



Short Communication

Electrochemically Activated Water and Its Impact in Severe Alcohol Intoxication

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Abstract

Introduction: Currently there is no specific antidote in cases of poisoning by alcohol. Therefore, is always searching for new means and methods for the treatment of alcohol poisoning. The combination of the pharmacological properties of electrochemically ionized fluid with a negative oxidation- reduction potential (ORP) allows predicting its therapeutic effect by alcohol poisoning.

Objectives: modeled acute alcohol poisoning in rats, treatment was carried out with a liquid with a negative redox potential. Investigated the behavioral activity in the test "open field" and morphological study of the liver. In rabbits, recorded heart rate and respiratory movement's intravenous administration of isotonic liquids with negative ORP.

Results: electrochemically activated water infusions to intact animals do not result in violations of electrical heart activity, increase emotional- locomotor activity and do not alter behavioral reactions. Addition of alcohol to ionized liquid leads to gradual increase of its ORP that might be a pathogenic factor for a living body. Only 20 out of 60 male- rats appeared to be alcohol-dependant in simulation of alcoholization. Volume of the consumed alcohol was more in its combination with water and less in its combination with water with negative redox potential; thus, use of it restrains alcohol intake in alcohol-dependant rats. The water with negative ORP decreases horizontal locomotor activity and emotional reactions in alcohol-dependent rats.

Conclusion: The reception of the electrochemically ionized fluid with a negative ORP therapeutic effect in the simulation of alcohol poisoning in rats. Intravenous electrochemically ionized fluid with a negative ORP and does not cause adverse reactions of cardiovascular and respiratory system.

Keywords

Ethyl alcohol; Ethanol; Ionized liquid; Redox potential; Physical parameters of drinking water; Liver, Central nervous system (CNS)

Introduction

Alcohol dependence is considered to be a severe progredient disease on the basis of pathological alcohol intake with the development of alcohol withdrawal syndrome. People abusing alcohols suffer from a wide range of disorders, liver damage being one of them, and behave a

socially. Therefore, search of means and methods reducing alcoholization of population and decreasing toxic impact of ethyl alcohol on a body appears to be an acute issue nowadays.

Ionized liquid with negative redox potential has antioxidant activity, capacity to accelerate tissue regeneration and improve immunity index [1]. Low toxicity of this fluid has been already proved [2]. These properties allow assuming a positive effect of ionized liquid with negative redox potential on the background of high alcohol doses.

The aim of the study was to determine an opportunity of positive effect of ionized liquid with negative redox potential in animals intoxicated by ethyl alcohol.

Materials and Methods

72 male and female white rats weighed 180-220 g and 30 male rabbits weighed 2900-3200 g were included into experiments.

The study design and maintenance of animals followed rules and regulations of laboratory practice and were performed in accordance with guidelines on experimental studies [3-5], the Order of the Ministry of Health and Social Development of the Russian Federation, August 23, 2010, No. 708n "About the approval of laboratory practice rules" (GLP) and the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Guidelines for accommodation and care of animals) (ETSN 124, Strasbourg, June 22, 1998).

Fresh ionized water with pre-determined parameters of oxidation reduction potential (ORP) and pH prepared immediately before each investigation was used in the study; the parameters were as follows: a catholyte with pH equal 8.3-9.0, ORP minus 480-510mV. An electrolyser "Karat" (model 40) (produced by OOO "SEL" – Russian abbreviation, - Voronezh, Russia, certificate of conformity №.POCC RU АЯ60.В21242№001338, Ministry of Healthcare of the Russian Federation) was used for this purpose. ORP value and liquid pH were estimated with a certified pH-meter and an ionometer "pH-150MI".

Formation of highly active de oxidants, such as OH^- , H_3O_2^- , $\text{H}_2\text{HO}_2^\bullet$, HO_2^- , O_2^- , takes place on a cathode. Due to cathode treatment water acquires alkali reaction, redox potential reduces up to minus 500 mV and more, surface tension and the amount of dissolved oxygen and nitrogen decrease, concentration of hydrogen and free hydroxyl groups rises, conductivity decreases, the structure of ion hydration shells and free water volume change [6].

To understand interaction of ionized liquid with negative ORP and water it is necessary to consider alterations of the ORP catholyte value when diluting a catholyte with alcohol. Alcohol was diluted with a catholyte in the ratios 1:100, 2:100, 3:100, 4:100 and 5:100. ORP was estimated in the obtained samples right after mixing and in an hour.

Study of the cardio-vascular system reactions to intravenous introduction of ionized liquid with negative ORP in the maximum permissible volumes was performed in 20 laboratory male and female chinchilla rabbits. Animals were divided into 2 groups: group I – control (isotonic solution NaCl 0.9%), group II – experimental (catholyte). Electrocardiogram findings and respiratory rates were registered in the experiment.

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General status, locomotor activity, reflexes and behavior were assessed when examining the cardiovascular system reactions to intravenous introduction of the investigated liquid in laboratory animals; respiratory rates (RR) and electrocardiogram (ECG) findings were registered using an electrocardiograph EKG 1T – 03M. RR and ECG findings were registered before the liquid introduction and 30 minutes, 60 minutes and 90 minutes after the liquid introduction.

Stimulation of acute alcohol intoxication was performed in 30 rats weighed 250 ± 20 g. Animals were divided into 3 groups, 10 species in each group. Group I – control (animals were introduced water for injections intravenously), group II – experimental (animals were introduced a catholyte). In the study (20 rats) acute alcohol intoxication was simulated by intraperitoneal introduction of 33% ethyl alcohol, dosage 0.8 LD₅₀ [7]. Injections of the investigated liquid were carried out in 30 minutes after the introduction of a challenging dose of ethyl alcohol. Animals were successfully observed for 7 days. General status of rats, their general behavioral reactions, locomotor activity, RR, papillary response were monitored and assessed over the whole period of the experiment. Based on these data the severity of alcohol intoxication was estimated; psycho-emotional status of rats was determined using an “open field” test.

In the period of formation of alcohol motivation 64 male rats were kept in usual conditions and had a free access to two bowls, one of them containing 20% ethanol solution, another – 10% glucose solution. Formation of experimental alcoholism was supported by the developing of the following criteria:

- 1) an individual volume of ingested alcohol per time unit constituted no less than 5 g/kg a day;
- 2) Percentage ratio of ingested alcohol to the total liquid volume was no less than 60%.

After estimating the initial level of water and alcohol intake animals were divided into two groups: alcohol-dependant and alcohol-intact. To study qualitative and quantitative alcohol motivation alcohol-dependant rats were offered to choose 10% glucose solution or 20% ethanol solution exposed for 60 minutes in specifically graduated drinking bowls. The amount of alcohol and water was estimated on the graduation of bowls before the animals were placed in individual cages and an hour after. On the completion of an individual test the body mass of animals was estimated; the volume of ingested liquid was given as the amount expressed by g/kg in an hour. Analysis of the research results performed by the authors demonstrated that only 20 out of 60 animals were alcohol-dependant. To study the impact of a catholyte on the developed alcohol motivation animals were divided into two groups: group I (n=10) had a free access to 20% ethanol solution and water; water was replaced by a catholyte in group II (n=10). Animals were kept in these conditions for two weeks; concurrently the evaluation of the liquid consumption in the conditions of free access to alcohol was carried out daily. Effect of electro activated water solutions on the exploratory activity of experimental animals in an “open field” test was studied in 21 male rats, which were divided into 3 groups. Group I, control, included 6 alcohol-intact rats; group II consisted of 8 alcohol-dependant animals demonstrating the regimen of alcohol preference; group III included 7 animals randomly chosen from experimental rats ingesting electroactivated water solution of a catholyte.

Morphometry of histological preparations was conducted using the computerized video-test-system consisting of a microscope

«Leica DME», a digital camera «Leica EZ3» and special software for obtaining and processing the morphometric data Leica Las EZ 2.0.0, MMC Multimeter (MMCSof), и Axio Vision (Carl Zeiss) и «Mecos-1».

Research results were statistically processed on PC Pentium V using software programs Excel 2010 (with XLSTAT-Pro - <http://www.xlstat.com>), Statistica 6.1, and parametric and non-parametric criteria [8].

Results

The authors obtained results of biological reactions of the cardiovascular system when intravenously injecting the liquid with negative ORP in experimental animals. In 30 minutes intravenous injection of the maximum permissible volume of the liquid with negative ORP resulted in bradypnoea by 28% ($p < 0.05$) in rabbits. In 90 minutes RR finding returned to the initial value in experimental groups, whereas in the control group RR value raised by 24% in comparison with the initial value ($p < 0.05$). RR and ECG findings did not practically alter during the whole period of observation when introducing the ionized liquid with negative ORP.

General status, locomotor activity, reflexes, behavior of animals was usual and no changes were registered in comparison with the initial level.

The research results obtained prove that in the group of intact-rats, who were introduced a catholyte, horizontal locomotor activity (a “cage” finding) has increased in 7 times by the end of the experiment; the maximum increase of activity was observed on the first day (in 20 times). Vertical locomotor activity has also increased by the end of the experiment: “climbing” – in 1.5 times, “rearing” – in 1.5 times, “grooming” – in 5.5 times. The maximal increase of activity was observed for “climbing” in 3 times on the first day, for “grooming” in 30 times on the fifth day, for “rearing” in 3 times on the fifth day. Therefore, introduction of a catholyte results in a mild stimulating effect in intact animals.

Experiments with dilutions were carried out to study an opportunity to alter body fluids under the influence of alcohol. Dilution of ethyl alcohol with a catholyte in the proportions 1:100-1:400 resulted in gradual increase of ORP by 15 ± 8 mV in average ($p < 0.05$); ORP increased by 46% when diluting ethyl alcohol with a catholyte in the proportion 5:100 ($p < 0.05$). No significant changes of parameters were observed when storing catholyte samples containing alcohol for an hour in comparison with the initials catholyte parameters. However, ORP tended to increase due to the increased amount of added alcohol. Thus, alcohol in small proportions 1:100-3:100 slightly influenced parameters of ionized liquids with various ORP, but accelerated processes of solutions relaxation; this fact was supported by the increased ORP of ionized liquids containing alcohol after a certain period of time.

The level of consumption of alcohol-containing solution by alcohol-dependent animals constituted is 5.57 g/kg during 24 hours in average as a result of 60-minute-stay in the drinking camera in the conditions of functional rest. On the background of catholyte intake animals of group II also being alcohol-dependant reduced alcohol consumption that constituted 4.2 g/kg during 24 hours in average, i.e. 24.6% less.

To more fully appreciate a possible effect of electroactivated water solution of a catholyte on the status of alcoholized animals the authors attempted to monitor the resulted exploratory behavior of animals.

The impact of a catholyte on the level of the expressed emotional reactions of fear and anxiety, exploratory and locomotor activity of experimental animals was evaluated in the “elevated plus maze” test. 30 2-month male rats weighed 180-220 g were divided into 3 groups: group I (control) consisted of 10 alcohol-intact rats; group II included 10 alcohol-dependent animals demonstrating the regimen of alcohol preference; group III included animals having free choice of 20% alcohol solution and a catholyte.

The time spent in the closed/ open arms and the central zone, as well as the number of closed- and open-arm entries, frequency of looking into and out of the cameras (exploratory activity) and standing on the hind legs in the field (rearing), the number of grooming acts and defecations were taken into account when performing the test.

During this test rats from the group III (free choice) choosing alcohol solution demonstrated reduced time of stay in the closed arms by 19.7% ($p < 0.01$) in comparison with the control group of alcohol-intact rats; this fact proved anxiolytic effect of ethanol. In addition, the time of stay in the central zone and open arms increased in 9.4 ($p < 0.01$) and 5.8 ($p < 0.01$) times, respectively. The number of entries into all sections of a maze also increased; that supported increased locomotor activity under the influence of ethanol solution. Thus, the number of the closed-arm entries increased in 2.4 times ($p < 0.01$), the central zone entries – in 5.6 times ($p < 0.01$), the open-arm entries – in 7 times ($p < 0.01$). The increased number of “looking into” (in 6.5 times ($p < 0.05$)) and “rearing” (in 16.7 times ($p < 0.01$)) revealed when testing the animals of group II proved anxiolytic effect of ethanol in comparison with lack of such an effect in alcohol-intact animals. Moreover, in animals of group III irregular locomotor activity significantly decreased and their exploratory activity became more regular.

Study of behavioral reactions of animals after introduction of ionized liquids with negative ORP on the background of experimental acute alcohol intoxication using “open field” test demonstrated decreased effect of alcohol intoxication and improvement of the central nervous system activity. More expressed action and significantly increased level of emotional, horizontal locomotor activity and exploratory activity was estimated in case of catholyte introduction in comparison with decreased findings in alcoholized animals.

Hepatic alterations morphologically classified as “hepatosis” resulted from simulation of chronic alcohol hepatitis; expected infiltration, as well as inflammation, were not revealed but were expressed in dystrophy of various degrees in the form of alcoholic hepatosis (adipose degeneration, signs of albuminous degeneration). Morphological analysis of the liver of animals receiving infusions of ionized liquid with negative ORP demonstrated decrease of damaging impact of alcohol in the model of chronic alcoholic hepatitis; in addition there was proved its hepato-protective effect.

Conclusion

Therefore, the conclusion can be made:

1. Catholyte infusions to intact animals do not result in violations of electrical heart activity, increase emotional-loco-motor activity and do not alter behavioral reactions.
2. Addition of alcohol to ionized liquid leads to gradual increase of its ORP that might be a pathogenic factor for a living body.

3. Only 20 out of 60 male-rats appeared to be alcohol-dependant in simulation of alcoholization.
4. Volume of the consumed alcohol was more in its combination with water and less in its combination with a catholyte; thus, use of catholyte restrains alcohol intake in alcohol-dependant rats.
5. A catholyte decreases horizontal locomotor activity and emotional reactions in alcohol-dependant rats.
6. Ionized liquid with negative ORP has hepato-protective effect and significantly reduces damage of hepatocytes caused by alcohol.

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