The Effect of Ethanol Intoxication on the Spectral Characteristics for Blood Components of White Rats

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Abstract

The present paper is devoted to studying, with the aid of different organic dyes, the transmittance spectra of hemoglobin and immunoglobulin G extracted from the blood of laboratory rats, which have been chronically intoxicated with ethanol. The differences in the spectra are detected, when compare with those for the control group. It is shown that the presence of ethanol in blood probably leads to uncoiling partially the hemoglobin molecules. The essential difference is also found in the transmission spectra of immunoglobulin-dye solution prepared from the blood of the control-group animals and those of the animals of the first generation. The IgG of blood plasma of the first-generation rats probably includes a larger quantity of free remains, which could couple with the intoxicated agents. The small difference between the IgG-dye solution spectra for the animals given to drink ethanol during 6 months and the control-group animals is quite possibly referred to decreasing the IgG concentration after a durable ethanol intake.

Key words: transmission spectra, alcohol intoxication, laboratory rats, hemoglobin, immunoglobulin G.

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Introduction

The toxic effects of ethanol have been connected with irregularity of neuro-humoral and immunity systems, which is determined by the changes in functional properties of biopolymers [1]. Let us remind that the ethanol impairs significantly the deformability of erythrocytes and decreases their filterability [2]. From these points of view, it is reasonable to consider the toxic influence of ethanol as the ability to modulate the conformational state of blood erythrocytes and plasma proteins. In general, the blood is a heterogeneous system able to store the information about the processes passing in the organism. However, the mechanisms of protein interaction with the ethanol have not been clear up to now. The presence of ethanol in the blood leads to the changes in polarizability of plasma molecules. On the other side, it is difficult to make the direct measurements of these changes [3]. Usually, different dyes are used for this, which are dissolved with the proteins. After dissolving, the non-occupied remains of proteins are bound with dyes. As a result, one can come to qualitative conclusions about the free protein remains. In case of studies of the hemoglobin extracted from the blood of ethanol-intoxicated group (IG) of animals and those of the control group (CG), it seems to be possible to compare

the influence of different dye adding while using the optical transmittance spectra. For example, the "bromthymol blue" dye [4] adding to hemoglobin could affect the spectra via the change in the conformation level induced by ethanol in this hemoglobin, which should have been invisible without the dye adding. The "cibacron blue" (F3GA) dye adding could induce the conformational change of the IgG [5]. The present work represents a further development of our previous studies [6].

Experimental

The experimental studies were carried out on the white rats (females) bred with the average weight of 200g. The rats of the IG were placed into individual cages under the condition of a free access of 15% water solution of ethanol. The volume of the used liquid and the weight of animals were controlled daily.

After the 10-day test, the rats with a clear alcohol motivation (average daily usage of ethanol being 7.1g/kg of weight) were placed into separate cages for the further forcible alcohol intoxication. The blood sampling from the tail vein was performed for the further investigations. The IG group of animals was divided into three subgroups: six months of ethanol consumption, three months of ethanol consumption (one month of withdrawal), and the first generation (three-month age and ethanol consumption). The rats were copulated in the age of four months and after a month of ethanol consumption. The intake of the ethanol continued during the gestation period in females. In order to have the reference data, the blood of the CG-animals, without any ethanol intoxication, was also studied. The heparin was used as anticoagulant. After the centrifugation of the whole heparinizated blood, the supernatant (plasma) was separated. The red blood cell hemolization was accomplished with the 30mM phosphate buffer (pH 7.36). The hemolisate was used for the optical transmittance studies.

The light transmission spectra were measured in the spectral range of 0.4-0.8μm. To study the conformational changes of IgG and Hb, we used respectively the cibacron blue (0.1M of "cibacron blue" dissolved in 0.1M acetate buffer; pH 4.8) and the "bromthymol blue" (10^{-5}M) dyes.

Results and discussion

The transmission spectra of the hemolisates are presented in Figure 1 for the three groups of animals (that of six-month ethanol consumption, the CG and the first generation (three-month-old)). As anticipated, no essential differences are observed in these spectra. Figure 2 represents the transmission spectra of the hemolisate-dye

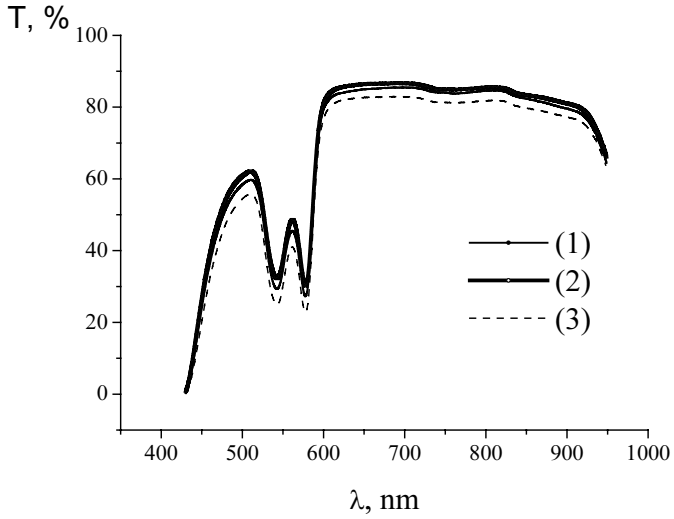

Fig. 1. Optical transmission spectra of the hemolisates of blood erythrocytes of the white rats: (1) the case of six-month ethanol consumption, (2) the CG, and (3) the first generation (three-month age).

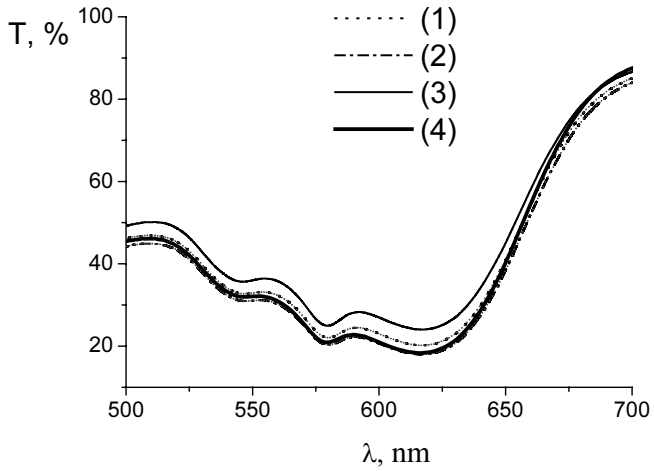

Fig. 2. Optical transmission spectra of the hemoglobin-dye ("bromthemol blue"): curve 1 – the first generation (three-month age), 2 –three-month ethanol consumption and one-month withdrawal, 3 – six-month ethanol consumption and 4 – the CG.

("bromthymol blue") solution for the cases of hemolisates extracted from the blood of the four groups of animals: (1) the first generation (the age of three months), (2) the group referred to three-month ethanol consumption combined with the one-month withdrawal, (3) the group referred to six-month ethanol consumption, and (4) the CG. As seen from Figure 2, there are no apparent differences in the light transmittances of the mentioned solutions for the CG (4) and the group (1), as well as for the CG (4) and the group (2). However, there is a pronounced difference in the transmittances for the CG (4) and the IG (3). The transmission at 615nm (i.e., the absorption line of "cibacron blue") in the CG spectrum is less than that in the spectrum of the IG (3) hemolisate. It means that for the CG there is a less number of binding sites on the surface of the globules with the dye probe. It is obvious that the difference in bonding should be related to the changes in the native conformation of hemoglobin. Quite probably, the presence of ethanol in the blood would lead to partial uncoiling of the hemoglobin molecules. This is also readily understood if one reminds a protective function of organism – ensuring the conditions for a free access of oxygen molecules to the hem for binding the iron ions.

Figure 3 shows the transmission spectra of the immunoglobulin-dye ("cibacron blue") solution prepared from the blood taken from rats of the following groups: (1) the first generation (the age of three months), (2) the CG, (3) the IG related to three-month ethanol consumption and the further one-month withdrawal, and (4) the IG related to six-month ethanol consumption. The "cibacron blue" dye has been used as a particular dye probe for binding the IgG. As seen from Figure 3, the transmission spectra differ for CG (2) and the group (4). Moreover, there exists an essential difference in the spectra of solutions prepared from the blood taken from the CG (2) and the group (1). It is clear that the IgG of the first-generation rats includes a larger quantity of free remains, the latter being ready for coupling with the acetaldehyde intoxicated agents. It is quite possible that, similarly to hemoglobin [7], the immunoglobulin can be modified by the acetaldehydes. The small difference of the IgG-dye spectra for the group intoxicated for six months and the CG is quite possibly attributed to decrease in the IgG concentration after a durable ethanol intake.

Conclusions

Basing on the results of the present study, one can conclude that the presence of ethanol in the blood may quite probably lead to partial uncoiling of the hemoglobin molecules. It also looks reasonable from the point of view of the protective function of organism, which should

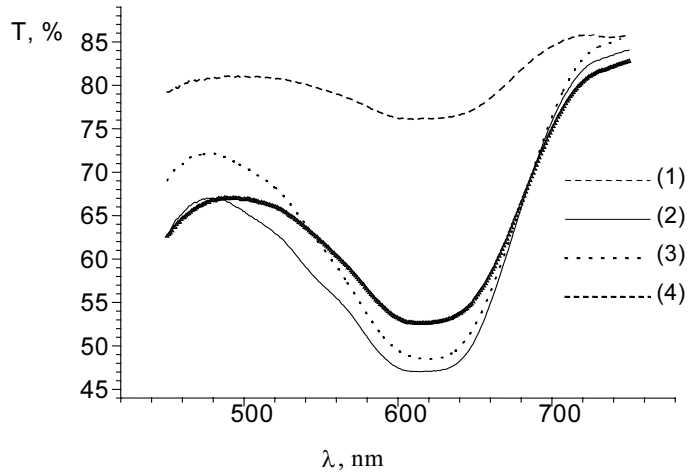

Fig. 3. Optical transmission spectra of the immunoglobulin-dye ("cibacron blue"): curve 1 – the first generation (three-month age), 2 – the CG, 3 –three-month ethanol consumption and one-month withdrawal, 4 – six-month ethanol consumption.

provide the conditions for a more free access of the oxygen molecules to the hem in order to bind the iron ions.

We have also found that there exists essential difference between the spectrum of IgG-dye solution prepared from the blood of the CG animals and that of the animals of the first generation. It is clear that the IgG of the first-generation rats should possess a larger quantity of free remains, which could couple with the intoxicated agents. A slight difference in the spectra of IgG-dye solutions prepared from the blood of animals having consumed ethanol during six months and the CG rats is quite possibly concerned with decreasing the IgG concentration after a durable ethanol intake.

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