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ANTIOXIDATIVE ACTION OF ANTICATARACT REMEDIES

L. V. Chasovnikova, V. E. Formazyuk,
V. I. Sergienko, and Yu. A. Vladimirov

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One possible trigger mechanism leading to the development of senile cataract, in the modern view [4], is destruction of components of the lens by active forms of oxygen. Consequently, their antioxidative action may be an important aspect of the pharmacologic activity of anticataract agents.

The aim of this investigation was to determine whether the anticataract remedies widely used at the present time in ophthalmology, and certain other drugs which have not yet achieved popularity, are in fact antioxidants. Oxidative processes can develop directly in the lens, and they may also involve components of the aqueous humor which bathes the lens. Quantity of aqueous humor in the eyes of animals is insufficient for the study of antioxidative properties of anticataract agents. For that reason rat serum, which contained high-density lipoproteins and fatty acids in the form of complexes with serum albumin [6], which are lipid-containing components common to both fluids, was used as the test object.

EXPERIMENTAL METHOD

The reaction system in which control oxidation of the rat serum was carried out consisted of 10 ml of buffer (0.105 M KCl, 0.02 M KH_2PO_4, pH 7.45), 0.4 ml of serum, and 1 ml of 0.05 M $FeSO_4$. The reaction was carried out at room temperature with constant mixing. To determine the antioxidative activity of the therapeutic preparation it was added to the reaction mixture in an amount sufficient to reveal the range of concentrations in which a change in the antioxidative activity of the preparation takes place from maximal effect of complete suppression of the reaction to absence of effect. After fixed time intervals samples of 0.2 ml were taken and mixed with 0.8 ml of 30% TCA. After centrifugation (20 min, 10,000g) 0.8 ml of supernatant was mixed with a 0.5% solution of 2-thiobarbituric acid (TBA) and kept for 1 h at 90-95°C. The level of TBA-active products was determined on a "Hitachi-323" spectrophotometer (Japan) at the absorption maximum of 534 nm [1]. The quantity of total lipids was measured as in [11].

EXPERIMENTAL RESULTS

In the first stage of the work oxidation of rat serum, induced by ferrous ions, was studied. For this purpose, the rate of accumulation of TBA-active products in the reaction system was measured for 2 h. It was found that the kinetic curves characterizing sera of different animals differ very substantially (Fig. 1), as a result of differences in the state of activity of the antioxidative system in the blood of these animals. Characteristically, the wide scatter of the experimental data did not correlate with the very small fluctuations in the content of total lipids, which we determined in parallel tests in the sera of these animals. The total lipid content was 3.5 $\pm$ 0.4 mg/ml. Curves characterizing accumulation of TBA-active products for the same serum were found to remain very close together for 5-7

Research Institute of Physicochemical Medicine, Ministry of Health of the RSFSR, Moscow.
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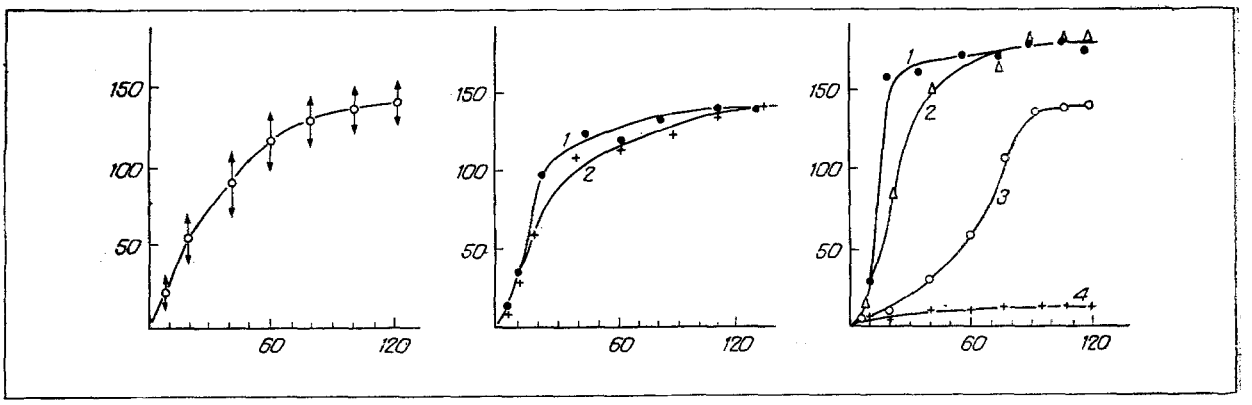

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Kinetics of accumulation of TBA-active products during oxidation of rat serum. Number of tests: 15. Here and in Figs. 2 and 3: abscissa, duration of incubation of sample (in min); ordinate, concentration of TBA-active products (in mM).

Fig. 2. Kinetics of accumulation of TBA-active products during oxidation of serum depending on its keeping time. 1) On day of taking blood; 2) the same serum 7 days later.

Fig. 3. Kinetics of accumulation of TBA-active products in presence of different concentrations of acetylsalicylic acid. 1) Control, 2) 1:50, 3) 1:20, 4) 1:10.

days (Fig. 2). For that reason, after having recorded the control kinetic curve for serum, we worked with it for 2-4 days and compared the results of testing the drugs (i.e., their ability to block the oxidative action of $FeSO_4$) with that curve alone. For example, Fig. 3 gives a series of kinetic curves characterizing the antioxidative action of acetylsalicylic acid together with the control curve. All curves, it will be seen, exhibit saturation, which is determined by the greatest depth of oxidation which is possible with the given initial relationship between the components. For the control experiment, when the preparation was absent from the system, the degree of inhibition of the reaction was considered to be zero. The experimental data are expressed as percentages of inhibition of the reaction by the drug relative to the control.

The experimental results are given in Table 1. The anticataract preparations such as senkatalin, vitaiodurol, etc., were used without further dilution, acetylsalicyclic acid was used in a concentration of 0.2 mg/ml, carnosine – 2.5 mg/ml, and α-tocopherol – 5% (in oil). α-Tocopherol was used as the control antioxidant, its antioxidative effect being closely similar to that described in [8]. As Table 1 shows, the antioxidative activity of the drugs, which have already achieved widespread popularity worldwide, differs very greatly. Senkatalin, for instance, exhibited quite a strong antioxidative action. Vitaiodurol and quinax in general were without this property, and katachrom occupied an intermediate position between them. Acetylsalicyclic acid, the positive effect of which in cataract has been questioned in the majority of publications [9, 10], is a fairly strong antioxidant. Its antioxidative properties were studied previously [2, 3], and we merely confirmed this fact once again. The preparation Baineiting (produced in China), whose anticataract action has proved to be quite strong, also is a powerful antioxidant. We confirm the anticataract effect of this drug by means of a test in vitro [7], based on inhibition of opacity of rat lenses in the presence of the drug. Judging by this test, the therapeutic effect of this drug is comparable with that of senkatalin.

The antioxidative action of components of senkatalin and Baineiting, namely the solvent, included in the pack with the drug, and tablets which, in this case, were dissolved in distilled water, the volume of which corresponded to the volume of the solvent, was tested separately. In both cases the antioxidative effect of the preparations could be completely ascribed to the substances included in the tablets. Carnosine (alanyl-histidine dipeptide), whose anticataract effect is ascribed to its antioxidative properties [5], is a not very powerful antioxidant in the concentration recommended for use by patients (comparable with katachrom).

TABLE 1. Antioxidative Effect of Anticataract Drugs

Drug	Quantity of drug in reaction mixture	Dilution	Degree of inhibition, %
Senkatalin	1 ml	1:10	95
»	0,5 ml	1:20	90
»	0,3 ml	1:33	30
»	0,1 ml	1:100	0
Solvent of senkatalin	1 ml	1:10	0
Senkatalin tablets in water	1 ml	1:10	90
» » »	0,5 ml	1:20	80
» » »	0,3 ml	1:33	40
» » »	0,1 ml	1:100	0
Katachrom	1,5 ml	1:7	45
»	1 ml	1:10	38
»	0,5 ml	1:20	28
»	0,2 ml	1:50	12
Acetylsalicylic acid	1 ml	1:10	95
» »	0,5 ml	1:20	25
» »	0,2 ml	1:50	0
Quinax	1 ml	1:10	0
Baineiting	1 ml	1:10	95
»	0,5 ml	1:20	85
»	0,3 ml	1:33	27
»	0,1 ml	1:100	18
Solvent of Baineiting	1 ml	1:10	0
Tablets of Baineting in water	1 ml	1:10	90
Carnosine	1 ml	1:10	60
»	0,5 ml	1:20	45
»	0,2 ml	1:50	20
α -Tocopherol	25 µl	1:400	90
»	15 µl	1:660	75
»	5 µl	1:2000	65
»	2 µl	1:5000	50

The therapeutic effect of anticataract drugs used at the present time in ophthalmology is thus determined not only by the antioxidative properties of the drugs. It is possible that this property may partially explain the therapeutic action of drugs such as senkatalin, katachrom, and certain others. Meanwhile, there exists a group of drugs whose therapeutic effect in general is unconnected with the presence of such properties (quinax, vitaiodurol).

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