Spectroscopic Study of Haemoglobin Ligand Forms and Erythrocyte Membrane Dynamics at Alcohol Intoxication of White Rats

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Received: 31.03.2004

Abstract

The results of spectroscopic studies are presented for desoxy-, oxy-, carboxy-, sulf- and methhaemoglobins of white rats and their posterity under the conditions of a durable (1 to 6 months) alcohol intoxication. The changes in the dynamics of oxy- and methhaemoglobin are found for the animals having consumed 15%-ethanol during 1-4 months. It is also found that structural and functional properties of erythrocyte membranes have changed for the white rats that consumed ethanol during 4-6 months. The increased sensitivity of erythrocytes to the action of haemolytic agents is detected for the posterity of alcoholized parents. The obtained results show that alcohol intoxication influences not only the structural and functional properties of periphery blood erythrocytes but also the nemogenous system.

PACS: 42.66.-p, 33.55.-b

Key words: spectroscopy, haemoglobin, ligand forms, erythrocytes, alcoholism

Alcoholism is a part of complicated and broad medical and social problem of psychic and somatic disorders associated with the alcohol usage. In connection with a wide spread of alcoholism and the related psychosomatic disorders, the knowledge about biochemical mechanisms of its formation and development is quite important, at least from the viewpoint of its after-effect prediction and the ways for its prevention [1]. One of the most important stages of alcoholism is hypoxia that appears as a result of perturbation of external breathing, as well as oxygen transportation and its assimilation by tissues. It is known that pathological phenomena that accompany alcoholism are concerned with acetaldehydes, which originate from ethanol oxygenation. The acetaldehyde can interact with proteins and enzymes and induces their modification. Non-enzyme protein modification leads to disturbance of protein conformation and, as a result, a disturbance of its function. It is also known that acetaldehyde can create complexes with the blood serum albumin, the modified iron transferin-mediator and haemoglobin-oxygen-transportation protein [1-3].

The acetaldehyde can have indirect effects, e.g., stimulating the peroxide lipid oxidation [4]. Owing to the peroxide lipid oxidation, we have the increased level of active metabolites, such as mulonic dialdehyde, as well as the other aldehydes and cetons that manifest cytotoxic influence and are actively modifying agents [5,6]. These processes induce hyper-production of oxygen in the mitochondria and creation of strong oxidizer, the super oxide anion (O_2^-).

The latter participates in oxygenation of fatty acids, the components of phospholipids.

Thus, the ethanol, when having entered into the organism, causes, directly or indirectly, a gorge of secondary metabolites which induce a disturbance of cytoskeletons of cells, membranes of cell organelles, modify biologically important macromolecules and change their functions [3,7].

The aim of the present work consists in studying comparatively the structural state of erythrocyte membranes for the blood sampled from laboratory rats that have been durably intoxicated with ethanol, as well as the spectroscopic studies for the haemoglobin and its ligands.

Materials and methods

The white outbred female and male rats with the average weight of 200-300g were used in the experiments, together with their posterity having the weight of 150-170g. The rats were separated with the method of inclination to ethanol usage. For the creation of ethanol dependence, the rats were forced to drink a 15% water solution of ethanol, instead of water. The rats where separated into 7 groups – a control group and six experimental groups joining the animals that consumed ethanol during 1-6 months. The posterity rats (F_1) originated from alcoholized rats also consumed 15% ethanol solution. The blood was taken from the tail vein or after decapitating the animals.

The rate of desoxy-, oxy-, carboxy-, sulf- and methforms of haemoglobin was determined in hemolysates of the whole blood (Tabl. 1). The hemolysation process was carried with K-, Na-phosphate buffer at pH 7.36, 3mM. The absorption spectra were recorded with the aid of spectrophotometer "Specord M-40" in the spectral range of 400-700nm. The calculations were performed for the five wavelengths, using the relative values of the absorption coefficients [8].

Stability of erythrocyte membranes was studied with the method of acid erythrograms by *Terskov and Hitelzon* [9]. Erythrocytes were separated from the whole blood plasma with centrifugation (2000 rpm) during five minutes. The erythrocyte suspension was wetted three times with 0.9% solution of NaCl. The hemolysation was done at 0.004 HCl. The measurements were conducted with photoelectrical calorimeter KFK-3. The erythrograms were plotted using the results of those measurements.

Table 1
The hemoglobin ligand forms dynamic (%).

	Ligand forms				
	RHb	HbO2	HbCO	SHb	MetHb
Control group (6 rats)	0.01±0.005	93.24±0.2.47	2.06±1.12	3.22±3.03	1.47±1.75
Intoxicated group 1month.(3 rats)	0.01±0.004	87.14±1,47	2.66±1.05	5.42±0.49	4.76±0.23
Intoxicated group 2month (7 rats)	0.01±0,004	88.35±2,70	2.78±0.77	4.20±0.81	4.88±0.89
Intoxicated group 4month (4 rats)	0.01±0.003	90.87±1,12	3.33±0.35	2.30±0.55	3.46±0.50
Intoxicated group 5 month (3 rats)	0.01±0.005	97.08±0,82	2.10±0.07	0.01±0.01	0.81±0.80
Intoxicated group 6 month (3 rats)	0.025±0.005	92.66±2.92	3.4±0.19	3.28±1.21	1.65±1.40
F1 (6 rats)	0.01±0.005	93.15±2.33	4.14±0.22	1.45±1.36	1.06±0.21

Results and discussion

Since the processes in organs and tissues feeding with oxygen are linked with both the dynamics of haemoglobin saturation by oxygen and the erythrocyte stability, the studies of physical and chemical properties of erythrocyte membranes acquire especial importance. It is known that the alcohol intoxication is accompanied with the activation of free-radical lipid oxygenation, i.e. the components of erythrocyte membranes [7,10]. This should reflect itself in the stability, functionality and life duration of the erythrocytes.

We have studied the stability of erythrocyte membranes using the acid erythrogram method. Our data show that the maximum hemolysation of blood for the control group of rats occurs at 5.1±0.1 min (see Table 1 and Fig. 1).

According to the obtained results (Table 1), the consumption of ethanol during the first 4 months leads to decreasing oxyhaemoglobin content and increasing methhaemoglobin content in the blood of rats. After 5-6-month alcohol intake, the values of those parameters return to their normal level. Thus, one can conclude that the oxidation of hem iron and the increase in the methhaemoglobin can be a result of oxidation-reduction reactions induced by the ethanol metabolites. Normalization of the ligand form rate for the rats given to a more durable ethanol intake and even for those of the next generation is associated, most probably, with the activation of enzyme systems (acetaldehyde dehydrogenase, etc.), participating in decreasing the level of acetaldehyde. It is, probably, at this stage that the mechanisms for adaptation to alcohol intake activate [10-13].

The studies for hemolysation hardness may provide some information on the stability of erythrocytes. We have found that the maximum hemolysation of blood for the control group rats occurs at 5.1±0.1 min (see Table.2 and Fig. 1). The corresponding time parameter for the group including the rats that consumed ethanol during 1-3 months is located in the same range, while the duration of total hemolysation is somewhat longer. In case of the rats that consumed ethanol during 4-6 months, the time of maximum hemolysation and the time for total hemolysation are essentially reduced (from 4.5±0.2min down to 2.3±0.2min). At the same time, a fast destruction of erythrocytes has been

Table 2. The erythrogram parameters of blood for the rats of the control group and those intozicated with the ethanol (15% C_2H_5OH)

Groups of animals according to durability of alcohol consumption	Number of animals	Maximum hemolysation, min	Total hemolysation, min	Maximum hemolysation, %
Control group	6	5.1+0.1	8.1 ±0.2	38,52±1,90
Durable consumption of ethanol				
1 month	5	5.1+0.2	9.4±0.6	36,05±3.10
2 months	3	5.5±0.5	9.7±0.7	31.94±3.50
3 months	3	5.0±0.1	8. 2±1.3	37.30±3.70
4 months	3	4.5 ±0.2	7,3 ±0.7	39.06±1,30
5 months	3	2.3 ±0.2	6,5 ±0.7	35.71 ±0.80
6 months	4	4.6±0.3	8.9±0.6	21.37±4.0
The next generation (F_1)				
F_1 - 4 months [1]		3.25±0.10	8.9±0.4	20.90±2.4
F_1 - 4 months [2]		3.40±0.20	7.7±0.5	24.09±3.3
F_1 - 6 months [3]		3.2±0.2	9.00±0.7	18.83±2.2

[1] Posterity of the parents that consumed ethanol during 2 months
[2] Posterity of the parents that consumed ethanol during 3 months
[3] Posterity of the parents that consumed ethanol during 2 months

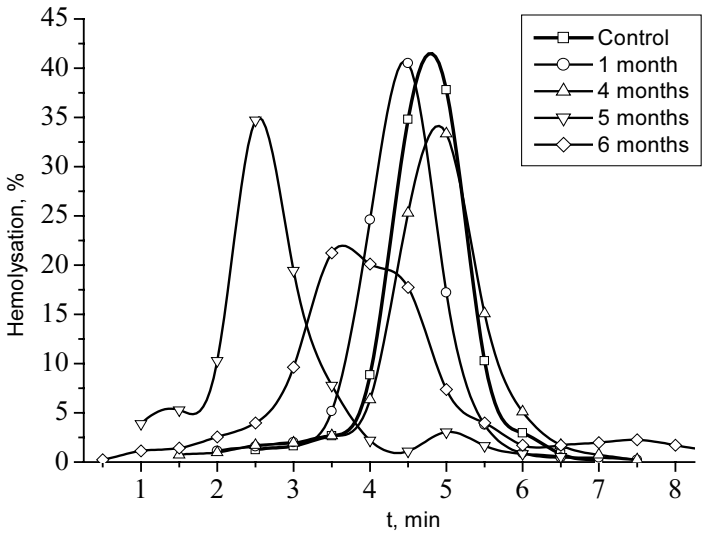

Fig. 1. Erythrograms of blood for the control group of rats and the rats that consumed ethanol during a long time.

observed for the next generation of rats that consumed alcohol during 4-6 months (3.2±0.2min), while the total hemolysation takes quite long time (8.4-9.8 min).

Conclusions

It follows from the obtained results that the prolonged intake of ethanol leads to decrease in the erythrocyte life duration and increasing inhomogeneity of their population. Hence, one can draw the conclusion that the alcohol intoxication induces a disturbance of both structural and functional states of erythrocytes, as well as the oxygen-transportation function of haemoglobin.

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