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Effectiveness assessment of sulfur-containing amino acids in rats with experimental “alcohol withdrawal syndrome” with modified zoosocial interaction methods

V.I. Petrov[1], N.A. Osadchenko[1], A.S. Tarasov[1,2], A.M. Dotsenko[1], E.I. Morkovin[2]

[1] Volgograd State Medical University,
1, Pavshikh Bortsov Sq., Volgograd, Russia, 400131
[2] Scientific Center of Innovative Medicines,
39, Novorossiyskaya Str., Volgograd, Russia, 400087

E-mail: n.a.osachenko@gmail.com

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The aim of the work was to compare the effects of ademethionine, acetylcysteine and taurine on the zoosocial behavior of rats in a post-intoxicated state after an acute ethanol poisoning.

Materials and methods. The study was conducted on male rats of the Wistar line. The post-intoxication state was modeled by a single injection of ethanol (3 g/kg, intraperitoneally). 30 min after awakening, the animals were injected with a physiological solution, acetylcysteine (1 g/kg), ademetionine (100 mg/kg) or taurine (40 mg/kg). A zoosocial interaction test was performed 30 min after the administration.

Results. Among the behavioral indicators investigated, the following were validated: the number of acts of freezing, their duration, the number of acts of sniffing in front, the number of acts of avoidance and the number of vertical stances without support (p <0.05 between the values of the negative and positive control groups in all cases). The administration of acetylcysteine, ademetionine and taurine reduced the number of freezing acts by 53.64, 7.27 and 24.51%, respectively (p <0.05 when compared with the indicator index in the animals from the positive control group in all cases). The administration of acetylcysteine and taurine reduced the number of avoidance acts by 50 and 10%, respectively (p <0.05 when compared to that of the animals from the positive control group in both cases). All amino acids normalized the communicative performance, although it did not differ from that of the animals from the positive control group (p >0.05). Alcoholization reduced the number of vertical stances by 65% (p <0.001 when compared with that in the animals from the negative control group), and when followed by the administration of ademetionine and taurine, the reduction was 38 and 36%, respectively (p <0.05 when compared to that in the animals from the negative control group).

Conclusion. According to the data obtained, sulfur-containing amino acids, primarily those that had central effects, normalized neuronal functions, positively influencing a complex behavior of rats. Taking into account the results of the previous studies, it was possible to conclude that the therapeutic effect of ademetionine and taurine in the context of a post-intoxication state is mediated by their central effects, which are not so pronounced in comparison with acetylcysteine.

Keywords: ethanol; acetylcysteine; taurine; ademetionine; preclinical studies

Abbreviations: AB – alcoholic beverage; AWS – alcohol withdrawal syndrome; LPO – lipid peroxidation; mNSS – modified Neurological Severity Scores.

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Оценка эффективности серосодержащих аминокислот у крыс с экспериментальным «алкогольным похмельем» с помощью методики зоосоциального взаимодействия

В.И. Петров[1], Н.А. Осадченко[1], А.С. Тарасов[1,2], А.М. Доценко[1,2], Е.И. Морковин[2]

[1] Федеральное государственное бюджетное образовательное учреждение высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации, 400131, Россия, г. Волгоград, пл. Павших Борцов, д. 1

[2] Научный центр инновационных лекарственных средств с опытно-промышленным производством федерального государственного бюджетного образовательного учреждения высшего образования «Волгоградский государственный медицинский университет» Министерства здравоохранения Российской Федерации, 400087, Россия, г. Волгоград, ул. Новороссийская, д. 39

E-mail: n.a.osachenko@gmail.com

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Цель. Сравнить влияние адеметионина, ацетилцистеина и таурина на зоосоциальное поведение крыс, находящихся в постинтоксикационном состоянии после острого отравления этанолом.

Материалы и методы. Исследование проведено на крысах-самцах линии Wistar. Постинтоксикационное состояние моделировали однократным введением этанола (3 г/кг, внутрибрюшинно). Через 30 мин после пробуждения животным вводили: физиологический раствор, ацетилцистеин (1 г/кг), адеметионин (100 мг/кг) или таурин (40 мг/кг). Через 30 мин после введения проводили тест зоосоциального взаимодействия.

Результаты. Среди исследуемых показателей поведения были приняты во внимание: количество актов замирания, длительность следования, количество актов обнюхивания спереди, количество актов избегания и количество вертикальных стоек без опоры (p <0,05 между показателями групп отрицательного и положительного контроля во всех случаях). Введение ацетилцистеина, адеметионина и таурина снижало количество актов замирания на 53,64, 7,27 и 24,51% соответственно (p <0,05 при сравнении с показателем у животных из группы положительного контроля во всех случаях). Введение ацетилцистеина и таурина снижало количество актов избегания на 50 и 10% соответственно (p <0,05 при сравнении с показателем у животных из группы положительного контроля в обоих случаях). Все аминокислоты нормализовали показатели коммуникативности, несмотря на то, что они не отличались от показателей у животных из группы положительного контроля (p >0,05). Алкоголизация снижала количество вертикальных стоек на 65% (p <0,001 при сравнении с показателем у животных из группы отрицательного контроля), а при последующем введении адеметионина и таурина снижение составило 38 и 36% соответственно (p <0,05 при сравнении с показателем у животных из группы отрицательного контроля).

Заключение. Согласно полученным данным, серосодержащие аминокислоты, в первую очередь те, что обладали центральными эффектами, нормализовали функции нейронов, положительно влияя на сложное поведение крыс. Учитывая результаты предыдущих исследований, удалось заключить, что терапевтическое действие адеметионина и таурина в контексте постинтоксикационного состояния опосредовано их центральными эффектами, не столь выраженными в сравнении с ацетилцистеином.

Ключевые слова: этанол; ацетилцистеин; таурин; адеметионин; доклинические исследования

Список сокращений: АН – алкогольные напитки; ПС – похмельный синдром; ПОЛ – перекисное окисление липидов; mNSS – модифицированная шкала неврологического дефицита (modified Neurological Severity Scores).

INTRODUCTION

In the modern society, alcohol abuse is common due to an increased stressor load as well as the availability of alcoholic beverages (ABs)[1,2]. The consumption of ABs in excessive amounts leads to the formation of an alcohol withdrawal syndrome (AWS). This condition is defined as “a set of mental and physiologic symptoms that a person experiences after a single episode of an alcohol consumption in large quantities, developing against the background of the absence of ethanol in the blood” [1]. According to WHO’s 2018 Global Status Report on Ethanol Consumption, 18.2% of the world population consumes ABs in the sufficient quantity to develop AWS[3]. In their systematic review, Gunn C. et al. (2018) presented the evidence supporting the negative impact of AWS on the performance of tests of short-

[1] Clinical Guidelines. Alcoholic liver disease in adults. Clinical guidelines rubric. Available from: https://cr.minzdrav.gov.ru/recomend/711_1. Russian

[2] Federal State Statistics Service of Russian Federation. Available from: https://rosstat.gov.ru/folder/210/document/13218. Russian

[3] WHO (World Health Organization). Global status report on alcohol and health 2018. Available from: https://www.who.int/publications/i/item/9789241565639. Russian

and long-term memory, the attention concentration, and a psychomotor reaction speed [2]. Later, this observation was confirmed by Palmer et al. (2020) in a study involving students taking a test to assess memory performance and other cognitive functions the day after taking NA in large quantities [3]. Later, this observation was confirmed by Palmer E.O.C. et al. (2020) in a study involving students taking a test to assess the memory performance and other cognitive functions the day after taking ABs in large quantities [3].

AWS requires research and the development of therapy, as it is a widespread condition that reduces many aspects of quality of life and creates a risk of injury both for the person experiencing it and for the people around him. Against the background of this condition, the risk of traumatization when driving a car or at work increases [4, 5], and the quality of sleep decreases [6].

The state of AWS in humans is characterized by a possible presence of 47 symptoms [7], therefore, when modeling this pathology in animals for the subsequent assessment of the effect of drugs on this state, a significant number of behavioral tests should be used, in which various aspects of motor, emotional and cognitive spheres of the psyche are evaluated [3]. Since the postintoxication state in animals is characterized by an impaired locomotor function [8], the Combs and D'Alecy neurological deficit scales [9] and modified Neurological Severity Scores (mNSS) [10, 11] are used to assess its severity. The Morris Water Maze test [12] and the Open Field test [12] are suitable for assessing the effect of AWS and the effect on cognitive functions (mnestic, an exploratory activity) [12] and "Open Field" [10, 13]. The "Open Field" test is also aimed at studying an anxiety activity [13], i.e. to assess the emotional sphere of the psyche, which is influenced by AWS.

In Russia, for the therapy of AH, drugs and dietary supplements based on acetylsalicylic and/or succinic acids, sorbents to be used during the alcohol consumption to prevent the occurrence of AWS, and symptomatic drugs other than those mentioned above – arginine glutamate drugs, herbal drugs are used. Thus, the hangover therapy to date has been limited to symptomatic and prophylactic approaches[4].

AWS is a special intoxication case, which is accompanied by a decrease in glutathione stores and a toxic liver damage, leading to neurological disorders. The hypothesis of the study is that sulfur-containing amino acids, which are participants in the glutathione metabolism, can be used to treat AWS. Repurposing of known and studied drugs for new indications is a promising way to find solutions for the treatment of common pathologies.

[4] State Register of Medicines of Russian Federation. Available from: https://grls.minzdrav.gov.ru/Default.aspx. Russian

For acetylcysteine, in addition to mucolytic action, antioxidant properties [14] and an NO-ergic activity [15] have been proven; however, the results of a clinical trial on the efficacy of acetylcysteine in AWS were mixed [16]. Taurine had a positive effect on the course of experimental neurological deficits caused by poisoning [17], ischemia [18], brain injury [19], and hemorrhage [20]. The role of this sulfonic acid in the course of neurologic deficits has been confirmed in clinical studies [21]. Ademethionine exerts various pharmacological effects on the central nervous system, in particular, it affects the metabolism of monoamine neurotransmitters and receptor systems [22, 23], which is also reflected in the behavioral performance. The metabolism of these sulfur-containing amino acids involves the formation of glutathione, the deficiency of which is a central link in the pathogenetic chain of alcohol withdrawal syndrome. On this basis, the administration of sulfur-containing amino acids was hypothesized to prevent a glutathione depletion and an alleviate oxidative stress leading to the impaired metabolic activity of the liver and impaired neuronal functioning.

THE AIM of the study was to compare the effects of ademethionine, acetylcysteine, and taurine on the zoosocial behavior of rats in a postintoxicated state after acute ethanol poisoning.

MATERIALS AND METHODS

Experimental animals

The study was performed on 40 male Wistar rats with a body weight of 300–450 g, obtained from the laboratory animal nursery Rappolovo (Russia). The animals were kept in the standard vivarium conditions under a light-dark cycle of 12/12 h, the temperature of 20±2°C and the humidity of 40-60%. The animals received water and food *ad libitum*.

Ethical approval

All experimental studies were conducted in accordance with the Rules of Laboratory Practice approved by the order of the Ministry of Health of Russia No. 708n dated 23 August 2010, in strict compliance with the European Convention for the Protection of Vertebrate Animals Used for Experiments or Other Scientific Purposes (Directive 2010/63/EC). The protocol was approved by the Regional Independent Ethical Committee at Volgograd State Medical University (IRB 00005839 IORG 0004900 (OHRP) protocol No. 132 dated 20 May 2019).

Study design

A total of 80 animals were used; 40 of them were intact and the other 40 were used in the experimental series. Five groups of 8 animals each were formed. The animals from the positive control and experimental

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groups were injected with a 20% aqueous ethanol solution at a dose of 3 g/kg, once intraperitoneally, and immediately after awakening (a sleep duration was 5±0.5 h), they were administered one of three drugs: acetylcysteine (1 g/kg, intragastrically once), ademethionine (100 mg/kg, intragastrically once) or taurine (40 mg/kg, intragastrically once), or a saline solution in the appropriate volume (in the positive and negative control groups). The doses for the administration had been chosen based on the literature data and according to the results of the experiments conducted previously [24–27]. The animals from the negative control group were injected with a physiologic solution in an appropriate volume instead of ethanol, and the physiologic solution was administered instead of drugs. The scheme of the study design is presented in Figure 1.

Studied compounds

The following drugs in a powder form were used in this work: acetylcysteine (Zambon, Italy), ademethionine butanedisulfonate (VEROPHARM LLC, Russia), taurine (Supptrue Taurine, Russia).

Assessment of zoosocial behavior

The assessment of the zoosocial behavior according to Petrov V.I. methods [28] was started 30 min after the administration of the tested drugs. Since the interaction between two animals is evaluated in the experiment, 40 healthy animals which were pairs for the tested rats, were used for the test. After typing the animals by body weight, animal was matched with a pair for a resident–intruder interaction among the 40 additional animals.

The test setup was a 97×97 cm square open field without burrows, located in a dark room and illuminated with red light [29]. A pair of animals – first intact (resident), then an animal from the experimental group (intruder) – was placed in the center of the setup in turn and observed for 10 min.

The following components of the animal behavior were assessed: anxiety, sociability, negativity, exploratory activity, and aggressiveness. An anxiety was evaluated by the number of acts of freezing and the number of acts of short grooming. A communicative behavior was assessed by the duration of following the intact animal, the number of sniffing acts (front, side, tail, and anus), and the number of allo- and autogrooming acts. The avoidance behavior was assessed by the number of acts of movement away from the resident. The exploratory behavior was assessed by the number of upright stances with and without a wall support. The aggressiveness was assessed by the number of acts of approaching from aside.

Statistical processing

The statistical processing was performed by methods of descriptive and analytical statistics. The distribution of quantitative values was evaluated using the Shapiro–Wilk test. The intergroup differences were assessed by a one-way analysis of variance using the Newman–Keuls post hoc test. All the data were presented as the mean and standard error of the mean (unless otherwise indicated). The differences in the categorical data were evaluated by the chi-square test. The differences between the indicators in the group were considered statistically significant at p <0.05.

The results were processed and analyzed using Microsoft Excel 2019 (Microsoft Corporation, USA) and GraphPad Prism 5 statistical package (Dotmatics, USA).

RESULTS

The obtained results and their statistical processing are presented in Table 1.

Anxiety

The numbers of freezing acts in the animals from the negative and positive control groups were 13.75±2.96 and 22.5±5.21 (p <0.01), respectively. In the animals administrated with acetylcysteine, ademethionine, or taurine after the alcoholization, these values were 6.38±3.16, 12.75±9.59 and 10.38±7.11, respectively (p <0.01 in all cases when compared with the number of animals in positive control group).

The numbers of short grooming acts in the animals from the negative and positive control groups were 4.38±2.07 and 7.88±4.19, respectively (p >0.05). Thus, the alcoholization had no statistically significant effect on the number of acts of short grooming. In the animals from acetylcysteine, ademethionine, and taurine groups, the indices were 2.63±2.13, 3.86±2.85 and 5.5±4.93, respectively. The index in the animals from the acetylcysteine group was statistically significantly lower than in the animals from the positive control group (p <0.05). Since the assessment of this behavioral component did not pass validation (negative and positive controls did not differ), it is not possible to interpret the effect of acetylcysteine as anxiolytic. The results of the anxiety assessment are presented in Figure 2.

Communicativeness

The average duration of following the resident in animals from the negative and positive control groups was 18.38±3.75 and 8.05±4.7 s, respectively (p <0.05). The value in the animals from the acetylcysteine, ademethionine and taurine groups did not differ statistically significantly from that in the animals from the control groups and occupied intermediate values: 9.55±5.54, 14.47±7.72 and 11.06±9.77 s, respectively.

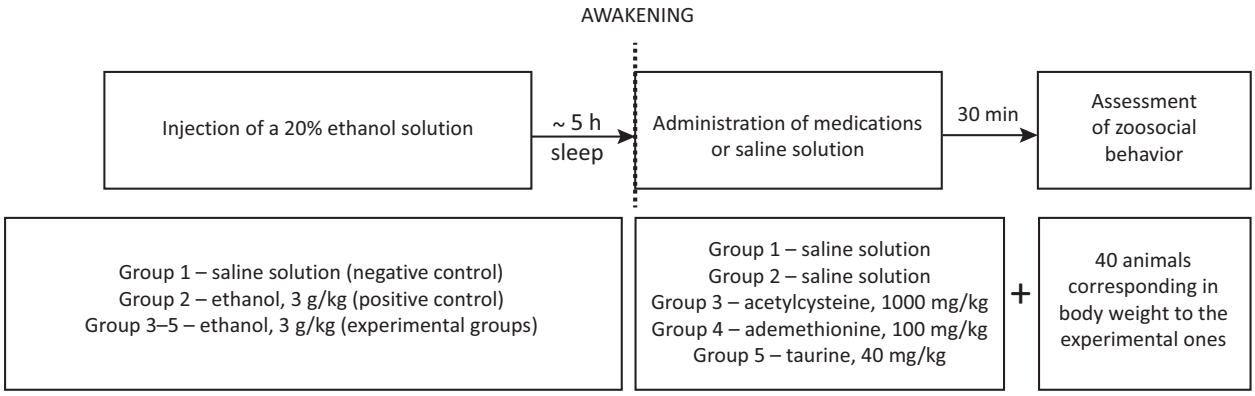

Figure 1 – Schematic study design to assess zoosocial behavior

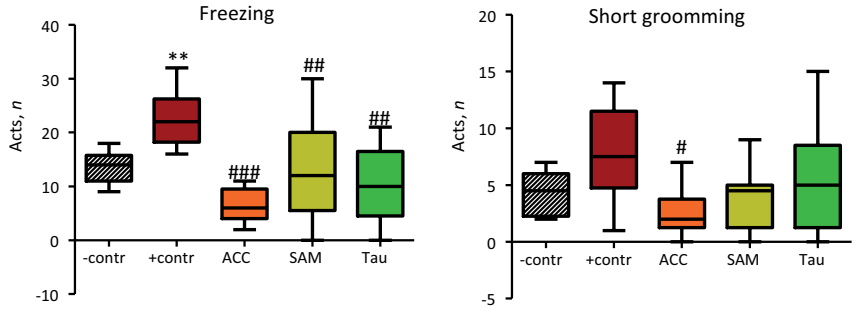

Figure 2 – Effect of acetylcysteine, ademethionine, and taurine administration in post-intoxication phase on anxiety indices in zoosocial interaction in ethanol-intoxicated animals

Note: -contr – negative control group; +contr – positive control group; ACC – animals administered with acetylcysteine; SAM – animals administered with S-ademethionine; Tau –animals administered with taurine; ** – statistically significant difference from the animals from the negative control group at p <0.01; # – statistically significant difference from the animals from the positive control group at p <0.05; ## – statistically significant difference from the animals from the positive control group at p <0.01; ### – statistically significant difference from the animals from the positive control group at p <0.001; the data are presented as median, standard deviation (box) and minimum and maximum values (whiskers).

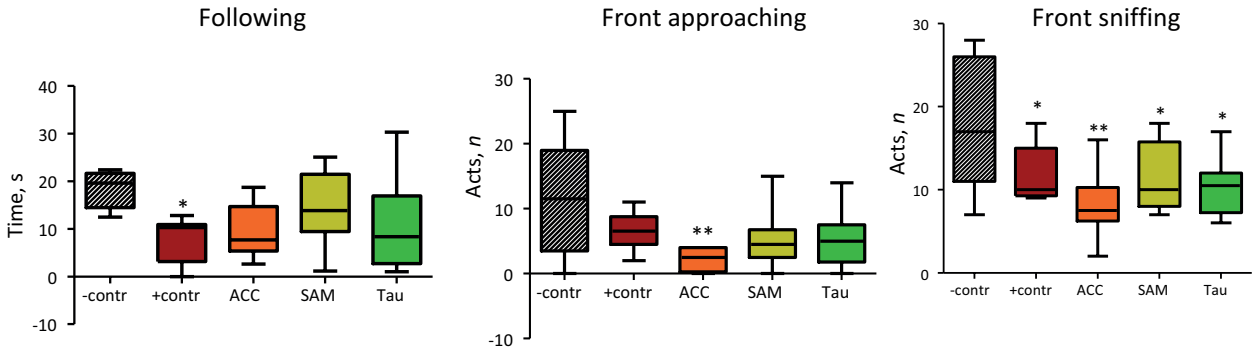

Figure 3 – Effect of acetylcysteine, ademethionine, and taurine administration in post-intoxication phase on communicative indices in zoosocial interaction in ethanol-intoxicated animals

Note: -contr – negative control group; +contr – positive control group; ACC – animals administered with acetylcysteine; SAM – animals administered with S-ademethionine; Tau – animals administered with taurine; * – statistically significant difference from the negative control group animals at p <0.05; ** – a statistically significant difference from the animals from the negative control group animals at p <0.01; the data are presented as median, standard deviation (box) and minimum and maximum values (whiskers).

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Table 1 – Results of measuring behavioral indicators reflecting neurological deficits and impaired zoosocial interaction

Behavioral component	Negative control	Positive control	Acetylcysteine	Ademethionine	Taurine
Anxiety			Acts of freezing, *n*		
	13.75±2.964	22.50±5.210 (+63.64%)**	6.375±3.159 (–53.64%)###	12.75±9.588 (–7.27%)##	10.38±7.110 (–24.51%)##
			Acts of short grooming, *n*		
	4.375±2.066	7.875±4.190 (+80%)	2.625±2.134 (–40%)*	3.875±2.850 (–11.43%)	5.500±4.928 (+25.71%)
Communicativeness			Duration of the following, s		
	18.38±3.752	8.046±4.696 (–56.22%)*	9.548±5.536 (–48.05%)	14.47±7.715 (–21.27%)	11.06±9.771 (–39.83%)
			Acts of front approaching, *n*		
	11.50±8.635	6.625±2.825 (–42.39%)	2.250±1.753 (–80.43%)**	5.375±4.47 (–53.26%)	5.375±4.34 (–53.26%)
			Acts of front sniffing, *n*		
	17.88±7.661	11.75±3.412 (–34.28%)*	8.125±4.051 (–54.56%)**	11.50±4.243 (–35.68%)*	10.38±3.503 (–41.95%)*
			Acts of side sniffing, *n*		
	7.875±2.357	6.000±1.604 (–23.81%)	6.875±2.031 (–12.70%)	6.250±3.196 (–20.63%)	9.250±2.550 (+17.46%)
			Acts of anus sniffing, *n*		
	10.63±6.391	6.375±3.815 (–40.03%)	4.125±1.458 (–61.19%)	5.750±4.950 (–45.91%)	6.625±4.838 (–37.68%)
			Acts of tail sniffing, *n*		
	10.38±8.535	10.13±4.998 (–2.41%)	4.250±3.370 (–59.06%)	7.625±4.470 (–26.54%)	6.750±3.576 (–34.97%)
			Acts of allogrooming, *n*		
	1.000±0.5345	0.3750±0.7440 (–62.5%)	0.8750±0.8345 (–12.5%)	0.6250±0.7440 (–37.5%)	0.6250±0.9161 (–37.5%)
			Acts of autogrooming, *n*		
	3.750±2.493	1.750±1.389 (–53.33%)	3.875±2.031 (+3.33%)	3.250±1.982 (–13.33%)	5.250±5.392 (+40%)
Negativity			Acts of avoidance, *n*		
	2.500±1.195	4.125±1.553 (+65%)*	1.250±1.035 (–50%)###	3.250±1.488 (+30%)	2.250±1.165 (–10%)#
Exploratory behavior			Acts of rearing with support, *n*		
	28.50±6.655	27.63±9.211 (–3.05%)	17.13±7.180 (–39.89%)	23.38±8.959 (–17.96%)	24.63±9.380 (–13.58%)
			Acts of rearing without support, *n*		
	27.00±8.401	9.375±4.749 (–65.28%)***	10.00±8.089 (–62.96%)***	16.63±9.724 (–38.41%)*	17.25±8.172 (–36.11%)*
Aggression			Acts of side approaching, *n*		
	3.125±1.808	1.125±0.991 (–64%)	2.375±1.685 (–24%)	2.375±1.598 (–24%)	1.500±1.309 (–52%)

Note: * – p <0.05 when compared with animals from the negative control group; ** – p <0.01 when compared with animals from the negative control group; *** – p <0.001 when compared with animals from the negative control group; # – p <0.05 when compared with animals from the positive control group; ## – p <0.01 when compared with animals from the positive control group; ### – p <0.001 when compared with animals from the positive control group; the data are presented as an arithmetic mean and a standard deviation (a percentage decrease).

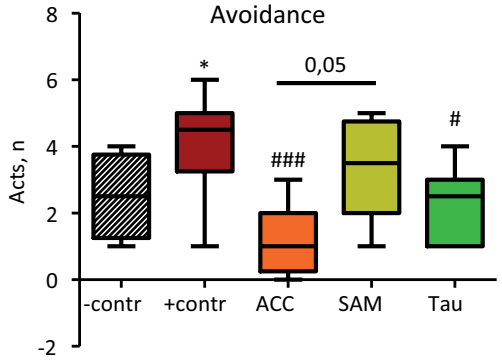

Figure 4 – Effect of acetylcysteine, ademethionine, and taurine administration in the post-intoxication phase on the number of avoidance acts of zoosocial interaction in ethanol-intoxicated animals

Note: -contr – negative control group; +contr – positive control group; ACC – animals administered with acetylcysteine; SAM – animals administered with S-ademethionine; Tau – animals administered with taurine; * – a statistically significant difference from the animals from the negative control group at p <0.05; # – a statistically significant difference from the animals from the positive control group at p <0.05; ### – a statistically significant difference from the animals from the positive control group at p <0.001; compared groups are indicated by a horizontal line; the data are presented as median, standard deviation (box) and minimum and maximum values (whiskers).

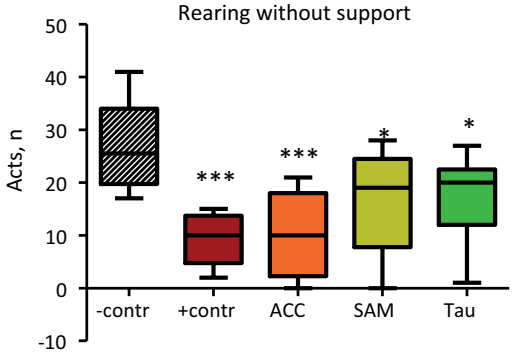

Figure 5 – Effect of acetylcysteine, ademethionine, and taurine administration in post-intoxication phase on the number of rearings without support during zoosocial interaction in ethanol-intoxicated animals

Note: -contr – negative control group; +contr – positive control group; ACC – animals administered with acetylcysteine; SAM – animals administered with S-ademethionine; Tau – animals administered with taurine; * - a statistically significant difference from the animals from the negative control group at p <0.05; *** – a statistically significant difference from the animals from the negative control group at p <0.001; the data are presented as median, standard deviation (box) and minimum and maximum values (whiskers).

The number of front approach acts in the animals from the negative and positive control groups were 11.5±8.64 and 6.63±2.83, respectively (p >0.05). Thus, the alcoholization had no significant or statistically significant effect on approaching from the front. The index in the animals from the acetylcysteine group was even lower than in the animals from the positive control group and amounted to 2.25±1.75 (p <0.01 when compared to the index in the animals from the negative control group). At the same time, the number of front sniffing acts as an anxiety score passed the validation. The values in the animals from the negative and positive control groups were 17.88±7.66 and 11.75±3.41 acts (p <0.05). The scores in the animals from the experimental groups were comparable to the mean score in the animals from the positive control group and were 8.13±4.05 (p <0.01 when compared to the intact animals), 11.5±4.24 and 10.38±3.5 (p <0.05 when compared to the intact animals in both cases) acts for acetylcysteine, ademethionine and taurine, respectively. The results of the communicative assessment are summarized in Figure 3.

Negativity (avoidance behavior)

The number of avoidance acts characterizing the negativity of behavior in the animals from the negative and positive control groups was 2.5±1.2 and 4.13±1.55, respectively (p <0.05). The index in the animals from the acetylcysteine group was lower than in the animals from the control groups and amounted to 1.25±1.04 (p <0.001 when compared to the index in the animals from the positive control group). The index in the animals from the ademetionine group occupied an intermediate position between the index in the animals from both control groups and did not differ statistically significantly from either of them, amounting to 3.25±1.49 acts (p >0.05, but p <0.05 when compared with the index in the animals from the acetylcysteine group). In the animals administered with taurine, the mean was statistically significantly different from that of the animals from

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the positive control group (p <0.05) and amounted to 2.25±1.17 acts. The results of measuring the number of avoidance acts are presented in Figure 4.

Exploratory behavior

The number of unsupported stances in the animals from the negative and positive control groups were 27±8.4 and 9.38±4.75, respectively. In the animals from acetylcysteine, ademethionine and taurine groups, the values were 10±8.09 (p <0.001 when compared to negative control), 16.63±9.72 and 17.25±8.17 (p <0.05 when compared to the negative control). The results are summarized in Figure 5.

Among the indicators assessed in the zoosocial behavior test, the parameters showing the following behavioral components: anxiety (acts of freezing), sociability (following, front sniffing), negativity (acts of avoidance) and exploratory behavior (rearing without support), were validated.

The administration of acetylcysteine, ademetionine, and taurine reduced the number of acts of freezing by 72, 43, and 54%, respectively, when compared with the rate in the positive control group. The duration of following the resident increased by 19, 80 and 38%, respectively. The number of sniffing acts decreased in all treatment groups, but was greatest in the acetylcysteine group.

DISCUSSION

Metabolic therapy agents with antioxidant, hepato- and neuroprotective properties can correct the course of toxic neuropathies [30]. The choice of drugs for the study was conditioned by the presence of sulfur atom in the molecule of ademethionine, acetylcysteine and taurine and their ability to form disulfide bonds.

Acetylcysteine is a derivative of L-cysteine, which is a precursor of the antioxidant tripeptide glutathione. The basis of a mucolytic action of acetylcysteine is its ability to discharge disulfide bonds of mucoproteins, which leads to the liquefaction of sputum. There are reasons to believe that due to its antioxidant properties, acetylcysteine may have an antiapoptogenic effect [31–33]. It has been suggested that acetylcysteine normalizes a glutamate neurotransmission in various brain structures [34]. The use of acetylcysteine to improve the condition after an acute alcoholization may have a dual effect – an improvement of the substance processing in the liver and a protective effect on the nervous system, which helps to reduce the desire to use ABs.

Taurine is a 2-aminosulfonic acid, a sulfonic acid that is widely distributed in living organisms and involved in many metabolic processes. Most mammals, including adult humans, are capable of self-synthesizing taurine. They obtain it directly from food of the animal origin in the amounts sufficient to meet metabolic needs[5]. Taurine deficiency is observed in various diseases, especially in diabetes mellitus and cardiovascular diseases. Against the background of the taurine administration to the animals with experimental diabetes mellitus, an increase in the glycogen content in the liver and an increase in the glucose utilization by muscles were observed [35]. Taurine can bind to lipid hydroperoxides that disrupt the integrity of the vascular epithelium, which prevents cell apoptosis and the development of endothelial dysfunction [36].

S-adenosylmethionine, an intermediate product of taurine synthesis, acts as a carrier of methyl groups in the body, which allows it to be used as a drug for the treatment of both hepatobiliary disorders and some types of depression [37–40].

In previous studies on the effects of sulfur-containing amino acids on biochemical parameters in the post-intoxication state, it was found out that acetylcysteine, ademethionine and taurine restored a liver function by normalizing the levels of aspartate aminotransferase and glutathione. Acetylcysteine had the most pronounced positive effect on these parameters, reducing an aspartate aminotransferase activity by 16% and increasing glutathione reserves by 16% relative to the parameters in the positive control group. Ademethionine and taurine decreased the aspartataminotransferase activity by 9 and 11%, respectively. Both drugs had a clear positive effect on the recovery of glutathione stores, increasing them by 11% in both cases [41].

As a part of the evaluation of neuro- and hepatoprotective effects of sulfur-containing amino acids in the conditions accompanied by a decrease in glutathione reserves, a spectrum of behavioral assessments was previously used: an elevated cruciform maze, an open field, an adhesive test, a test of a conditioned passive avoidance response, Morris water maze test, as well as Combs and D'Alecy scales and mNSS. According to the results of the earlier studies, the use of acetylcysteine, ademetionine and taurine in the rats undergoing a acute alcohol intoxication partially corrects behavioral disorders. The most pronounced effect was exerted by ademethionine, increasing the mean Combs and D'Alecy score relative to the index in the animals with experimental ABs without treatment. A motor activity in the "open field" test among the alcoholized animals was also the highest in the ademetionine group [10].

Since the suppression of neuropsychiatric changes in animals after an acute alcoholization coincided with an increase in the amount of glutathione stores in the

[5] Froger N. Taurine Deficiency and the Eye. Handbook of Nutrition, Diet and the Eye. V.R. Preedy, J. Sahel, S. Picaud editors. Academic Press; London: Elsevier. 2014;51:505–13. DOI: 10.1016/B978-0-12-401717-7.00051-4

liver, it was concluded that the action mechanism of all the studied drugs is mediated by a hepatoprotective action.

However, AWS in humans is a multifactorial condition, so a zoosocial interaction test was performed to assess behavioral components not accounted for by the above-mentioned methods. Despite the fact that only anxiety, sociability, negativity, and exploratory kinds of activity have been validated, the results obtained for these behavioral components correlate with the results of standard behavioral testing, adding an additional clarification to the existing ideas about modeling and treatment of AWS in an *in vivo* experiment.

Among the compounds studied, ademethionine had the most pronounced effect on the communicative behavior, while acetylcysteine worsened the condition, which is presumably due to the central effects of ademethionine and taurine, which acetylcysteine does not possess [23, 42, 43]. Acetylcysteine significantly suppressed the avoidance behavior, reducing the number of avoidance acts by 70% (*vs* 21 and 45% in the ademetionine and taurine groups), and its administration also resulted in a reduction in the number of freezing acts. Taurine most pronouncedly enhanced the exploratory component of behavior. The administration of taurine, ademetionine and acetylcysteine increased the number of unsupported vertical stands by 84, 77 and 7%, respectively.

The post-intoxication state, in addition to the previously detected anxiogenic effect and suppression of the exploratory activity, impaired the animal communication, enhancing the avoidance-related behavior (negativity). According to the results of the previous experimental series, acetylcysteine has a significantly more pronounced effect on the biochemical component of a postintoxication neuropsychiatric failure than ademethionine and taurine.

According to the data obtained in this experimental series, sulfur-containing amino acids, primarily those with central effects, normalized a neuronal function, positively affecting the complex behavior of rats.

Limitations of the study

As mentioned above, AWS in humans is characterized by the presence of 47 symptoms, all of which cannot be modeled and evaluated in animals. Therefore, preclinical studies of the described condition do not allow a direct extrapolation of the findings to humans. Clinical trials are required to develop an effective therapy for AWS.

CONCLUSION

Among the studied compounds, ademethionine had the most pronounced effect on the communicative behavior, while acetylcysteine worsened the condition, which is presumably due to the central effects of ademethionine and taurine, which acetylcysteine does not possess. Acetylcysteine significantly suppressed the avoidance behavior, reducing the number of avoidance acts by 70% (*vs.* 21 and 45% in the ademetionine and taurine groups), and its administration also resulted in a reduction in the number of freezing acts. Taurine most pronouncedly enhanced the exploratory component of behavior. The administration of taurine, ademetionine and acetylcysteine increased the number of unsupported vertical stands by 84, 77 and 7%, respectively.

Taking into account the data obtained in the zoosocial interaction test, it was possible to clarify that the contribution of the central effects of ademethionine and taurine was greater than the metabolic ones in the context of the studied condition.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have made equal and equivalent contributions to the preparation of the publication. All authors confirm that their authorship complies with the ICMJE international criteria (all authors made a significant contribution to the conceptualization, research and preparation of the article, read and approved the final version before the publication). Vladimir I. Petrov – idea of the experiment, methodology and scientific guidance; Nazar A. Osadchenko, Alexander S. Tarasov, Anna M. Dotsenko – literature review, collection of materials, conducting experiments, writing and editing the article; Evgeny I. Morkovin – search of sources for the literature review, statistical processing of the results.

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AUTHORS

Vladimir I. Petrov – Doctor of Sciences (Medicine), Professor, Academician of the Russian Academy of Sciences, Head of the Department of Clinical Pharmacology and Intensive Care, Volgograd State Medical University of the Ministry of Health of Russia; chief freelance specialist – clinical pharmacologist of the Ministry of Health of the Russian Federation; Honored Scientist of the Russian Federation; Honored Doctor of the Russian Federation. ORCID ID: 0000-0002-0258-4092. E-mail: brain@sprintnet.ru

Nazar A. Osadchenko – full-time postgraduate student of the Department of Clinical Pharmacology and Intensive Care at Volgograd State Medical University. ORCID ID: 0000-0002-7398-2186. E-mail: n.a.osadchenko@gmail.com

Alexander S. Tarasov – Junior Researcher at the Laboratory of Neuropsychotropic Drugs of the Scientific Center for Innovative Medicines of Volgograd State Medical University. ORCID ID: 0000-0002-8581-1595. E-mail: tarasov.pharm@ya.ru

Anna M. Dotsenko – Assistant at the Department of Fundamental Medicine and Biology, part-time postgraduate student of the Department of Clinical Pharmacology and Intensive Care at Volgograd State Medical University, Junior Researcher at the Laboratory of Genomic and Proteomic Research of the Scientific Center for Innovative Medicines of Volgograd State Medical University. ORCID ID: 0000-0003-3324-3351. E-mail: ev8278@mail.ru

Evgeny I. Morkovin – Candidate of Sciences (Medicine), Associate Professor, Head of the Laboratory of Neuropsychotropic Drugs at the Scientific Center for Innovative Medicines of Volgograd State Medical University. ORCID ID: 0000-0002-7119-3546. E-mail: e.i.morkovin@gmail.com