Essential and nonessential amino acids in appropriate and small for gestational age fetuses with congenital cytomegalovirus infection

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Summary

The aim of the study was to evaluate the correlation between valine and glycine, representatives of essential and nonessential amino acids, in appropriate and small fetuses for gestational age with congenital cytomegalovirus (CMV) infection.

Umbilical venous cord blood was obtained by cordocentesis at 22 to 29 weeks' gestation from 18 women (11 in appropriate for gestational age (AGA) -A, and 7 in small for gestational age (SGA) -B) fetuses with CMV infection. Plasma amino acids were measured with a Beckman M 121 amino acid analyzer.

Maternal valine level was 136.0 mmol/l; fetal valine in AGA and SGA fetuses: 219 and 189 mmol/l, respectively. Fetomaternal valine ratio was significantly lower in the SGA group (1.39 mmol/l-SGA, 1.61 mmol/l AGA, $t = -6.9$ $p<0.001$). The glycine level in maternal blood was 139.0 mmol/l; fetal in SGA and AGA fetuses 137 mmol/l and 176 mmol/l, respectively. The fetomaternal glycine ratio was also significantly lower in the SGA group than in AGA, 1.01 and 1.27, respectively ($t = -2.96$, $p<0.001$). Valine/glycine maternal and fetal ratio did not show any difference between groups.

In the congenital CMV infected fetuses with intrauterine growth retardation there were decreased valine and glycine levels compared to the congenitally CMV infected fetuses with normal intrauterine growth. There was a lower fetal concentration of these amino acids compared to the maternal level in SGA fetuses. A decreased glycine level compared to the valine level has also been found in congenitally CMV infected fetuses with intrauterine growth retardation.

Key words: Cytomegalovirus; Glycine; Valine; Prenatal diagnosis; Congenital infection; IUGR.

Introduction

It is well known that the synthesis of fetal proteins takes place in the fetus itself from the free amino acids transported from the maternal circulation through the placenta. The level is higher in the fetal than in the maternal circulation. Active transport of the amino acids in against the concentration gradient. Fetal amino acids are very similar to the adult ones and the majority of the amino acids are essential for the fetus until the complete maturation of the enzymatic system [1, 2].

There are several factors that influence these systems. One of the most important is the maternal/fetal gradient. While the elevation of ramified neutral amino acids in maternal blood leads to the elevation of the fetal level, that influence has not been shown on the non-ramified and alkaline amino acids. The level of the transfer does not depend on whether some amino acids are essential or not. The blood flow also has a great influence on the level of amino acid transfer.

The aim of the study was to evaluate the correlation between valine and glycine, representatives of essential and nonessential amino acids, in appropriate and small fetuses for gestational age with congenital cytomegalovirus infection.

Received July 1, 1997
revised manuscript accepted for publication September 10, 1997

Material and Methods

Umbilical venous cord blood was obtained by cordocentesis at 22 to 29 weeks gestation from 18 women referred to our clinic for possible fetal CMV infection in which subsequent analysis detected fetal CMV infection.

To determine the effect of cytomegalovirus (CMV) infection on valine and glycine metabolism, in appropriate (group A) and small (group B) for gestational age fetuses with congenital cytomegalovirus infection, we performed cordocentesis in 18 patients with proven congenital CMV fetal infection. All the mothers were healthy, had negative screening for antinuclear factor, and did not show serologic evidence of recent toxoplasmosis, rubella, herpes simplex virus or syphilis infection. Gestational age was calculated by Nagele's rule and confirmed by an ultrasonographic scan in early pregnancy in the referring hospital.

Eleven cordocenteses were performed in appropriate for gestational age (A) and seven in small for gestational age (B) fetuses with CMV infection, according to the technique described by Daffos *et al.* [3]. In group B fetal abdominal circumference at the time of cordocentesis was within our reference range for gestation, while in group A the fetal abdominal circumference was below the 5th percentile of our reference range. All of the infants (from group B) were small for gestational age at delivery, while those from group A were appropriate.

Cordocentesis was performed as an outpatient procedure without maternal fasting or sedation. The umbilical cord vessel was identified ultrasonographically as an umbilical vein or artery by the turbulence produced after the injection of 100 to 200 µl of normal saline solution. The fetal origin of blood was subsequently confirmed by Kleinhauer Betke testing and deter-

mination of particle volume (Coulter Counter S Plus II). Maternal blood was taken from an antecubital vein immediately before fetal blood sampling.

Fetal blood was collected into heparinized syringes with a sample collected in lithium heparine tubes. For determination of plasma amino acids, 300 µl of blood was centrifugated (2000 g at 4°C for 10 minutes) and the plasma was frozen and stored at -20°C for subsequent analysis. Plasma amino acids were measured with a Beckman M 121 amino acid analyzer. The intra-assay coefficient of variation was 6.6%.

After informed consent of the patient, an extra 0.2 ml of fetal blood was obtained for analysis. The extra fetal blood taken for this study was <1% of the feto-placental blood volume. Approval from the Ethical Committee was obtained both for the indication of cordocentesis and for the extra blood taken from the mothers and fetuses.

The data were analyzed by t-test and linear regression analysis.

Table 1. — *Values of the maternal and fetal valine and glycine levels, the maternal/fetal ratio of the analyzed amino acids as well as the valine/glycine ratio in fetal blood.*

	A (SD)	B (SD)
Valine (mol/l)		
Mother	136.4 (27.9)	135.8 (17.1)
Fetus	219.4 (28.2)	189.4 (28.7)*
Fetus/Mother	1.61 (0.39)	1.39 (0.14)*
Glycine (mol/l)		
Mother	139.0 (39.5)	136.1 (37)
Fetus	176.1 (54.5)	137.2 (47)*
Fetus/Mother	1.27 (0.14)**	1.01 (0.11)**
Valine/Glycine		
Mother	0.98	0.99
Fetus	1.24	1.38

* – P<0.05
** – P<0.001

Results

Eighteen patients with congenital CMV infections were analyzed (group A, 11; group B, 7).

Table 1 shows the values of the maternal and fetal valine and glycine levels, the maternal/fetal ratio of the analyzed amino acids as well as the valine/glycine ratio in fetal blood.

Maternal valine level was 136.0 mmol/l; fetal valine in AGA and SGA fetuses: 219 and 189 mmol/l, respectively. Fetomaternal valine ratio was significantly lower in the SGA group (1.39 mmol/l - SGA, 1.61 mmol/l AGA, $t = 6.9$ $p < 0.001$). Glycine level in maternal blood was 139.0 mmol/l; fetal in SGA and AGA fetuses 137 mmol/l and 176 mmol/l, respectively. Fetomaternal glycine ratio was also significantly lower in the SGA group than in the AGA, 1.01 and 1.27, respectively ($t = 2.96$, $p < 0.001$). Valine/Glycine maternal and fetal ratio did not show any difference between groups.

Discussion

In normal fetuses the concentration of amino acids in fetal plasma depends on the maternal level. The total concentration of amino acids does not change during gestation. Some of the amino acids, like phenylalanine, methionine or tyrosine show significant decrease during pregnancy. Glycine is the only acid that shows a significant elevation in the fetus during pregnancy. Since the maternal concentrations of this neural, non-essential amino acid that has a central role in different metabolic pathways do not change, it is probable that this finding implies higher placental production.

During gestation there is a decrease in the fetal/maternal ratio of all amino acids. This most probably implies that there is higher fetal/placental utilization of amino acids. In maternal blood there is an elevation of certain amino acids - alanine, serine, leucine, cysteine, threonine, taorine and glutamate. In some of the amino acids - fenylalanine and ornitine - a concentration decrease during pregnancy has been found.

The maternal blood concentration of the majority of amino acids does not change during pregnancy. In the analyzed group of SGA fetuses, a significantly lower concentration of fetal valine, as a representative of essential amino acids, has been found. This implies that in the mechanism of intrauterine growth retardation due to CMV infection, the disturbance of amino acids is an important pathophysiological factor. In the majority of amino acids fetal concentration significantly correlates with maternal concentration. The decrease of fetal/maternal ratio of valine shows that the decrease of fetal valine concentration is a result of not only a disturbance in the fetus but also a disturbance in the transport mechanism through the placenta.

Glycine is a neutral, non-essential amino acid which has a central role in a variety of metabolic pathways. Glycine exhibits significant umbilical uptake, but little uterine uptake in the sheep model [4].

The use of multiple tracer methodology coupled with measurement of net tracer fluxes into and out of fetal and placental tissues can be used to delineate amino acid metabolism in considerable detail. Such studies demonstrate that even essential amino acids can be oxidized extensively by the fetus. Glycine metabolism is unique in several ways; there is a large umbilical uptake of glycine without a measurable uterine uptake. Glycine is oxidized within the fetal liver and used for serum production. The interorgan exchange of amino acids between the fetal liver and placenta is clearly of major importance for serine and glycine metabolism and is likely to be of major importance for most nonessential amino acids [5]

It has already been said that the increase of the fetal glycine concentration that is normally found during pregnancy can be explained by elevated placental production. Lack of this increase - lower glycine concentrations found in congenitally CMV infected SGA fetuses, can be explained by derangement in placental glycine production, because of specific CMV placentitis. It is also possible that CMV leads to the derangement in glycine active transport through the placental barrier.

The increased ratio of the essential amino acid, valine to the non-essential glycine, found in the SGA of the

infected fetuses implies that the degree of glycine metabolism derangement is greater than the valine's.

In congenitally CMV infected fetuses with intrauterine growth retardation there are decreased valine and glycine levels compared to congenitally CMV infected fetuses with normal intrauterine growth. There is a lower fetal concentration of these amino acids compared to the maternal level in SGA fetuses. A decreased glycine level compared to the valine level has also been found in congenitally CMV infected fetuses with intrauterine growth retardation.

References

[1] Enders R. H., Judd R. M., Donohue T. M., Smith C. H.: "Placental amino acids uptake. III. Transport systems for neutral amino acids". *Am. J. Physiol.*, 1976, *230*, 706.

[2] Cetin I., Pardi G.: "Fetal metabolism of amino acids". *J. Perinat. Med.*, 1994, *22*, Suppl. 1, 35.

[3] Daffos F., Capella-Pavlovsky M., Forestier F.: "Fetal blood sampling during pregnancy with use of a needle quided by ultrasound. A study of 606 consecutive cases". *Am. J. Obstet. Gynecol.*, 1985, *153*, 655.

[4] Lemons J. A., Adcock E. W., Jones M. D., Naughton M. A., Meschia G., Battaglia F. C.: "Umbilical uptake of amino acids in the unstressed fetal lamb". *J. Clin. Invest.*, 1976, *58*, 1428.

[5] Battaglia F. C.: "New concepts in fetal and placental amino acid metabolism". *J. Anim. Sci.*, 1992, *70*, 3258.

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