Research Article

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Results of a Multicenter Study on the Efficacy and Safety of Inosine Glycyl-Cysteinyl-Glutamate Disodium in the Treatment of Acute Ethanol Poisoning

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ABSTRACT The article presents results of a multicenter, randomized, double-blind, placebo-controlled clinical trial on the efficacy and safety of inosine glycyl-cysteinyl-glutamate disodium (Molixan®) in the treatment of acute severe ethanol poisoning.

THE AIM OF THE STUDY To evaluate the efficacy and safety of inosine glycyl-cysteinyl-glutamate disodium in a new dosage regimen in the treatment of severe ethanol poisoning.

MATERIAL AND METHODS The material of the study is the data of 133 patients with severe ethanol poisoning. Inclusion criteria: age from 18 to 60 years, 1–2 coma stage (Glasgow-Pittsburgh coma scale of 14–27), ethanol in the blood of more than 2.5%. The patients were randomized into 2 groups: the Study Drug group (SD group) — 67 patients who, in addition to the standard therapy, were injected with the study drug - inosine glycyl-cysteinyl-glutamate disodium in a dose of 3.0 mg/kg; and the Placebo group — 66 patients who, in addition to the standard therapy, were injected with 0.9% sodium chloride solution in a dose of 3.0 mg/kg. Comparative intergroup and intragroup analyses were carried out according to the main clinical, laboratory parameters and EEG monitoring data.

RESULTS The study showed a positive effect of the study drug — a reduction in the coma period was noted (from 137 (75; 180) minutes to 78 (50; 155) minutes ($p<0.001$)), higher values of the Glasgow-Pittsburgh scale were recorded after 3 and 6 hours from the start of the therapy ($p<0.01$), a reduction in the time of formation of EEG awakening patterns in patients with delta coma activity from 192.2 (161.9; 222.5) minutes to 112.5 (97.6; 127.6) minutes ($p<0.001$), a decrease in heart rate ($p<0.02$), a decrease in the number of complaints of weakness and dizziness ($p<0.005$), in patients with high ALT levels, the frequency of development and severity of tremor decreased ($p<0.01$). The hepatoprotective effect of the drug was revealed, it was manifested by a decrease in ALT ($p<0.001$), AST ($p<0.001$) and direct bilirubin ($p<0.03$); the effect of the drug on metabolic processes — a decrease in lactate ($p<0.02$), an increase in BE-ECF ($p<0.01$), glucose ($p<0.01$) 3 hours after drug administration, an increase in potassium after 24 hours ($p<0.03$). The analysis of safety data did not reveal statistically significant differences between the treatment groups, no serious adverse events were recorded.

CONCLUSION The study demonstrated the efficacy and safety of inosine glycyl-cysteinyl-glutamate disodium (Molixan®) in the treatment of severe ethanol poisoning in a single dose of 3.0 mg/kg administered intravenously.

Keywords: ethanol poisoning, disodium glycyl-cysteinyl-glutamate inosine, clinical trial, molixan, alcoholic coma, ethanol hepatotoxicity

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β – hCG – human chorionic gonadotropin
ALT – alanine aminotransferase
AST – aspartate aminotransferase
AP – alkaline phosphatase
APTT – activated partial thromboplastin time
BP – blood pressure
CTS – chemical-toxicological study
ECG – electrocardiography
EEG – electroencephalography
GGTP – gamma-glutamyl transpeptidase
GPCS – Glasgow-Pittsburgh Coma Scale
HR – heart rate
INR – international normalized ratio
IV – intravenous
RR – respiratory rate
SD – study drug

BACKGROUND

The high level of alcohol consumption causes a large number of severe ethanol poisonings, which makes it necessary to search for optimal and effective treatment regimens. For this reason, studies aimed at improving the treatment of alcohol poisoning, preventing complications and prompt rehabilitation of patients are of exceptional medical and social significance.

The feature of the toxic effect of ethanol is its multifactorial nature. The main damaging mechanism of ethanol is the toxic damage to the central nervous system, due to its membrane toxic effect and the effect on the neurotransmitter systems of the brain. The indirect toxic effect of ethanol is realized through the formation of toxic metabolites, a change in the ratio in cells of NAD^+/NADH (nicotinamide adenine dinucleotide/reduced form of NAD^+); oxidative stress, hypoxia, and immune status disorders, which primarily determine its hepatotoxic effects [1–3]. That is why the treatment of acute ethanol poisoning should be aimed primarily at restoring the functional activity of brain and liver cells.

Recently, encouraging results have been obtained in the study of the effectiveness of peptide compounds in critical conditions accompanied by a complex of the listed disorders, which allows considering the group of peptide drugs as promising ones in the treatment of acute alcohol poisoning [4–7]. Molixan® is one of the low-toxic peptide drugs approved for clinical use, which has cytoprotective, immunomodulatory and hepatoprotective effects. The hepatoprotective properties of the drug are of exceptional importance in the treatment of acute poisoning, since the liver plays a key role in ethanol metabolism and detoxification processes [8–12]. Due to the fact that ethanol poisoning often occurs against the background of long alcohol involvement, the disease can be complicated by withdrawal symptoms. Previous studies have revealed an effective effect of the study drug (SD) on the somatovegetative and neurological components of alcohol withdrawal syndrome, which suggests the possibility of reducing the frequency and severity of this complication in acute ethanol poisoning [9].

SD is an organic salt of biologically active metabolites inosine and peptide, glycyl-cysteinyl-glutamate. Glycyl-cysteinyl-glutamate normalizes the number of disulfide bonds in the structure of various types of cell surface receptors, increases the level of their internal activity, restores affinity for ligands: hormones, cytokines, growth factors and drugs. Inosine affects cellular functions through purine receptors A_1, A_{2A} and A_3, initiating a wide range of cytoprotective reactions in the cells of various organs during hypoxia, including the brain, heart and liver. Also, the therapeutic efficacy of the drug is ensured by the entry into cells of glutamate, glycine, cysteine and inosine, which molecules are actively used in metabolic processes aimed at maintaining cell viability in critical conditions. The metabolic concept of SD determines the possibility of its effectiveness in acute poisoning with neurotropic poisons, including ethanol.

Aim of study: to evaluate the efficacy and safety of the use of disodium glycyl-cysteinyl-glutamate (Molixan®) inosine in a new dosing regimen in the treatment of severe ethanol poisoning.

MATERIAL AND METHODS

The study was conducted on the basis of 6 clinical research centers of the Russian Federation from 2016 to 2018. The basis for the study was the official permission of the Ministry of Health of the Russian Federation No. 880 dated December 26, 2016, the Ethics Council (extract from the protocol No. 134 dated October 4, 2016) and local ethical committees. Inclusion criteria were male and female patients aged 18 to 60 years with severe acute ethanol poisoning; depression of consciousness in the range from 14 to 26 on the Glasgow-Pittsburgh coma scale (GPCS) (coma grade 1–2); the presence of ethanol in the blood at a concentration of more than 2.5 g/l; signed informed consent of the patient's legal representative or the opinion of the doctors' council on the possibility of involving the patient in the study. The study did not include patients with impairment of consciousness less than score 13 and more than score 27 according to the GCS; with coma of non-alcoholic etiology (toxic, traumatic, vascular, endocrine genesis); with severe comorbidities; patients with hemodynamic disorders; with acute respiratory failure requiring artificial lung ventilation; with body weight less than 50 or more than 120 kg; pregnant and lactating women, as well as patients who participated in any other clinical study within the last 3 months. The calculation of the sample size was carried out on the basis of the following conditions: the level of significance of statistical conclusions is 95%; the power indicator is 80%; the standardized difference between the studied parameters in the compared groups is in the range of 0.5–0.6 (the calculation was made on the basis of preliminary studies). Based on the above parameters, the sample size should be in the range of 80 to 120 patients. Two groups with equal number of participants were studied. It is the equal number of groups that provides maximum sensitivity.

A total of 133 patients were included in the study. After screening, patients were randomized into groups in a 1:1 ratio: the "Placebo" group (66 patients), where participants were treated with standard therapy and the introduction of placebo (physiological saline in 2 ml ampoules, in appearance and labeling, did not differ from SD), and the study drug group (67 patients), who were treated with standard therapy and administration of SD (inosine glycyl-cysteinyl-glutamate disodium). Groups were comparable on main indicators (Table 1).

Table 1

Comparison of the groups according to the main demographic indicators and the degree of exotoxicosis

Index	Placebo, *n* = 66	Study drug, *n* = 67	*p*	All groups, *n* = 133
Age, years	42 (34; 50)	42 (35; 50)	0.657	42 (35; 50)
Gender, male/female	59/7	65/2	0.081	124/9
Height, m	1.75 (1.7; 1.82)	1.75 (1.7; 1.8)	0.978	1.75 (1.7; 1.8)
Weight, kg	83 (75; 91)	82 (75; 90)	0.568	82 (75; 90)
Blood ethanol, ‰	3.71 (2.9; 4.7)	3.97 (3.02; 5.4)	0.203	3.8 (3.0; 5.2)
Urine ethanol, ‰	5.07 (3.69; 6.065)	5.4 (4.3; 6.9)	0.081	5.3 (4.1; 6.5)

Notes: The data in the Table are presented as a median, 1st, 3rd quartile Me (Q1; Q3). Comparative intergroup analysis—Mann-Whitney U test, Chi-square test

SD or placebo were administered in dose of 3.0 mg/kg once intravenously (IV) (bolus) in dilution with 0.9% solution sodium chloride in ratio on volume 1:1. The concomitant therapy was carried out in accordance with the Federal clinical guidelines "Toxic effect of alcohol" [14].

The study was carried out with double blindness. The procedure for unlocking codes was performed at the end of the clinical trial by simultaneously opening all the envelopes for statistical processing.

The duration of the patient's participation in the study was 24 hours. The study controlled the most significant hematological parameters: the content of hemoglobin, RBC, WBC, neutrophils, lymphocytes, monocytes, platelets (*Sysmex XN* 1000, *Sysmex Corporation*, Japan), aspartate aminotransferase (AST), ALT, alkaline phosphatase (AP), gamma-glutamyl transpeptidase (GGTP), total bilirubin, direct bilirubin, glucose, creatinine, total protein, urea, lactate (*Cobas c* 501, *Roche Diagnostics*, Switzerland); activated partial thromboplastin time (APTT), international normalized ratio (INR) (*Sta Compact*, *Stago Diagnostica*, France); indicators of the gas, electrolyte and acid-base balance of the blood: pH, pCO_2, pO_2, TCO_2, BE, sO_2, lactate, HCO_3-, Fe, Na^+, K^+, Ca_2^+, Cl^- (*Cobas b* 221, *Roche Diagnostics* , Switzerland); chemical-toxicological examination of blood and urine (Khromatek, Russia), instrumental monitoring of vital functions — heart rate (HR) and respiratory rate (RR), blood pressure (BP) (*Patient Monitor B* 30, *China*), electrocardiography (ECG)) (*ECG* - 9620M, *Japan*); assessed the complaints of patients during the period of recovery of consciousness. The scope of the examination of patients is presented in the flowchart (Fig. 1).

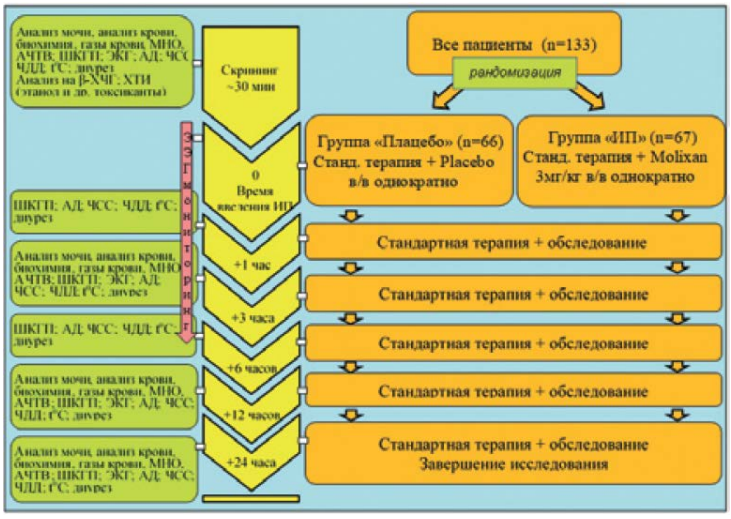

Fig. 1. The study block diagram

Notes: BP — blood pressure; APTT — activated partial thromboplastin time; IV— intravenously; INR — international normalized ratio; ß- hCG — human chorionic gonadotropin; CTT — chemical and toxicological testing; RR — respiratory rate; HR — heart rate; GPCS — Glasgow–Pittsburgh Coma Scale; ECG — electrocardiography

When analyzing the results of the study, the medical records of patients with initially high levels of ALT, AST, and GGTP were analyzed separately (Table 2).

Table 2

Distribution of patients by groups and subgroups

	Placebo, *n*	Study drug, *n*
Main groups, *n*	66	67
Among them:		
Patients with high levels of ALT, *n*	20	32
Patients with high GGTP levels, *n*	25	36
Patients with high levels of AST, *n*	30	36
Patients who underwent EEG monitoring, *n*	25	25

Notes: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGTP, gamma-glutamyl transpeptidase; EEG - electroencephalography

The primary endpoint of the study was the dynamics of GPCS scores. The secondary endpoints were the dynamics of the bioelectrical activity of the brain (the recovery time of the EEG curve parameters (electroencephalography)), the incidence of complications during the treatment period, and the dynamics of changes in blood biochemical parameters.

The period of the patient's stay in a coma was recorded in minutes. The dynamics of recovery of consciousness was controlled by an instrumental method: in 50 patients, the bioelectrical activity of the brain (EEG monitoring) was monitored from the moment the drug was administered to the patient's recovery from the coma. Bioelectrical activity was recorded using a Mizar-EEG-201 encephalograph (OOO Mizar, Russia) in standard monopolar and bipolar leads according to the 10–20 system.

Statistical processing was performed using the *Microsoft Excel* and *Statistica* 10 packages. The normality of data distribution was assessed using the Shapiro–Wilk test ($n \leqslant 50$). Given the nature of the distribution, which is different from normal, the median was determined, the 1st, 3rd quartiles in the form of *Me* (*Q*1; *Q*3). The comparison of quantitative data for unrelated samples was performed using the Mann–Whitney test and the Wilcoxon test for related samples. Fisher's exact test was used to compare categorical data between groups. The significance level where the null hypothesis of the absence of statistically significant differences was rejected was taken at $p < 0.05$.

RESEARCH RESULTS

Clinical efficacy. When analyzing the results, statistically significant differences were obtained between the groups in terms of the dynamics of GPCS indicators. In the group of patients treated with SD, statistically significantly higher values were noted on the scale after 3 and 6 hours from the start of therapy ($p < 0.01$) (Fig. 2).

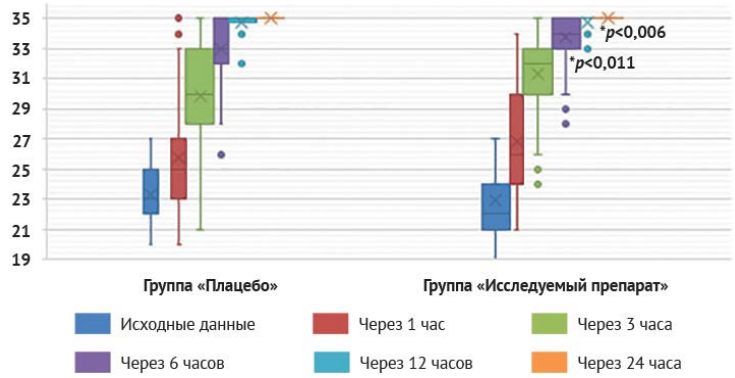

Fig. 2. Dynamics on the Glasgow–Pittsburgh Coma Scale in the Placebo group (n=66) and in the Study Drug group (n=67)
Notes: The data are presented as median, 1st, 3rd quartile Me (Q1; Q3); * — differences are statistically significant between the indicators (p<0.05)

A more rapid recovery of consciousness in patients was reflected in a reduction in the time spent by the patient in a coma. The moment of the patient's recovery from the coma was observed using the number of signs: the appearance of a purposeful reaction to pain stimuli, the establishment of speech contact and the

execution of simple commands by the patient. The median time spent in a coma in the placebo group was 137 (75; 180) minutes, and in the study drug group it was 78 (50; 155) minutes (p <0.004). The data of clinical observations were confirmed by the results of EEG monitoring. It should be noted that with a relatively similar clinical picture in terms of the parameters of the bioelectrical activity of the brain, the examined cohort of patients was not homogeneous. Based on the nature of the background EEG, the patients were divided into two groups: 1) patients with "theta-coma" pattern observed (28 patients); 2) patients with "delta-coma" pattern (22 patients). In patients in the group with a delta pattern on the EEG, the addition of standard therapy with the introduction of a SD statistically significantly increased the rate of awakening, the median time for the formation of an awakening pattern on the EEG was 112.5 (97.6; 127.6) minutes, while in the "Placebo" group 192.2 (161.9; 222.5) minutes (p <0.001) (Table 3).

Table 3

Comparative analysis of parameters of bioelectrical activity in patients with delta coma pattern at the time of admission in the Placebo group (n=10) and in the Study Drug group (n=12)

EEG parameters		Placebo (*n* =10)	Study drug (*n* =12)	*p*
Level of consciousness, Glasgow-Pittsburgh scale, score		23 (22; 25)	22 (21; 24)	0.213
Amplitude, μV		41.9 (36.8; 47.1)	45.1 (37.8; 51.2)	0.746
Total spectrum power, μV^2 s		61.9 (50.8; 72.1)	63.8 (52.2; 76.1)	0.926
Share of range in total power, %	Delta	44.9 (41.1; 50.2)	42.8 (40.1; 45.9)	0.118
	Theta	30.5 (28.1; 33.1)	29.9 (26.6; 33.2)	0.439
	Alpha	20.9 (16.7; 24.2)	20.7 (17.2; 23.8)	0.243
	Beta	5.5 (2.7; 8.2)	5.2 (2.3; 8.1)	0.287
SEF 50, Hz		4.8 (3.7; 5.9)	5.1 (3.9; 6.3)	0.126
Increase in *SEF* 50 during stimulation, Hz		0.8 (0.4; 1.2)	0.7 (0.4; 1.0)	0.131
Awakening pattern formation time, min		192.2 (161.9; 222.5)	112.5 (97.6; 127.6)	<0.001*

Notes: * - differences are statistically significant between the indicators (p<0.05). The data are presented as Me (Q 1; Q 3)

A significant proportion of patients had tachycardia upon admission. With the introduction of SD, a statistically significant decrease in heart rate was noted, which was confirmed by an increase in the *RR interval* on the electrocardiogram (Table 4).

Table 4

Changes in heart rate and RR interval duration in the study groups

Research phase		Placebo group, *n* =66	Comparison with original data, *p* *	Study drug group, *n* =66	Comparison with original data, *p* *	Intergroup comparison, *p* #
Initial values	*R-R*	0.72 (0.6; 0.87)	–	0.70 (0.62; 0.79)	–	0.514
	HR	88 (72; 100)	–	85 (77; 94)	–	0.645
In 1 hour	*R-R*	0.72 (0.6; 0.8)	0.943	0.70 (0.64; 0.8)	0.089	0.775
	HR	85.5 (77; 100)	0.612	85 (75; 93)	0.057	0.147
In 3 hours	*R-R*	0.72 (0.6; 0.84)	0.659	0.69 (0.6; 0.76)	0.175	0.325
	HR	90 (75; 100)	0.975	87 (77; 97)	0.233	0.784
After 12 hours	*R-R*	0.74 (0.62; 0.87)	0.236	0.72 (0.61; 0.80)	0.612	0.326
	HR	85 (75; 98)	0.121	85 (77; 98)	1.00	0.716
After 24 hours	*R-R*	0.74 (0.65; 0.84)	0.562	0.73 (0.67; 0.84)	0.003*	0.753
	HR	85 (75; 96)	0,149	80,5 (70; 90)	0,001*	0,131

Notes:The data in the Table are presented as median, 1st, 3rd quartile (Me (Q1; Q3)); * — comparative intergroup analysis - Mann-Whitney U test; # — comparative within-group analysis — Wilcoxon test with Bonferoni correction

In patients with elevated ALT levels, the difference between the groups in heart rate was observed 1 hour after the administration of the drug, while in the SD group the heart rate decreased statistically significantly from 92 (81; 100) to 88 (79; 96) bpm (p<0.056), and in the placebo group, there was a trend towards an increase in heart rate from 92 (84.5; 100) bpm to 96 (88; 103) bpm (p<0.422), which led to an intergroup difference (p<0.058) (Fig. 3).

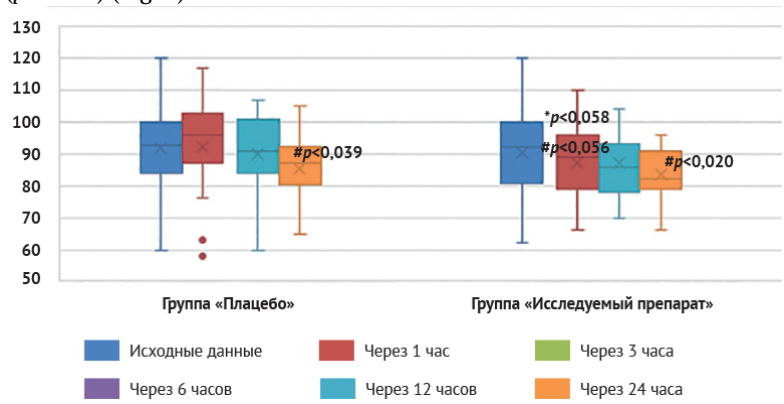

Fig. 3. Comparative analysis of heart rate dynamics in patients with initially higher-than-normal levels of ALT in the Study Drug group (n=32) and in the Placebo group (n=20)
Notes: the data are presented as median, 1st, 3rd quartile Me (Q1; Q3); * — intergroup differences; # — intragroup differences from the initial data

The study also assessed patient complaints after regaining consciousness. Statistically significant differences between the main groups in the number of complaints of weakness and dizziness were detected after 12 hours (Table 5). The mild weakness was reported by 16 patients (34.8%) in the study drug group and 30 patients (65.2%) in the placebo group; 21 patients (63.3%) in the study drug group and 12 patients (36.4%) in the placebo group complained of moderate weakness; 1 patient (14.3%) in the study drug group and 6 patients (85.7%) in the placebo group complained of severe weakness; there were no complaints of weakness in 28 patients (60.1%) in the study drug group and 18 patients (39.1%) in the placebo group. Dizziness was reported by 4 patients (21.1%) in the study drug group and 15 patients (78.9%) in the placebo group. Comparative intergroup analysis showed a statistically significant difference in these characteristics (p <0.01) (Table 6).

Table 5

The number of complaints of the main group patients in the somatogenic stage of poisoning

	In 6 hours				In 12 hours				In 24 hours			
	Placebo group (n=66)		Study drug group (n=67)		Placebo group (n=66)		Study drug group (n=67)		Placebo group (n=66)		Study drug group (n=67)	
	Weakness											
No	6	9.1%	13	19.4%	18	27.3%	28	42.4%	37	56.1%	42	64.6%
Mild	17	25.8%	17	25.4%	30	45.5%	16	24.2%	18	27.3%	10	15.4%
Moderate	24	36.4%	23	34.3%	12	18.2%	21	31.8%	11	16.7%	13	20%
Severe	19	28.8%	14	20.9%	6	9.1%	1	1.5%	0	0%	0	0%
	Dizziness											
No	44	67.7%	51	76.1%	51	77.3%	62	93.9%	61	92.4%	64	98.5%
Yes	21	32.3%	16	23.9%	15	22.7%	4	6.1%	5	7.6%	1	1.5%

Table 6

Comparative intergroup analysis of patient complaints

Complaints	In 6 hours	In 12 hours	In 24 hours
Weakness	0.12	0.0047	0.25
Dizziness	0,28	0,0051	0,10

Note: comparative intergroup analysis — χ^2 (Chi-square test)

As can be seen in Table 7 and 8, in addition to a decrease in the number of complaints of dizziness, in patients with an elevated ALT level, a decrease in the frequency of development and severity of tremor was noted. The severity of tremor was assessed in points: 0 points — no tremor; 1 point — mild tremor (invisible, but felt when touching the fingers); 2 points — noticeable tremor (noticeable with outstretched arms); 3 points — severe tremor (visible even if the patient's arms are not extended). Tremor of varying severity 6 hours after the start of observation was seen in 70% of patients with high ALT (14 patients) in the Placebo group, while in the Study Drug group, tremor was observedonly in 37.6% of patients (12 patients). Intergroup differences in this indicator were statistically significant in 6 hours after the initiation of therapy (p <0.05).

Table 7

Number of complaints of patients with higher-than-normal levels of alanine aminotransferase in the Study Drug (n=32) and Placebo (n=20) groups

	In 6 hours				In 12 hours				In 24 hours			
	Placebo grou (n =20)		Study drug group (n=32)		Placebo group (n =20)		Study drug group (n=32)		Placebo group (n=20)		Study drug group (n=32)	
	Dizziness											
No	13	65%	24	75%	14	70%	29	93.6%	18	90%	30	96.8%
Yes	7	35%	8	25%	6	30%	2	6.4%	2	10%	1	3.2%
	Tremor											
0 points	6	30%	20	62.5%	7	35%	18	58.1%	11	55%	21	67.7%
1 point	5	25%	6	18.8%	8	40%	7	22.6%	3	15%	6	19.4%
2 points	8	40%	6	18.8%	4	20%	6	19.4%	6	30%	3	9.7%
3 points	1	5%	0	0%	1	5%	0	0%	0	0%	1	3.2%

Table 8

Comparative intergroup analysis of complaints of patients with higher-than-normal levels of alanine aminotransferase in the Placebo (n=20) and Study Drug (n=32) groups

Complaints	In 6 hours	In 12 hours	In 24 hours
Dizziness	0.443	0.025	0.309
Tremor	0.015	0.155	0.277

Note: comparative intergroup analysis - χ^2 (Chi-square test)

ASSESSMENT OF LABORATORY PARAMETERS

The study revealed a positive effect of SD on a number of biochemical parameters. At high values of ALT, the administration of the drug led to their statistically significant decrease, while in the "Placebo" group there was no decrease in the level of ALT. Thus, in the group of patients receiving PI, it decreased from 80 (52; 112) to 64 (43; 89) U/l per day (p<0.001), while in the "Placebo" group it practically did not change — 78 (52; 120) U/l (at admission) — 78 (48; 107) U/l (in a day) (p<0.21) (Fig. 4).

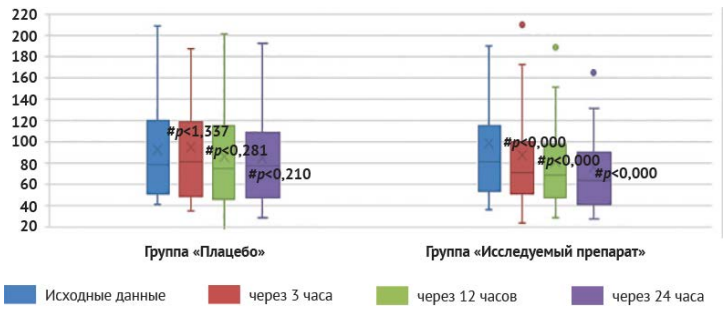

Fig. 4. Dynamics of alanine aminotransferase level in patients with its initially higher-than-normal values in the Placebo (n=20) and Study Drug (n=32) groups

Notes: The data are presented as median, 1st, 3rd quartile Me (Q1; Q3); * — intergroup differences; # — intragroup differences from the initial data

In patients with initially elevated ALT, SD administration also showed a statistically significant decrease in AP levels from 119 (97; 170) to 98 (71; 160) U/l (p <0.001) and GGTP from 101.5 (60; 211) to 89 (66; 182) U/L (p<0.001). There was no statistically significant decrease in the levels of these enzymes in the placebo group (p<0.18; p<0.77).

In patients with elevated levels of AST, SD administration showed a statistically significant decrease in the level of this enzyme from 100.5 (58.2; 122.5) to 73.6 (41; 100) U/l, (p<0.001), while there was no statistically significant decrease in the Placebo group: 73.3 upon admission (55.5; 122.3) U/l, 65.7 a day later (50.4; 161.7) U/l (p<1.42) (Fig.5).

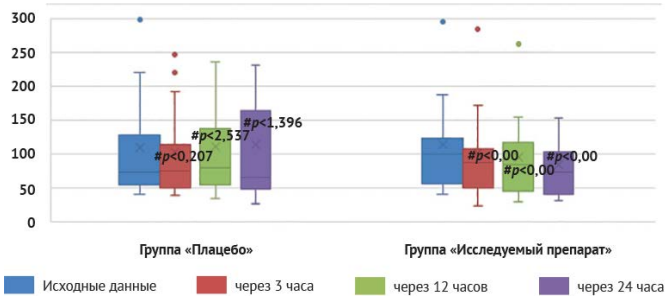

Fig. 5. Dynamics of aspartate aminotransferase (AST) level in patients with its higher-than-normal values in the Placebo (n=20) and Study Drug (n=32) groups

Notes: The data are presented as median, 1st, 3rd quartile Me (Q1; Q3); * - intergroup differences; # - intragroup differences from the initial data

The study showed a positive effect of SD on the level of direct bilirubin. It is known that direct bilirubin increases with toxic damage to the liver, including alcoholic genesis. The mechanism of liver damage when exposed to ethanol is a combination of its direct hepatotoxic effects and the action of its metabolites. Acetaldehyde is highly toxic to hepatocytes because it forms many adducts with structural proteins, enzymes, and DNA (deoxyribonucleic acid). Acetaldehyde, binding to tubulin, damages the microtubules of the cytoskeleton, preventing the excretion of synthesized substances, including direct bilirubin [15, 16]. As a result of a violation of the processes of excretion of direct bilirubin with bile, its concentration in the blood increases. The study showed that 3 hours after SD administration, the level of direct bilirubin decreased statistically significantly from 2.5 (1.5; 3.2) to 1.8 (1.15; 2.88) mmol/l (p<0 04), while in the placebo group the concentration of direct bilirubin increased. It is important to note that after 24 hours the level of direct bilirubin in the study drug group increased to 5 (3.2; 6.2) µmol/l, while in the placebo group the increase was more significant, 5.6 (3. 7; 7.3) µmol/l (Fig. 6).

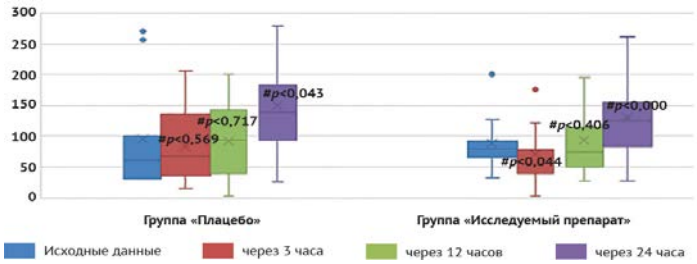

Fig. 6. Dynamics of direct bilirubin level in patients with higher-than-normal values of alanine aminotransferase (ALT) in the Placebo group (n=20) and in the Study Drug group (n=32)

Notes: The data are presented as median, 1st, 3rd quartile Me (Q1; Q3); * — intergroup differences; # — intragroup differences from the initial data

With the introduction of SD, a more rapid correction of metabolic acidosis was noted. The presence of metabolic acidosis was indicated by base deficiency (decrease in BE-ECF). Low BE-ECF values upon admission were found in the main groups: in 30 patients (47.6%) in the study drug group and in 26 patients (39.4%) in the placebo group. In the study drug group, BE-ECF values were statistically significantly different from baseline in 3, 12, and 24 hours (p<0.01, p<0.01, p<0.03), while those in the placebo group were statistically there was no significant change. One of the causes of metabolic acidosis in severe ethanol poisoning was an increase in the blood level of lactate, which was detected in 82 patients (61.7%). With the introduction of SD in 3 hours, its statistically significant decrease was observed (p <0.021), while in the Placebo group it was statistically insignificant (p <0.28). Also, 3 hours after SD administration, a statistically significant increase in glucose levels was noted (p<0.01), while in the Placebo group there were no significant changes in this indicator. After 12 hours,

the glucose level decreased statistically significantly in both groups relative to baseline values. The study also noted the positive effect of PI on the electrolyte composition of the blood. In 56 patients (42.1%), hypokalemia was detected upon admission. A statistically significant increase in blood potassium levels was only obtained in the study drug group after 24 hours (p<0.03). In the Placebo group, potassium did not increase statistically significantly (p=1.51) and remained low in another 30% of patients. Thus, in terms of electrolyte metabolism, the effectiveness of SD was manifested in a statistically significant correction of hypokalemia (Table 9).

Table 9

Comparative intergroup and intragroup analysis of metabolic parameters in patients with acute ethanol intoxication

Indicator, unit of measure	Placebo group, *n* =66	Study drug group, *n* =67	Intergroup comparison, *p*
Initial data			
BE-ECF, mmol/l	1.1 (-3.9; 2.7)	-1.5 (-4.8; 1.4)	0.167
Lactate, mmol/l	2.6 (2.1; 3.5)	3 (2.2; 3.7)	0.297
Glucose, mmol/l	6.0 (5.1; 6.5)	5.5 (4.8; 6.1)	0.057
K+, mmol/l	3.5 (3.2; 3.87)	3.8 (3.29; 4.05)	**0.029**
In 3 hours			
BE-ECF , mmol/l	1 (-2.3; 3.3)	0.5 (-2.3; 2.7)	0.412
Comparison with original data	0.098	**0.009**	
Lactate, mmol/l	2.5 (1.7; 3.4)	2.8 (2.1; 3.5)	0.227
Comparison with original data	0.28	**0.021**	
Glucose, mmol/l	5.5 (4.3; 7.3)	5.9 (5; 7.5)	0.213
Comparison with original data	2.943	**0.014**	
K+, mmol/l	3.4 (3.04; 3.65)	3.6 (3.3; 3.9)	**0.009**
Comparison with original data	0.15	0.09	
In 12 hours			
BE-ECF, mmol/l	1.15 (-1.6; 3.6)	0.3 (-2.5; 2.4)	0.112
Comparison with original data	0.073	**0.008**	
Lactate, mmol/l	2.5 (1.8; 3.35)	2.9 (2.1; 3.51)	0.437
Comparison with original data	0.13	0.10	
Glucose, mmol/l	5.2 (4.4; 6.1)	4.9 (4.6; 5.4)	0.411
Comparison with original data	**0.014**	**0.001**	
K+, mmol/l	3.42 (3.07; 4)	3.61 (3.17; 4.12)	0.101
Comparison with original data	2.353	1.901	
In 24 hours			
BE-ECF, mmol/l	0.8 (-2.2;2.5)	0.6 (-2.7;1.6)	0.317
Comparison with original data	0.453	**0.032**	
Lactate, mmol/l	1.95 (1.2; 2.75)	1.9 (1.2; 2.6)	0.891
Comparison with original data	0.001	0.001	
Glucose, mmol/l	5.6 (4.91; 7.2)	5.7 (5.05; 6.5)	0.813
Comparison with original data	2.632	1.453	
K+, mmol/l	3.57 (3.19; 3.86)	3.87 (3.4; 4.2)	**0.003**
Comparison with original data	1.511	**0.033**	

Note: The data are presented as median, 1st, 3rd quartile Me (Q1; Q3)

The analysis of blood gas parameters in the main groups did not reveal statistically significant intergroup and intragroup differences. However, when analyzing individual values, multidirectional changes in PO_2 and PCO_2 were observed . Hypoxemia upon admission was revealed in 19 patients (28.4%) in the study drug group and in 12 patients (18.2%) in the placebo group. In 3 hours, hypoxemia is registered only in 13 patients (19.4%) in the "Study Drug" group, while in the "Placebo" group, the number of patients with hypoxemia increased to 17 patients (25.8%). Hypercapnia was detected at admission in 60 patients (32 patients (47.8%) in the study drug group and in 28 patients (42.4%) in the placebo group). In 3 hours, hypercapnia was observed in only 26 patients (38.8%) of the "Study Drug" group, and in the "Placebo" group the number of such patients increased to 32 people (48.5%) That is, in 3 hours the number of patients with hypoxemia and hypercapnia in the "Study Drug" group decreased, while in the "Placebo" group, on the contrary, a negative trend was observed as the number of patients with blood gas disorders increased.

Another observation was made when assessing changes in the coagulogram. APTT increased at baseline in 3 patients in the "Study Drug" group and in 5 patients in the "Placebo" group. In 3 hours, the number of patients with high APTT increased to 7 in the "Study Drug" group, and decreased to 2 in the "Placebo" group. This may indicate a possible hypocoagulant effect of the drug and requires a more detailed study.

SAFETY ASSESSMENT

All patients included in the study were included in the safety analysis. No serious adverse events, including deaths, were reported during the study period. Also, there was no hyperthermia, local hyperemia, burning, swelling at the injection site.

A total of 406 adverse events were reported during the study, 187 in patients in the "Study Drug" group and 219 in the "Placebo" group ($p<0.11$). There were no statistically significant differences between the groups in the frequency of abnormalities ($p<0.11$, Bernoulli test) and in the number of patients ($p<0.69$, Fisher's exact test). Of the laboratory criteria, the most common abnormalities were an increase in the levels of glucose and direct bilirubin in the blood, its decrease in creatinine. Hyperglycemia was revealed in 27 patients (38%) in the "Study Drug" group and in 24 patients (35%) in the "Placebo group", with no statistically significant differences between the groups ($p<0.5$). An increase in direct bilirubin was observed in 19 patients (26.8%) in the "Study Drug" group and in 16 patients (23.5%) in the "Placebo" group, with no significant differences between the groups ($p<0.48$). A decrease in creatinine was found in 16 patients (22.5%) in the "Study Drug" group and in 17 patients (25%) in the "Placebo" group, with no statistically significant differences between the groups ($p<0.74$). An increase in the erythrocyte sedimentation rate was detected in 13 patients (18%) from the "Study Drug" group and in 21 patients (30.5%) from the "Placebo" group ($p<0.13$). In the general analysis of urine, leukocyturia, erythrocyturia and proteinuria were detected in a large number of patients, while proteinuria was observed in 20 patients (28.2%) from the "Study Drug" group, while in the "Placebo" group, proteinuria was revealed in a statistically significantly more number of patients — 33 patients (48.5%) ($p<0.03$). It is important to note that the condition of all patients participating in the study improved clinically, and the deviations of laboratory analyzes that were identified during the study were clinically insignificant.

According to the ECG data, there was no slowdown in atrioventricular and intraventricular conduction after the administration of the drug. There were no signs of prolongation of the processes of depolarization and repolarization of the ventricles, thus, the risk of developing fatal ventricular arrhythmias did not increase. The use of the drug is not associated with an increase in ectopic activity of the atria and ventricles.

It should be noted that the preparation of inosine glycyl-cysteinyl-glutamate disodium solution for intravenous and intramuscular administration is approved for medical use by the Pharmacological Committee, pr. No. 10 dated 12.07.2001 (registration No. 001355/02-14.07.11) and has been used in clinical practice over 20 years.

DISCUSSION OF THE RESULTS

The data obtained in the change in laboratory parameters during SD therapy explain its positive effect on the clinical picture. The pharmacological activity of SD ensures the restoration of a functionally active conformation of a wide range of surface cell receptors, which predetermines the restoration of the effectiveness of the action of mediators and hormonal factors. In particular, normalization of the sensitivity of the surface cell receptors of the bronchi and blood vessels to catecholamines restores the effective functioning of the physiological mechanisms of normalization of the ventilation-perfusion ratio in the lungs, which leads to improved oxygenation of blood and tissues and, accordingly, faster awakening of patients. The stimulating effect of catecholamines on the processes of lipolysis determines the increased release of free fatty acids, which become the main energy substrate during the normalization of tissue oxygenation, helping to reduce glucose

consumption by cells and the formation of lactate. The lactate, in turn, is actively metabolized by hepatocytes with the formation of glucose entering the blood (Cori cycle). A decrease in glucose consumption by cells and its entry from hepatocytes predetermine a transient increase in blood glucose levels at the peak of SD action. The ability of SD to influence the processes that predetermine an increase in the level of prostacyclin, leveling the negative effect of catecholamines on the vascular system and the normalization of microcirculation is significant. A higher concentration of potassium in the blood, associated with the pharmacological activity of SDs, enhances the polarization of membranes, which leads to stabilization of the cell membranes. This mechanism may underlie the normalization of the electrical activity of the brain, a decrease in heart rate, the occurrence of tremors, sweating, and other general somatic changes.

The improvement of blood oxygenation in combination with full microcirculation in the liver tissue predetermines the full functional activity of hepatocytes, which is accompanied not only by the active utilization of lactate, but also by an increase in the detoxifying function of the liver, thus contributing to the acceleration of the inactivation of ethanol and its metabolites, and ultimately providing a faster restoration of consciousness and improvement of well-being in the post-intoxication period of poisoning.

CONCLUSION

The study showed the efficacy and safety of the drug inosine glycyl-cysteinyl-glutamate disodium (Molixan®) in severe acute ethanol poisoning at a dosage of 3.0 mg/kg IV diluted with 0.9% sodium chloride solution in a ratio of 1:1 by volume. The drug is well combined with the main drugs used in the treatment of acute alcohol intoxication, accompanied by alcoholic coma. The analysis of the obtained results showed that the most significant positive effect of the drug was registered 3 hours after its administration, which indicates the expediency of its repeated administration during the first day of the disease.

1. The use of the drug inosine glycyl-cysteinyl-glutamate disodium (Molixan®) at a dose of 3.0 mg/kg in the complex therapy of acute alcohol intoxication accompanied by alcoholic coma led to a statistically significant reduction in the period of the patient's stay in a coma from 137 (75; 180) up to 78 (50; 155) minutes (p<0.004) and higher values of the Glasgow-Pittsburgh scale 3 and 6 hours after initiation of the therapy (p<0.01), which indicates an accelerated recovery of consciousness when using the study drug. This is confirmed by the results of electroencephalographic monitoring, which revealed a statistically significant 1.7-fold reduction in the period of awakening pattern formation (p<0.001). Rapid awakening in patients with the introduction of the drug is not accompanied by adverse changes in the recovery period, the "rebound phenomenon" is not typical.

2. When using the study drug, the heart rate normalized, which was confirmed by electrocardiogram data: an increase in the *RR interval* from 0.70 (0.62; 0.79) to 0.73 (0.67; 0.84) (p<0.03) and a decrease in heart rate from 85 (77; 94) to 80 (70; 90) bpm (p <0.01, statistically significant) 24 hours after the start of therapy. In patients with an initially high level of alanine aminotransferase, a statistically significant decrease in heart rate was recorded as early as 1 hour after drug administration: from 92 (81; 100) to 88 (79; 96) bpm (p <0.05), while in the Placebo group, it increased from 92 (84.5; 100) to 96 (88; 103) bpm (p<0.42), which was reflected in a statistically significant intergroup difference in heart rate (p<0 .05).

3. The clinical efficacy of the study drug is manifested in a statistically significant decrease in the frequency of patient complaints of weakness and dizziness in 12 hours (p<0.005). In patients with elevated levels of alanine aminotransferase, the frequency and severity of tremor development after 6 hours from the start of therapy (p<0.01) and the number of complaints of dizziness after 12 hours (p<0.03) statistically significantly decreased.

4. The hepatoprotective effect of the drug was revealed, which in patients with an initially elevated level of transaminases manifests itself: in a statistically significant decrease in the level of alanine aminotransferase from 80 (52; 112) to 64 (43; 89) U/l in 24 hours (p <0.001); aspartate aminotransferase from 101 (58; 123) to 73.6 (41; 100) U/l (p <0.001); alkaline phosphatase from 119 (97; 170) to 98 (71; 160) U/l (p<0.001); gamma-glutamyl transpeptidase from 102 (60; 211) to 89 (66; 182) U/l (p<0.001) and direct bilirubin 3 hours after drug administration from 2.5 (1.5; 3.2) to 1.8 (1.15; 2.88) mmol/l (p<0.04).

5. The effect of the drug on metabolic processes is manifested by a statistically significant decrease in lactate levels from 3.0 (2.2; 3.7) to 2.8 (2.1; 3.5) mmol/l (p <0.02), decrease in *BE-ECF deficiency* from 1.5 (-4.8; 1.4) to 0.5 (-2.3; 2.7) mmol/l (p<0.01) and glucose from 5.5 (4.8; 6.1) to 5.9 (5; 7.5) mmol/l (p<0.01) 3 hours after its administration.

6. A positive effect of the study drug on the electrolyte composition of the blood was established, which manifests itself in a statistically significant increase in the level of potassium in the blood from 3.8 (3.3; 4.1) mmol/l to 3.9 (3.4; 4.2) mmol/l (p<0.03).

7. In 3 hours, the number of cases of hypoxemia and hypercapnia decreases and the number of patients with hypocoagulation increases, but without a statistically significant difference from the initial data on blood oxygen and carbon dioxide tension and activated partial thromboplastin time. The data obtained require further study of the effect of the drug on the gas composition and the blood coagulation system.

8. During the study, no serious adverse events and statistically significant differences between the groups in the frequency of deviations from the norm of laboratory and clinical parameters were observed, which proves the safety of using the drug inosine glycyl-cysteinyl-glutamate disodium in the treatment of acute severe ethanol poisoning at a daily dosage of 3.0 mg/kg and intravenous administration.

REFERENCES

1. Zakhari S. Overview: how is alcohol metabolized by the body? *Alcohol Res Health* . 2006;29(4):245–254. PMID: 17718403
2. Shikalova IA. *Osobennosti terapii ostrykh otravleniy etanolom u bol'nykh's alkogol'noy zhirovoy distrofiey liver: cand. med. sci. diss. synopsis.* Saint-Petersburg; 2015. (In Russ.) Available at: http://www.toxicology.ru/docs/dissov/shikalova_avtoref.pdf [Accessed Jul 5, 2022]
3. Busanov DV, Afanasiev VV, Miroshnichenko AG, Basharin VA, Petrova NV, Alkhimenkov NG, et al. Molixan and Cytoflavin Administration to Comatose Patients Caused by Alcohol Consunption in the Emergency Unit. *Emergency Medical Care* . 2018;19(2):28–33. (In Russ.) https://doi.org/10.24884/2072-6716-2018-19-2-28-33
4. Halyutin DA, Hovpachev AA, Grebenyuk AN, Reynyuk VL, Antushevich AE, Kolobov AA. Therapeutic Efficacy of New Neuropeptides and Hepatoprotector Molixan at Acute Poisonings With Ethanol. *Toxicological Review*. 2015;(2):10–17. (In Russ.)
5. Muzurov KV, Antushevich AE, Antonov VG, Afanasiev VV, Buzanov DV, Petrova NV, et al. Molixan Efficasy in Acute Severe Corvalol Poisoning. *Emergency Medical Care.* 2017;18(2):73–77. (In Russ.) https://doi.org/10.24884/2072-6716-2017-18-2-73-77
6. Orlov YV, Bugaev PA, Khalimov YS, Basharin VA, Sinyachkin DA, Antushevich AE. The Effectiveness of Glutathione Disulfides in the Treatment of Toxic Hepatitis and Liver Fibrosis. *Voenno-medicinskij žurnal*. 2018;339(11):21–27 (in Russ.) https://doi.org/10.17816/RMMJ73093
7. Halyutin DA, Solovyeva TS, Reinyuk VL, Hovpachev AA, Chirski VS, Antushevich AE, et al. Morphological Evaluation in Experiment of Brain Damages in Acute Poisoning With Ethanol and Their Correction With Peptide Drugs. *Toxicological Review*. 2016;(3):15–21. (In Russ.) https://doi.org/10.36946/0869-7922-2016-3-15-21
8. Bugaev PA, Basharin VA, Antushevich AE. Comparative effectiveness of Molixan and Glutoxim in the model of toxic liver lesions with anti-tuberculosis drugs. *Reviews on Clinical Pharmacology and Drug Therapy*. 2019;17(2):49–54 (in Russ.) https://doi.org/10.17816/RCF17249-54
9. Vinnikova MA, Krenkel NT, Titkov MS, Tsareva IS, Ablova VV. Inosine Glycyl-Cysteinyl-Glutamate Disodium (Molixan®) in the Treatment of Severe Alcohol Withdrawal Syndrome: Results of a Randomized, Blind, Placebo-Controlled Trial. *Journal of Addiction Problems*. 2019;3(174):48–63. https://doi.org/10.47877/0234-0623_2019_3_48
10. Ostapenko YuN (ed.) *Toksicheskoe deystvie alkogolya. Federal'nye klinicheskie rekomendatsii.* Moscow; 2018. Available at: https://drive.google.com/file/d/1_Li5mfMUOiFRYC4DxMLKimxjWkr_BlLw/view [Accessed Jul 7, 2022]
11. Tuma DJ. Role of malondialdehyde-acetaldehyde adducts in liver injury. *Free Radic Biol Med*. 2002;32(4):303–308. PMID: 11841919 https://doi.org/10.1016/S0891-5849(01)00742-0
12. Wondergem R, Davis J. Ethanol increases hepatocyte water volume. *Alcohol Clin Exp Res*. 1994;18(5):1230–1236. PMID: 7531405 https://doi.org/10.1111/j.1530-0277.1994.tb00110.x

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