Comparison of Antioxidants in the Ability to Prevent Cataract in Prematurely Aging OXYS Rats

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The biological model of prematurely aging OXYS rats is proposed for evaluation of anticataract activity of preparations. Pathological changes in the lens develop in 2-month-old OXYS rats. By the 6th month of life cataract morbidity rate attains 100%. Adrusen Zinco, Mirtilene Forte, blueberry extract, and vitamin E (Russian and from Sigma) possessing antioxidant properties and given with food decreased the number of OXYS rats with cataract. The preparation from blueberry Mirtilene Forte and blueberry extract normalized the content of lipid peroxidation products in the blood. Blueberry extract manufactured in Russia decreased the index of lipid atherogenicity that was high in OXYS rats.

Key Words: *OXYS rats; experimental senile cataract; anticataract activity of antioxidants*

Cataract is the most common cause for vision impairment and loss. The disease is associated with oxidative damage to the lens. Antioxidants are extensively used to delay the development of this disorder. Synthesis of new preparations for the therapy and prevention of cataract is an urgent problem of modern medicine. Biological models are required to evaluate objectively the efficiency of preparations. There are several models to study the etiology, pathogenesis, and prevention of age-related cataract. They include Emory mice [6], prematurely aging SAM-R/3 mice [5], and UPL Sprague-Dawley [15] and SCR rats (Shumiya Cataract Rat) [12]. Prematurely aging OXYS rats are proposed to perform these experiments in Russia. This strain was bred by selection and inbreeding of Wistar rats sensitive to the cataractogenic effect of galactose in the beginning of the 1970s (Institute of Cytology and Genetics) [3,10]. OXYS rats are characterized by early involution of internal organs and emotional and cognitive disorders typical of aging humans and animals. These disturbances are related to increased sensitivity of OXYS rats to oxidative stress [1,10]. Pathological changes in the lens develop in 2-month-old OXYS rats. By the 6th month of life, cataract morbidity rate in these animals reaches 100%. It should be emphasized that in 41% OXYS mice both eyes are involved [2]. In Wistar rats of the same age cataract morbidity rate does not exceed 5%. Moreover, there are no Wistar rats with damage to both eyes. Here we evaluated the ability of antioxidant preparations synthesized in Russia and other countries to prevent the development of cataract in OXYS rats. Experiments were performed with Mirtilene Forte (dry blueberry extract, *Vaccinum mirtillus*; Societa Industia Farmaceutica), Adrusen Zinco (complex of vitamin E, zinc, copper, selenium, and polyunsaturated fatty acids; Societa Industia Farmaceutica), vitamin E (α-tocopherol acetate; Sigma and Uralbiofarm), and blueberry extract (Sibbiotekh). We determined the effects of these preparations on biochemical indexes of the blood generally accepted in clinical practice for the diagnostics of oxidative stress and study of lipid metabolism.

MATERIALS AND METHODS

Experiments were performed on male OXYS (n=120) and Wistar rats (n=30) at the Laboratory of Animal

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Breeding (Institute of Cytology and Genetics). The animals fed a standard diet. OXYS rats were divided into 5 groups. One of the test preparations was given with food over the 3rd, 5th, 7th, and 9th months of life. The animals daily received Mirtilene Forte (230 mg/kg, group 1), blueberry extract (230 mg/kg, Sibbiotekh, group 2), α-tocopherol acetate (25 mg/kg, Sigma, group 3; Russia, group 4), and Adrusen Zinco (1 g/kg: 340 mg proteins, 260 mg carbohydrates, 350 mg lipids, 23 mg zinc, 1 mg copper, 88 μg selenium, and 24 mg vitamin E, group 5). The lenses of 10-month-old rats were examined using a SL 30 slit lamp (Opton) and an automatic image recording system. Pupillary dilation was induced with 1% homatropine hydrobromide. The animals were decapitated. Serum content of secondary products of free radical oxidation (malonic dialdehyde, MDA; other carbonyl compound) was estimated in the reaction with thiobarbituric acid. The amount of trimethine complexes was measured on a Hitachi 557 spectrophotometer at 532 nm [14]. Tocopherol concentration was determined on an MPF-4 spectrofluorometer (Hitachi) [15]. Serum lipid composition (total cholesterol, TCH; triglycerides; and α-cholesterol, α-CH) was assayed with enzymatic colorimetric methods (CHOD-PAP and GPO-PAP) and precipitating reagent (FLUITEST HDL-CHOL system, Biosub). The index of atherogenicity was calculated as follows: (TCH — α-CH)/α-CH. The results were analyzed by one-way ANOVA (STATISTICA 5.5 software).

RESULTS

Ophthalmologic examination revealed changes of the lens in 18% Wistar rats and 100% control OXYS rats aging 10 months. It should be emphasized that 58% OXYS rats had cataract of both eyes. Biomicroscopy of the lens revealed zonular, punctate, and spindle cataracts in the cortex and/or core of the lens. Adrusen Zinco, Mirtilene Forte, and α-tocopherol (Sigma) decreased the number of OXYS rats with cataract by 3.7, 2.7, and 1.4 times, respectively (Fig. 1). The preparation Adrusen Zinco was most effective in this respect. Cataract morbidity rate decreased by 2.4 times in animals receiving blueberry extract (Russia). Moreover, the number of rats with damage to both eyes was 8-fold lower than in the control. In animals receiving α-tocopherol (Russia) these indexes decreased by 1.6 and 2.6 times, respectively.

OXYS rats had increased level of lipid peroxidation (LPO) products in blood serum (Table 1). Only blueberry extracts manufactured in Italy and in Russia were capable of decreasing the concentration of LPO products in the serum. Serum tocopherol concentration did not differ in Wistar and control OXYS rats. Treatment with vitamin E manufactured by Sigma and Uralbiofarm increased serum tocopherol concentration by 28 and 38%, respectively. Moreover, tocopherol concentration tended to increase in animals receiving blueberry extracts (Table 1). The content of triglycerides and TCH was similar in control OXYS and Wistar rats. However, the index of atherogenicity in these animals increased by 1.6 times due to lower content of α-CH (maximum efficiency; F=8.7, p=0.007). Only blueberry extract manufactured in Russia markedly decreased the atherogenicity index in OXYS rats.

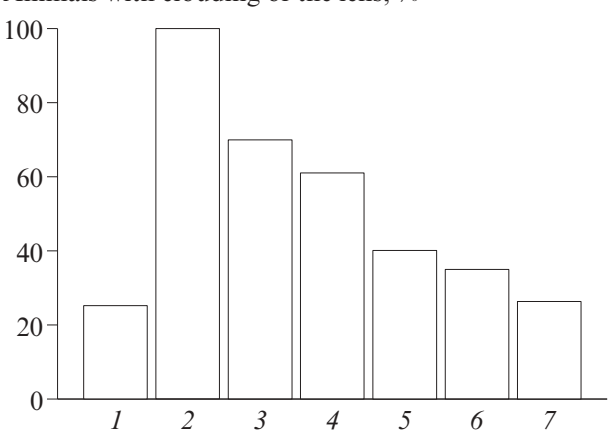

Fig. 1. Cataract morbidity rate in 10-month-old Wistar and OXYS rats: Wistar rats (*1*); control OXYS rats (*2*); OXYS rats receiving α-tocopherol acetate (Sigma, *3*), α-tocopherol acetate (Russia, *4*), blueberry acetate (*5*), Mirtilene Forte (*6*), and Adrusen Zinco (*7*).

The increase in blood MDA content serves as a clinical marker of oxidative stress. The decrease in MDA level reflects the efficiency of test preparations. It is not surprising that blood content of LPO products is high in OXYS rats with cataract. We found that only blueberry preparations decrease serum concentration of LPO products. However, a correlation was found between cataract morbidity rate and the mean content of MDA in animals of 7 groups (r=0.85, p=0.14).

It is absolutely clear that free radicals are involved in the pathogenesis of cataract. However, the role of antioxidant supply to the organism remains unclear. Large-scale epidemiological studies evaluate the relationship between vitamin E content and morbidity rate of cataract in humans [12]. As differentiated from lutein, treatment with food α-tocopherol does not decelerate vision impairment in patients with cataract [9]. We showed that the preparation Adrusen Zinco is highly effective in rats. It contains not only α-tocopherol, but also omega-3 polyunsaturated fatty acids and essential antioxidant microelements (zinc, copper, and selenium).

Blueberry is a traditional medicine used to improve vision acuity. The number of pharmacological preparations and biological additives (*e.g.*, blueberry) for the therapy of eye diseases constantly increases.

TABLE 1. Effect of Antioxidants on the Amount of LPO Products and Tocopherol and Indexes of Lipid Metabolism in the Serum from OXYS Rats (*M*±*m*)

Index	Wistar rats	OXYS rats					
		control	Adrusen Zinco	Mirtilene Forte	blueberry extract	α-tocopherol (Uralbiofarm)	α-tocopherol (Sigma)
MDA, nmol/ml	5.18±0.51	7.35±0.47* *p*=0.008	5.82±0.42 *p*=0.068	5.41±0.58⁺ *p*=0.042	6.19±0.19⁺ *p*=0.040	7.13±0.51	6.10±0.61
Tocopherol, μg/ml	15.2±1.3	16.80±0.85	17.70±1.33	22.43±2.40 *p*=0.056	23.1±2.4⁺	19.5±1.4 *p*=0.042	21.5±1.4⁺ *p*=0.039
Triglycerides, mmol/liter	1.14±0.12	1.28±0.13	1.22±0.14	1.19±0.09	1.12±0.13	1.27±0.12	1.37±0.09
Cholesterol, mmol/liter	2.22±0.18	2.00±0.09	2.14±0.13	1.73±0.11	1.75±0.15	2.19±0.17	2.17±0.19
α-Cholesterol, mmol/liter	1.35±0.11	1.09±0.06* *p*=0.036	1.07±0.09	0.98±0.05	1.10±0.09	1.22±0.11	1.13±0.09
Atherogenicity index	0.55±0.07 *p*=0.002	0.88±0.08*	0.84±0.05	0.801±0.070 *p*=0.01	0.62±0.05⁺	0.82±0.07	1.07±0.17

Note. Significant differences: *compared to Wistar rats; ⁺compared to the control.

However, there are no objective methods for evaluation of the efficiency of blueberry during the therapy and prevention of cataract. Our results show that long-term preventive treatment with blueberry extracts decreases cataract morbidity rate in OXYS rats and normalized the concentration of LPO products in the blood. Moreover, the preparation manufactured in Russia normalizes the index of lipid atherogenicity. These effects are probably related to high antiradical activity of blueberry flavonoids [7,8] and ability of these compounds to prevent age-related mitochondrial dysfunction in OXYS rats (most probable cause of preterm aging) [4]. The data show that antioxidants have different efficiency in preventing the development of cataract in OXYS rats. These rats can be used to evaluate anticataract activity of medicines and develop new approaches to the prevention and therapy of a disease.

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