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Research article

Lutein and its oxidized forms in eye structures throughout prenatal human development

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ABSTRACT

The presence of carotenoids in the vitreous body, retina, lens, retinal pigment epithelium together with choroid (hereinafter RPE), and ciliary body and iris together with choroidal stroma (hereinafter CBI) was studied throughout the second trimester of prenatal development of the human eye. It has been found that the vitreous body, retina, and RPE contain lutein and its oxidized forms. Zeaxanthin was not found in the tissues studied. The presence of lutein in the vitreous body is transient and no longer detected after 28 weeks of gestation. Lutein was not detected in the lens and CBI, but its oxidized forms were found. The presence of carotenoids in different tissues of the eye in the course of normal eye development and the antioxidant role of carotenoids are discussed.

1. Introduction

Carotenoids, lutein and zeaxanthin (yellow pigments), are contained in almost all structures of the definitive human eye: retina, retinal pigment epithelium, lens, choroid, iris, ciliary body, and the matrix of the subretinal space. The only eye structure completely devoid of carotenoids is the vitreous body (Chan et al., 1998; Bernstein et al., 2001; Bernstein, 2002; Jewell et al., 2001; Sommerburg et al., 2000).

Lutein and zeaxanthin are natural antioxidants usually present in the human diet and selectively accumulated in the retina (macula) and the lens, in which they are assigned with an important protective function (Maci, 2010; Barker et al., 2011). First, they serve as a cutoff light filter protecting the photoreceptive cells of the retina and the cells of the retinal pigment epithelium from the hazard of the blue and violet light damaging effect. Second, having strong antioxidant properties and the ability to intercept reactive oxygen species, lutein and zeaxanthin prevent in the retina and the lens the development of peroxidation and photooxidation processes of lipids and proteins (Jewell et al., 2001; Mares-Perlman et al., 2002; Maci, 2010).

Macula (yellow spot) is a specialized structure of the retina of humans and primates, in the center of which a region of highest visual acuity – foveola – is located. It is the macula that contains the highest concentration of lutein and zeaxanthin – more than 70% of their total content in the human eye (Handelman et al., 1988). And it is the macula that is susceptible to degenerative diseases, especially in people more than 65 years old. Cataract, or clouding of the lens, is one of the leading causes of blindness in people over 40 years old all over the world (Abdel-Aal et al., 2013).

It has been shown that full-value carotenoid diet reduces the hazard of development of macular retinal degeneration and

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cataract (Moeller et al., 2008; Maci, 2010; Barker et al., 2011). Given this fact, increasing attention is paid to the carotenoid diet of not only adult humans, but also children after birth, nursing mothers, and mothers in pregnancy (Jewell et al., 2001; Connor et al., 2008a; Connor et al., 2008b).

In recent years, questions have been posed about the role of carotenoids in the formation of eye structures, particularly the macula, in prenatal human development (Zimmer and Hammond, 2007; Yakovleva et al., 2007; Panova et al., 2013, 2015).

Detection of lutein and zeaxanthin in the region of incipient macula in 17–20-week fetuses (Bone et al., 1988) generates a need to study the presence of carotenoids not only in the retina, but also in other structures of the human eye in the course of its prenatal development. During this period, carotenoids can act as valuable antioxidants. The developing structures of the eye are closely surrounded by oxygen-rich blood vessels. Indeed, retinal pigment epithelium, epithelia of ciliary body and iris develop surrounded by choroid vessels. The lens develops in close surrounding of the vascular tunic of the lens (*tunica vasculosa lentis*). In the vitreous body, hyaloid vessels are present, which adjoin the retina. In the retina, beginning at the 14th week of gestation, its own blood vessels are actively formed (Mann, 1949). Therefore, the proximity of vessels and active processes of the formation of lens fibers, nerve fibers, and particularly the retinal photoreceptors outer segments, which require easily oxidizable polyunsaturated fatty acids to form biological membranes, create the hazard of development of destructive oxidation processes. Hence, the antioxidant system of the eye is undoubtedly important for the eye development.

In this regard, the aim of the present work was to study the vitreous body, retina, lens, RPE together with choroid (hereinafter RPE), and ciliary body and iris together with choroidal stroma (hereinafter CBI) throughout the second trimester of prenatal development of the human eye, for the purpose of finding carotenoids in these tissues.

2. Experimental

2.1. The research materials

The objects of the study were the vitreous body, retina, lens, RPE, CBI obtained from eyes of human abortuses. Fetuses of 12–20 weeks of gestation and autopsy material obtained upon autopsy of dead fetuses, nonviable after premature birth at 31 weeks, were supplied to the Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences, from licensed institutions of the Ministry of Health of the Russian Federation, acting within the framework of the law of the Russian Federation about protection of the health of citizens and according to the approved list of medical indications. The age of fetuses was determined by an obstetrician.

To take out vitreous body, cornea was cut out along the limbus and was completely removed. Then lens with vitreous body was taken out by forceps and vitreous body was separated from lens. After removing retina, RPE and CBI were taken out.

Vitreous bodies of adult humans at the age of 21, 25, and 48 years were isolated from cadaverous eyes after cornea had been taken for transplantation. Human cadaverous eyes were obtained from the Eye Tissue Bank in the S.N. Fyodorov Eye Microsurgery Complex (Moscow, Russia) with permission for research in accordance with local ethical requirements (for details see section “Ethical Statement” in the previously published work (Feldman et al., 2015).

2.2. UV spectroscopy

Since carotenoids have characteristic absorption spectra, to detect them, we measured the absorption spectra of the native vitreous body (with a UV-1700 spectrophotometer, Shimadzu, Japan) in a 1 cm cell in the spectral range of 370–670 nm. Before measuring the absorption spectra, the vitreous bodies were weighed (on a BP 310 S balance, SARTOGOSM, Russia) for later use in determining the content of lutein and its oxidized forms in the studied tissues of the eye after the HPLC analysis (as the ratio of the mass of carotenoids in ng to the wet mass of the tissue in g). The vitreous bodies were taken out from eyes of fetuses of 15–28 weeks of gestation and, for comparison, from eyes of an adult (21 and 48 years old) human. A photograph of the vitreous body from an eye of 18 week gestation (Fig. 1) was made with a Canon EOS 550D camera.

We also measured the absorption spectra of supernatants obtained after centrifugation of the native vitreous bodies (in an Eppendorf 5417 R centrifuge, 12,500 rpm, 4 °C, 30 min) of 16–31 weeks of gestation and, for comparison, from the eyes of adult humans 25 years old. Previously we have shown the high albumin content in the vitreous body of human fetuses during the period studied (Panova et al., 2007). Based on the properties of albumin to bind carotenoids, converting them into a soluble state, we assumed that carotenoids would be also detected in the supernatants of the vitreous bodies. The measurements were performed on a UV-3101PC spectrophotometer (Shimadzu, Japan) in a 1 mm cell in the range of 300–600 nm.

2.3. High performance liquid chromatography (HPLC)

Extraction of carotenoids from the native vitreous body, retina, lens, RPE, and CBI of the eyes of human fetuses and the vitreous

Fig. 1. Photograph of the vitreous body from the eye of an 18 week fetus, placed in a test-tube.