DOI 10.1007/s10517-017-3673-6
Bulletin of Experimental Biology and Medicine, Vol. 162, No. 11, March, 2017 BIOPHYSICS AND BIOCHEMISTRY

Albumin in the Vitreous Body, Retina and Lens of Human Fetal Eye

I. G. Panova[1], A. S. Tatikolov[2], Yu. A. Smirnova[1], R. A. Poltavtseva[3], and G. T. Sukhikh[3]

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 162, No. 11, pp. 578-580, November, 2016
Original article submitted February 1, 2016

The content of serum albumin was evaluated in the vitreous body, retina, and lens of human fetuses of 14, 16, 17, 18 and 24 weeks of gestation. Albumin was detected in these tissues. PCR analysis revealed no albumin mRNA in the retina or in the lens, while in fetal liver (control) mRNA for this protein was expressed. These findings suggest that serum albumin is not synthesized by cells of retina and lens, but is captured by them. The presence of serum albumin in human eye tissues suggests its involvement in the metabolism, maintenance of tissue volume, and antioxidant reactions.

Key Words: *human fetuses; serum albumin; vitreous body; retina; lens*

Serum albumin (SA) is the main transport protein in the body. It acts as a transporter for hormones, lipids, metal ions, amino acids, carotinoids, drugs, *etc*. SA binds toxic metabolites for their further detoxication, which suggests that this protein plays an important role in protection of organs and tissues. Various endogenous and exogenous components are stored in systemic circulation and in tissues and delivered to the target organs in the SA-bound form. SA stabilizes blood plasma pH and generates intravascular oncotic pressure [3,8]. SA is the main plasma antioxidant [11].

In humans, SA is synthesized starting from the early stages of prenatal development. During the embryonic period, SA production begins in the yolk sac and embryonic liver [5]. In the second and third trimesters, SA is produced mainly by the fetal liver [6] and is transported to the target tissues via the circulatory system.

Little is known about SA distribution in the eye tissues during prenatal human development. In our previous study, we demonstrated high SA content in the vitreous body of human fetuses in the second trimester of pregnancy [1].

Here we studied SA content in the vitreous body, retina, and lens of the developing eye of human fetuses.

MATERIALS AND METHODS

Abortive human eye material (vitreous body, retina, and lens) was used in the study. The fetuses were brought to V. I. Kulakov Research Center for Obstetrics, Gynecology, and Perinatology from licensed institutions of Ministry of Health of Russia, operating within the Russian Federation legislation on health care and in accordance with the approved list of medical indications. The age of the fetuses corresponded to the terms determined by the obstetricians.

The cornea was cut off along the limbus, the vitreous body was removed together with the lens, and the lens was carefully separated. The retina was separated from the pigment epithelium. The retina and lens were washed in 0.9% NaCl, diluted 2-3 times with distilled water, and homogenized. The vitreous body and homogenates of the lens and retina were centrifuged (12,000 rpm, Eppendorf 5417R centrifuge, 4°C, 30 min), supernatants were collected and used for measurement of SA concentration with squarylium dye

[1]N. K. Koltsov Institute of Developmental Biology, Russian Academy of Sciences; [2]N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences; [3]V. I. Kulakov Research Center for Obstetrics, Gynecology, and Perinatology, Ministry of Health of Russian Federation, Moscow, Russia. ***Address for correspondence:*** pinag@mail.ru. I. G. Panova

probe [2]. SA concentration in the vitreous body and lens was measured on gestation weeks 14, 16, 17, 18, and 24 and in retina on weeks 14 and 24.

The expression of SA gene was analyzed in the retina and lens of 12-week-old fetuses; the liver of a 16-week fetus was used as the control. Total RNA was extracted from tissues using TRI Reagent mixture (Sigma). Libraries of cDNA were synthesized on total RNA using Sileks kit. PCR was carried out in a reaction mixture (25 μl) containing 0.625 U ColoredTaq polymerase, 1× buffer, 250 mM dNTP, 400 mM specific primers, and 0.5 μl cDNA. Eppendorf Mastercycler thermocycler was used. Reaction conditions: primer annealing temperature 58°C; number of cycles 30. PCR results were analyzed by horizontal gel electrophoresis in 2% agarose gel on 1× TAE-buffer. Ethidium bromide was used as a dye. Electrophoresis was performed in a Horizon 58 chamber (Life Technologies Inc.). The gel was photographed in a GelDoc XR transilluminator (Bio-Rad Laboratories) using Quantity One software (Bio-Rad Laboratories). cDNA libraries were preliminary normalized for gene *RPL18s*.

RESULTS

The dynamics of SA concentration in the vitreous body corresponded to that in the previous study, where high SA concentration was observed on prenatal weeks 16, 17, and 18 [1]. In the lens, SA concentration on week 14 was much higher than in other periods of investigation: by ~4 times in comparison with weeks 16, 17, and 18 and by 2 times in comparison with week 24 (Table 1). In the retina, SA concentration on weeks 14 and 24 differed insufficiently and was low, when compared to that in the lens.

PCR analysis (Fig. 1) revealed mRNA of SA only in the liver, but not in the retina and lens, which suggests that SA during development enters the retina and the lens from outside. The vitreous body located between the retina and lens and characterized by high SA content in the period of investigation could be a source of SA for these structures.

TABLE 1. Concentration of SA in the Vitreous Body, Reina, and Lens of Human Eye during the Prenatal Period ($\times 10^{-4}$ mol/liter)

Fetus age, weeks	Vitreous body	Retina	Lens
14	0.11	0.11	0.54
16	0.96	—	0.13
17	1.52, 1.29*	—	0.13
18	1.2	—	0.11
24	0.76	0.069	0.26

Note. *Data for the two vitreous bodes are shown, "—", not analyzed.

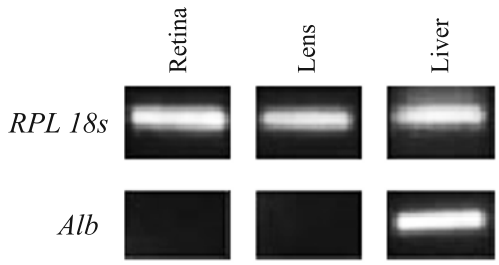

Fig. 1. PCR analysis of SA gene expression in the retina and lens of 12-week fetuses and in the liver of 16-week fetuses.

The physiological functions of SA in the eye tissues are now intensively studied and discussed. Such SA properties as delivery of macromoleculed to the tissues and organs and antioxidant function are necessary for the development of human fetal organs and tissues, including eye structures [7]. Important functions of SA include creation of oncotic pressure in the body fluids [4] and probably in the vitreous body. Elevated SA content in the vitreous body provides intraocular pressure, which is an important factor of eye growth and morphogenesis of its structures during prenatal development in humans [7]. SA is regarded as a biologically active molecule involved in the regulation of cell proliferation in the developing retina [12]. SA has an important neuroprotective effect on neurons and glial cells [9]. Polyunsaturated fatty acids essential for the formation of cell membranes in the retina and lens are transferred to these tissues in SA-bound form [10]. Due to its antioxidant properties, SA acts as protector from lipid peroxidation in the period of maximum growth of retinal blood vessels and axonogenesis, as well as active formation of lens fibers. Accumulation of knowledge about SA functions in the developing eye can provide the basis for new targeted drug delivery strategy in therapy of the fetal eye diseases, *e.g.* retinopathy of premature infants and congenital cataract.

Authors are grateful to A. A. Ischenko from Institute of Organic Chemistry, National Academy of Sciences of Ukraine for the squarylium dye probe.

This study was supported by Russian Foundation for Basic Research (grant Nos. 14-04-00745 and 16-03-00735).

REFERENCES

1. Panova IG, Tatikolov AS, Sukhikh GT. Correlation between the content of albumin and carotenoids in human vitreous body during prenatal development. Bull. Exp. Biol. Med. 2007;144(5):681-683.

2. Tatikolov AS, Panova IG, Ishchenko AA, Kudinova MA. Spectral and fluorescent study of the interaction of squarylium dyes, derivatives of 3H-indolium, with albumins. Biophysics. 2010;55(1):35-40.
3. Fanali G, di Masi A, Trezza V, Marino M, Fasano M, Ascenzi P. Human serum albumin: From bench to bedside. Mol. Aspects Med. 2012;33(3):209-290.
4. Hankins J. The role of albumin in fluid and electrolyte balance. J. Infus. Nurs. 2006;29(5):260-265.
5. Jauniaux E, Gulbis B, Jurkovic D, Campbell S, Collins WP, Ooms HA. Relationship between protein concentrations in embryological fluids and maternal serum and yolk sac size during human early pregnancy. Hum. Reprod. 1994;9(1):161-166.
6. Naval J, Calvo M, Laborda J, Dubouch P, Frain M, Sala-Trepat JM, Uriel J. Expression of mRNAs for alpha-fetoprotein (AFP) and albumin and incorporation of AFP and docosahexaenoic acid in baboon fetuses. J. Biochem. 1992;111(5):649-654.
7. Panova IG, Tatikolov AS, Stroeva OG. Albumins and carotenoids of the human fetal vitreous body and their morphogenetic role during midgestation. J. J. Ophthalmol. 2015;1(2):013.
8. Peters T Jr. All about Albumin: Biochemistry, Genetics, and Medical Application. San Diego, 1996.
9. Prajapati KD, Sharma SS, Roy N. Current perspectives on potential role of albumin in neuroprotection. Rev. Neurosci. 2011;22(3):355-363.
10. Sabah J, McConkey E, Welti R, Albin K, Takemoto LJ. Role of albumin as a fatty acid carrier for biosynthesis of lens lipids. Exp. Eye Res. 2005;80(1):31-36.
11. Taverna M, Marie AL, Mira JP, Guidet B. Specific antioxidant properties of human serum albumin. Ann. Intensive Care. 2013;3(1):4. doi: 10.1186/2110-5820-3-4.
12. Yang J, Klassen H, Pries M, Wang W, Nissen MH. Vitreous humor and albumin augment the proliferation of cultured retinal precursor cells. J. Neurosci. Res. 2009;87(2):495-502.