International Journal of BioMedicine 2(4) (2012) 296-299

INTERNATIONAL JOURNAL OF BIOMEDICINE

Basic Research

Lipid Transport through the Fetoplacental Barrier by the Fatty Acid-Binding Proteins in Pregnant Women with Herpes Virus Infection in the third Trimester

Michael T. Lucenko, PhD, ScD*, Irina A. Andrievskaya, PhD, ScD, Natalia A. Ishutina, PhD

Far Eastern Scientific Center of Physiology and Pathology of Respiration, Siberian Branch of Russian Academy of Medical Sciences Blagoveshchensk, Russian Federation

Abstract

In this study, the transport of the long chain polyunsaturated fatty acids (LCPUFAs) from the lacunar blood through the syncytiotrophoblast of the placental villi to the fetal cord blood via a saturable protein-mediated mechanism by the heart-type fatty acid-binding proteins (H-FABPs) has been examined. Exacerbation of the herpes simplex viruses (HSV-1) in the third trimester of gestation reduces the delivery of the fatty acid-binding proteins to the syncytiotrophoblast. During exacerbation of the HSV-1 infection, the selective transfer of the LCPUFAs across the syncytiotrophoblast basal plasma membrane into the fetal cord blood was observed. The supply of anti-inflammatory ω-3 PUFAs was reduced; however, the inflow of inflammatory arachidonic acid and other ω-6 PUFAs into the fetal blood was increased.

Key words: *fetoplacental unit, long chain polyunsaturated fatty acids, heart-type fatty acid-binding proteins, herpes virus infection*

Introduction

The transport proteins carry the molecules and ions in the blood plasma and across the membrane or within the cell. The transport proteins are also involved in the movement of enzymes, carbohydrates and lipids in the form of cholesterol and fatty acids (FA) [1-3,5,8-11]. They also facilitate the lipid exchange between the cell membranes. Each carrier protein is designed to recognize only one substance or one group of very similar substances. The fatty acid binding proteins (FABPs) constitute a family of cytosolic proteins exhibiting a high degree of structural homology. The FABPs show great affinity for non-covalent binding of the FA. These proteins are widely distributed and are present in the fatty-acid metabolizing tissues. The intracellular FABPs belong to a multigene family. H-FABPs participate in the uptake, intracellular metabolism and/or transport of long-chain fatty acids. They may also be responsible for the modulation of cell growth and proliferation, and membrane update by transferring the lipids from the basal to the outer membrane [2].

The objective of this research was to study the influence of HSV-1 infection on the transport of LCPUFAs by H-FABP from the maternal plasma across the fetoplacental barrier into the fetal cord blood and identify the features of the ω-3 PUFAs/ ω-6 transfer.

Material and methods

A total number of 15 pregnant women in the third trimester of gestation with exacerbation of HSV-1 infection (IgG antibody to HSV-1 of 1:12800) and 20 healthy pregnant women (as control) were examined. The placental homogenate and umbilical cord blood of the newborns from these women

***Corresponding author**: Prof. Michael T. Lucenko, PhD, ScD, Academician of RAMS, Head of Far Eastern Scientific Center of Physiology and Pathology of Respiration SB of RAMS, 95A, Gorky str., 675000, Blagoveshchensk, Russian Federation.*
E-mail: Lucencomt@mail.ru

were also investigated.

To obtain the fetal placental homogenate, the chorionic villi were cut into segments 2- to 3-cm in size, up to 1 mm thickness, using a scalpel. The tissue pieces were placed in a beaker containing 200 ml of saline and washed free of blood cells. They were then stirred with a magnetic stirrer for 15 min and dried on a filter paper. The tissue was ground and homogenized until smooth. The homogenate was frozen at -20 ° C during the day; later it was thawed for further analysis.

Fatty acid composition was determined by employing capillary gas-liquid chromatography on a "Kristall 2000 m" (Russia) gas chromatograph that was equipped with a Flame Ionization Detector. Counting and identification of the peaks was done using the hardware-software complex "Chromatek Analyst 2.5" according to the «Supelco» (USA) standards. Quantitative calculation of the chromatograms was performed by the internal normalization method by determining the peaks areas of the components analyzed and their proportion (in relative %) in the total peak areas of the methylation products of the higher fatty acids.

The HHV antibody titers were determined using the Microplate Reader Stat Fax - 2100 (USA) using CC "Vector-Best" sets. The phospholipase A2 activity and the H-FABP levels were determined by ELISA using the reagents of the «Cayman Chemical» (USA) and Human H-FABP ELISA Test kit, respectively.

The histochemical reaction to determine the lipid peroxidation activity in the syncytiotrophoblast and the endothelium of the umbilical cord blood vessels was performed according to Winkler-Schulze [4]. The quantitative peroxide content was measured by computer cytometry.

All the studies were conducted in line with the requirements of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects" (update 2000) and the Rules of Clinical Practice in the Russian Federation, approved by Order #266 of the Ministry of the Russian Federation of June 19, 2003.

Statistical processing of data was performed using Student's t and Fisher criteria.

Results

The results thus obtained showed that the H-FABP serum level in healthy pregnant women was 23.94 ± 3.1 ng /mL while in the placental homogenate it was found to be 29.11±2.7 ng / mL. The H-FABP level in the placental homogenate decreased up to 23.93 ± 2.7 ng / mL in pregnant women with an exacerbation of HSV-1 infection. Therefore, an exacerbation of the HSV-1 infection can reduce the lipid transfer through the placental villous fetoplacental barrier by decreasing the placental H-FABP level. Suppressing the lipid transfer from the lacunar blood through the syncytiotrophoblast of the placental villi may reduce the lipid level in the cord blood which is mandatory for the metabolic processes of the developing fetus. Studies have shown that the outer membrane of syncytiotrophoblast during the exacerbation of the herpes viral infection demonstrates an intense reaction to the peroxide fatty acids (Figure 1a); on cytophotometry study it was observed to be 29 c.u. The intensity of the reaction on the peroxide fatty acids in healthy pregnant women was found to be significantly lower, at 10.5 c.u. During the exacerbation of the HSV-1 infection, the H-FABPs deliver the saturated fatty acids (SFA) into the syncytiotrophoblast at 17% more than in a healthy pregnant woman; for example, the oleic acid level was noted to increase by 35% when compared with the controls (Table 1). Perhaps, the rise in the concentration of the oleic acid in the placental homogenate was a result of the selective transport of this fatty acid associated with the H-FABPs which provide the preferential transfer of long chain polyunsaturated FA such as docosahexaenoic, arachidonic, linoleic and linolenic fatty acids. Oleic acid effectively facilitates the absorption of the polyunsaturated fatty acids (PUFAs) entering the syncytiotrophoblast.

As the H-FABPs realize the transfer by sharing lipids of only one type and from one membrane to the other, it is only after that procedure is completed that the fatty acid may be detached into the environment. Therefore, it becomes significant to understand the nature of the fatty acid transfer across the basal membrane of the syncytiotrophoblast in the fetal cord blood.

During exacerbation of the HSV-1 infection, the transport of the SFAs in the umbilical cord blood increased by 25% more than in the control, while the PUFAs dropped by 11% (Table 1). This reduction of the PUFAs occurred mainly due to the decrease in the oleic acid and ω-3 PUFAs, while the ω-6 PUFAs, linoleic and arachidonic acids were seen to increase in the cord blood.

In the course of the HSV-1 infection exacerbation, the phospholipase A2 activity in the placental homogenate increased up to 0.80 ± 0.08 ng/mL vs 0.35 ± 0.06 ng/mL in the control. This was associated with an increase in the decomposition of the lipids moving in the syncytiotrophoblast. However, as shown in Table 1, not all the FA crossed the basal membrane of the syncytiotrophoblast in equal measure. In light of this fact, the structure of syncytiotrophoblast should be seen as a complex barrier system, in which the outer part is attacked by harmful substances from the lacunar placental blood.

In the outer membrane, an intense reaction on the peroxide in the fatty acids is observed (Figure 1a); therefore, it becomes available for the passage of the macromolecular structures.

At the same time, the basal membrane of the syncytiotrophoblast is seen more as a safety system, which, under similar conditions did not reveal an intense reaction to the peroxide fatty acids (Figure 1b).

Therefore, it can be concluded that only small molecule compounds can pass through the cord blood, although some of which, unfortunately, becomes damaging during the HSV-1

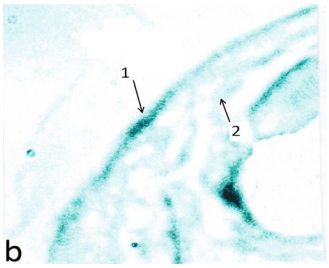


Figure 1
Syncytiotrophoblast of the placental villus in healthy pregnant women. 1- the outer membrane; 2- the basal membrane; reaction to the peroxide fatty acids according to Winkler-Schulze (magnification - 15 x 90.

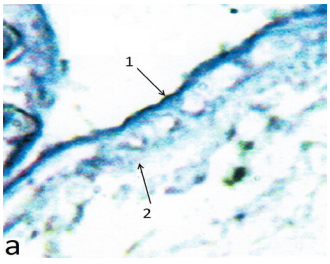
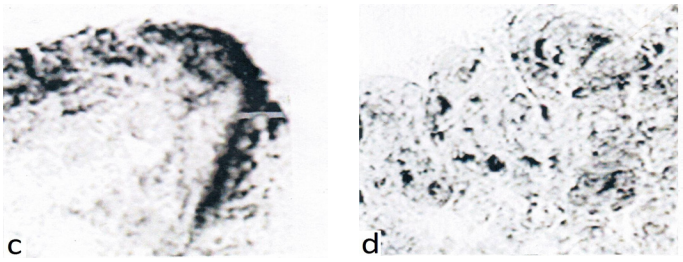


Figure 1
Syncytiotrophoblast of the placental villus after exacerbation of HSV-1 infection. 1- the outer membrane; 2- the basal membrane; reaction to the peroxide fatty acids according to Winkler-Schulze (magnification – 15×90.

infection. This is supported by a study showing that the vascular endothelium of the umbilical cord of newborns from mothers with an exacerbation of the HSV-1 infection during gestation revealed a greater response to the peroxide in the fatty acids that were associated with a high arachidonic acid level, which passes through the fetoplacental barrier (Figure 1 c, d).

Figure 1
Endothelium of the umbilical cord of newborns from mothers with an exacerbation of the HSV-1 infection during gestation; a greater response to the peroxide in the fatty acids.

Figure 1
Endothelium of the umbilical cord of newborns from healthy mothers.



Conclusion

In the fetoplacental unit, preferential transport of the essential lipids of the maternal plasma across the placenta assumes critical importance for fetal growth and development. The activation of the herpes viral infection during gestation with heavy aggression (IgG antibody to HSV-1 of 1:12800) inhibits the lipid transport through the fetoplacental barrier due the drop in the H-FABP levels. Simultaneously, during an exacerbation of the HSV-1 infection, substantial quantities of arachidonic acid and other ω-6 PUFAs penetrate through the fetoplacental barrier in the cord blood, which increases the lipid peroxidation in the vascular endothelium of the umbilical cord, precipitating the threat of inducing placental insufficiency.

Table 1
Fatty acid composition of the lacunar blood of pregnant in the 3rd trimester of gestation, maternal placental homogenates and umbilical cord blood of newborns from healthy mothers and mothers with an exacerbation of the HSV-1 infection during gestation

FA (wt% of the total FA)	Lacunar blood		Maternal placental homogenate		Umbilical cord blood	
	Control group	HSV-1 infection	Control group	HSV-1 infection	Control group	HSV-1 infection
Myristic ($C_{14:0}$)	1.80±0.20	2.52±0.15*	0.82±0.06	1.36±0.05*	1.10±0.05	1.48±0.06*
Pentadecanoic ($C_{15:0}$)	0.79±0.06	1.26±0.04*	0.53±0.04	0.71±0.06*	0.26±0.04	0.49±0.04*
Palmitic ($C_{16:0}$)	25.90±0.90	29.0±1.0*	30.10±2.20	32.30±1.80	18.50±1.10	24.0±0.86*
Palmitoleic ($C_{16:1}$)	2.86±0.08	3.24±0.08*	2.58±0.12	1.93±0.10*	2.34±0.12	1.82±0.10*
Margarinic ($C_{17:0}$)	0.94±0.07	1.38±0.08*	1.43±0.09	1.81±0.10 *	1.30±0.10	1.67±0.12*
Stearic ($C_{18:0}$)	13.80±1.0	17.30±0.90*	12.10±0.96	16.20±0.83*	11.0±0.90	15.10±0.83*
Oleic ($C_{18:1}$)	16.87±0.24	13.44±0.26*	10.30±0.87	13.90±0.80*	9.10±0.70	7.40±0.60
Linoleic ($C_{18:2}$)	7.10±0.22	8.30±0.17*	3.82±0.41	2.21±0.37 *	5.60±0.42	7.30±0.51*
Linolenic ($C_{18:3}$)	0.62±0.06	0.40±0.04*	0.28±0.06	0.16±0.04	0.43±0.04	0.29±0.05*
DGLA ($C_{20:3}$)	0.73±0.04	0.88±0.05*	0.69±0.08	0.82±0.07	0.67±0.05	0.81±0.08*
Arachidonic ($C_{20:4}$)	4.20±0.15	6.31±0.22*	4.66±0.30	5.96±0.42*	5.24±0.21	6.63±0.34*
Eicosapentaenoic ($C_{20:5}$)	1.40±0.07	0.96±0.06*	1.63±0.09	1.31±0.08*	1.36±0.12	0.83±0.06*
Docosahexaenoic ($C_{22:6}$)	8.90±0.21	6.34±0.16*	6.44±0.82	4.20±0.63*	3.82±0.35	2.37±0.30*

***Note:** * – statistical significance: p(t)<0.05, p(F) <0.05; IgG antibody titer to HSV-1 (1:12800);*
DGLA: Dihomo-γ-linolenic acid.

References

1. Antonov VK, Alexandrov SY. Bioorganic chemistry. M.: Medicine, 1977. [in Russia].

2. Barsukov AI. Results of science and technology. Issue Biophysics 1979; 11: 189-230. [in Russia].

3. Kotyk A, Janacek K. Membrane transport. M., 1980. [in Russia].

4. Lilly R. Histopathological techniques and practical histochemistry. Translated from English into Russian by VV Portugalova. M.: Mir 1969. [in Russia].

5. Nicholskiy NN, Troshin AS. Transport of sugars across cell membranes. L., 1973. [in Russia].

6. Titov VN. State of the lipid transport system in normal and its correction in certain lipid disorders. Abstract of PhD Thesis, 2004. [in Russia].

7. Titov VN. C-reactive protein-transfer vector of fatty acids to the cells that are directly implementing systemic inflammatory syndrome. Clinical laboratory diagnostics 2008; 5: 21-36. [in Russia].

8. Edidin M. Lipids on the frontier: a century of cell-membrane bilayers. Nature Reviews-Molecular cell biology

2003; 4:414-418.

9. Lev S. Non-vesicular lipid transport by lipid-transfer proteins and beyond. Nature Reviews-Molecular cell biology 2010; 11: 739-750.

10. Toll A. Plasma lipid transfer proteins. Ann Rev Biochem 1995; 64: 235-257.

11. Zilversmit D, Hughes M. Methods in membrane biology. Plenum, New York, 1976. P 211-259.