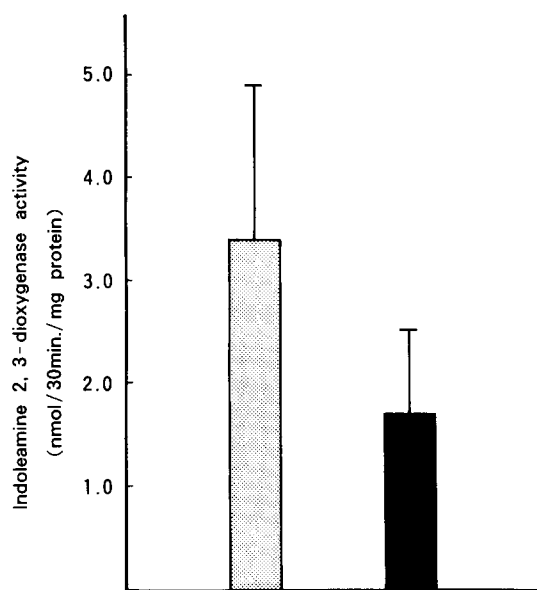


Fig. 3 The indoleamine 2, 3-dioxygenase activities in human placenta of neonates with intrauterine growth retardation ($n = 9$) and appropriate for date neonates (37–41 weeks of gestation) ($n = 21$). The indoleamine 2, 3-dioxygenase activity in the placenta of neonates with intrauterine growth retardation (■) was significantly ($p < 0.001$) lower than that of in appropriate for data (□). The data are expressed as the mean \pm SD.

retardation was significantly ($p < 0.001$) lower than that of appropriate for date group (3.4 ± 1.5 nmol/30 min/mg protein) (Fig. 3).

Discussion

We previously reported that plasma levels of tryptophan and most of its metabolites were significantly higher in umbilical blood than in maternal vein (8). This result seemed to suggest the importance of the kynurenine metabolites in fetuses and neonates because kynurenine was maintained at high levels in neonatal plasma as well (8). It has been reported that the initial reaction of tryptophan metabolism to kynurenine is catalyzed by tryptophan pyrrolase in the human adult liver (9,10). However, the tryptophan pyrrolase activity in the fetal liver and human placenta is reported to be very low (10). This suggests that the metabolism from tryptophan to kynurenine in the fetal life is catalyzed by other enzymes.

In the present experiment, we detected indoleamine 2, 3-dioxygenase (12, 13), in the placental tissue and determined the enzymatic activity in the placenta. It is already maintained the indoleamine 2, 3-dioxygenase activity of the placenta is the highest in human organs (12).

In our immunological staining using a monoclonal antibody, we identified indoleamine 2, 3-dioxygenase in syncytiotrophoblasts of the placental tissue. Furthermore, the indoleamine 2, 3-dioxygenase activity was determined at each week of gestation. The activity of indoleamine 2, 3-dioxygenase was first noticed at around 14 week of gestation, the time of the placental maturation, increased rapidly thereafter and maintained high levels up to near term.

When indoleamine 2, 3-dioxygenase activity of the placenta of neonates with intrauterine growth retardation was compared, activity appeared significantly lower. These results may suggest the importance of the kynurenine metabolism in the growth of fetuses.

The placenta is a metabolically active organ, and it is reported to play the role of the liver for fetuses (13–15). Results of our present experiment also suggest that indoleamine 2, 3-dioxygenase in the placenta plays the role of tryptophan pyrrolase in the liver of the adults.

In this paper, we have shown the role of the kynurenine metabolism in the fetal life and the relationship between placental indoleamine 2, 3-dioxygenase activity and kynurenine metabolism. But the significance of the kynurenine metabolism in fetuses remains obscure, and a suitable subject of future studies.

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