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Effect of combination hepatoprotective therapy with sulfur-containing drugs on oxidative homeostasis in the blood of patients with alcoholic hepatitis: A randomized prospective study

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ABSTRACT

Background. The study aims to evaluate the efficacy of combination hepatoprotective therapy for alcoholic liver damage. Given the difference in the mechanism of action and the experimentally demonstrated cytoprotective efficacy of ademetionine and lipoic acid, these drugs could be expected to potentiate each other's effects. **Objective.** To determine the effect of the combined use of ademetionine and lipoic acid on the cytolytic syndrome and oxidative metabolism in the blood of patients with alcoholic hepatitis. **Methods.** A randomized prospective study was conducted examining 30 patients diagnosed with alcoholic liver disease and 15 healthy individuals. The patients were treated at the Drug Abuse Clinic of the Krasnodar Krai Ministry of Health, whereas healthy volunteers were monitored at the Clinic of the Kuban State Medical University (2022–2024). The study included male patients aged 20 to 40 years with a diagnosis of alcoholic liver disease in the form of alcoholic hepatitis. Patients with other decompensated somatic and psychiatric disorders were excluded from the study. Via simple randomization with the use of random number tables, the patients were distributed into three groups. Group 2 patients received ademetionine (400 mg intravenously per day, Hepcifol). Group 3 patients were administered lipoic acid (600 mg intravenously per day, Octolipen). Group 4 patients received combination therapy with the administration of ademetionine and lipoic acid in the specified dosages and forms. The inpatient treatment lasted 15–18 days; on admission and prior to discharge, the patients had their blood samples taken. Blood serum was assayed for the activity of hepatocyte cytolysis markers and the concentrations of total protein, albumin, and bilirubin, as well as total antioxidant activity and thiol group content. The concentrations of glutathione and thiobarbituric acid reactive substances were determined in erythrocytes. The data were statistically processed using Statistica 10 (StatSoft, Inc., 2011). The differences between the parameters of the groups were considered statistically significant at $p < 0.05$. **Results.** Following a three-week course of treatment, the combination therapy with two sulfur-containing hepatoprotectors helped to achieve 1.7–2.1 times lower activity of alanine aminotransferase and gamma-glutamyltransferase in blood plasma as compared to the corresponding markers in the groups of patients receiving only one of the drugs. The therapy with sulfur-containing drugs was accompanied by tendencies toward normalization of free radical homeostasis. The maximum effects were achieved when lipoic acid was used alone or together with ademetionine. In this case, a 52–64% increase in the antioxidant activity of blood plasma was observed, with the concentration of thiobarbituric acid reactive substances decreasing by 28–36%. **Conclusion.** The combination therapy with the use of sulfur-containing hepatoprotectors helped to achieve the lowest possible enzyme activity (cytolytic syndrome markers) in patients with alcoholic hepatitis.

KEYWORDS: alcoholic hepatitis, ademetionine, lipoic acid, cytolytic syndrome, oxidative stress

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to be accountable for all aspects of the work, meaning that issues related to the accuracy and integrity of any part of the work are appropriately examined and resolved.
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Влияние комбинированной гепатопротекторной терапии серосодержащими препаратами на состояние окислительного гомеостаза в крови больных алкогольным гепатитом: рандомизированное проспективное исследование

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АННОТАЦИЯ

Введение. Работа направлена на оценку эффективности комбинированной гепатопротекторной терапии алкогольных повреждений печени. С учетом разного механизма действия и показанной в эксперименте цитопротективной эффективности адеметионина и липоевой кислоты можно было бы ожидать потенцирования эффектов друг друга. **Цель исследования** — определение особенностей влияния комбинированного использования адеметионина и липоевой кислоты на цитолитический синдром и состояние окислительного метаболизма в крови больных алкогольным гепатитом. **Методы.** Проведено рандомизированное проспективное исследование с участием 30 больных с диагнозом «алкогольная болезнь печени» и 15 здоровых индивидуумов. Больные проходили лечение на базе государственного бюджетного учреждения здравоохранения «Наркологический диспансер» Министерства здравоохранения Краснодарского края, здоровые добровольцы наблюдались на базе Клиники федерального государственного бюджетного образовательного учреждения высшего образования «Кубанский государственный медицинский университет» Министерства здравоохранения Российской Федерации в течение 2022–2024 гг. В исследование включали пациентов мужского пола в возрасте от 20 до 40 лет с диагнозом «алкогольная болезнь печени» в форме алкогольного гепатита. До исследования не были допущены пациенты с другими соматическими и психическими заболеваниями в стадии декомпенсации. Больные методом простой рандомизации с использованием таблиц случайных чисел были распределены в три группы. Больные группы № 2 получали адеметионин (400 мг внутривенно в сутки, «Гепцифол»). Больным группы № 3 вводили липоевую кислоту (600 мг внутривенно в сутки, «Октолипен»). Больные группы № 4 получали комбинированную терапию, включающую введение адеметионина и липоевой кислоты в вышеуказанных дозировках и формах. Продолжительность стационарного лечения составляла 15–18 суток, на этапе поступления больных и перед их выпиской осуществляли забор крови. В сыворотке крови определяли активность ферментов маркеров цитолиза гепатоцитов, концентрации общего белка, альбумина и билирубина, общую антиоксидантную активность и содержание тиоловых групп. В эритроцитах определяли концентрации глутатиона и продуктов реакции с тиобарбитуровой кислотой. Статистическую обработку данных выполняли с помощью программы StatSoft, Inc. (2011) Statistica, version 10. Статистически значимыми различия между значениями показателей групп считали при выполнении условия для уровня значимости $p < 0,05$. **Результаты.** Комбинированная терапия с использованием двух серосодержащих гепатопротекторов после трехнедельного курса лечения позволила добиться значений активности аланинаминотрансферазы и гамма-глутамилтрансферазы в плазме крови в 1,7–2,1 раза ниже значения соответствующих маркеров групп больных, получавших только один из препаратов. Проведение терапии с использованием серосодержащих препаратов сопровождалось тенденциями к нормализации состояния свободнорадикального гомеостаза. Максимальные эффекты были достигнуты при использовании липоевой кислоты самостоятельно или совместно с адеметионином. В этом случае наблюдался рост антиоксидантной активности плазмы крови на 52–64 %, концентрация реактивных продуктов тиобарбитуровой кислоты снижалась на 28–36 %. **Заключение.** Комбинированная терапия с использованием серосодержащих гепатопротекторов позволила добиться максимально низких значений активности ферментов — маркеров цитолитического синдрома у больных алкогольным гепатитом.

КЛЮЧЕВЫЕ СЛОВА: алкогольный гепатит, адеметионин, липоевая кислота, цитолитический синдром, окислительный стресс.

ДЛЯ ЦИТИРОВАНИЯ: Быков И.М., Ермакова Г.А., Попов К.А., Попова М.А., Завгородняя А.Г., Устинова Е.С. Влияние комбинированной гепатопротекторной терапии серосодержащими препаратами на состояние окислительного гомеостаза в крови больных алкогольным гепатитом: рандомизированное проспективное исследование. *Кубанский научный медицинский вестник.* 2024;31(6):15–27. https://doi.org/10.25207/1608-6228-2024-31-6-15-27

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КОНФЛИКТ ИНТЕРЕСОВ: один из авторов — профессор, доктор медицинских наук Быков И.М. является членом редакционной коллегии журнала «Кубанский научный медицинский вестник». Авторам неизвестно о каком-либо другом потенциальном конфликте интересов, связанном с этой рукописью.

ДЕКЛАРАЦИЯ О НАЛИЧИИ ДАННЫХ: данные, подтверждающие выводы этого исследования, можно получить у корреспондирующего автора по обоснованному запросу. Данные и статистические методы, представленные в статье, прошли статистическое рецензирование редактором журнала — сертифицированным специалистом по биостатистике.
СООТВЕТСТВИЕ ПРИНЦИПАМ ЭТИКИ: проведение исследования было одобрено на заседании экспертной комиссии Независимого этического комитета федерального государственного бюджетного образовательного учреждения высшего образования «Кубанский государственный медицинский университет» Министерства здравоохранения Российской Федерации (ул. им. Митрофана Седина, д. 4, г. Краснодар, 350063, Россия) протокол № 96 от 29.01.2021 г. Все описанные в статье работы проведены в согласии с принципами и правилами, разработанными и документированными в Хельсинкской декларации ВМА (64-я Генеральная ассамблея, Форталеза, 2013) и Федеральном законе Российской Федерации № 323-ФЗ от 21 ноября 2011 г. Обязательным критерием для включения испытуемых лиц в исследование было получение от них добровольного информируемого согласия в письменной форме.
ВКЛАД АВТОРОВ: И.М. Быков, Г.А. Ермакова, К.А. Попов, М.А. Попова, А.Г. Завгородняя, Е.С. Устинова — разработка концепции и дизайна исследования; Г.А. Ермакова, М.А. Попова, — сбор данных; И.М. Быков, К.А. Попов, А.Г. Завгородняя, Е.С. Устинова — анализ и интерпретация результатов; Г.А. Ермакова, К.А. Попов, М.А. Попова — обзор литературы, проведение статистического анализа, составление черновика рукописи и формирование его окончательного варианта; И.М. Быков, А.Г. Завгородняя, Е.С. Устинова — критический пересмотр черновика рукописи с внесением ценного замечания интеллектуального содержания. Все авторы одобрили финальную версию статьи перед публикацией, выразили согласие нести ответственность за все аспекты работы, подразумевающую надлежащее изучение и решение вопросов, связанных с точностью и добросовестностью любой части работы.
✉**КОРРЕСПОНДИРУЮЩИЙ АВТОР:** Быков Илья Михайлович, доктор медицинских наук, профессор, заведующий кафедрой фундаментальной и клинической биохимии федерального государственного бюджетного образовательного учреждения высшего образования «Кубанский государственный медицинский университет» Министерства здравоохранения Российской Федерации. Адрес: ул. им. Митрофана Седина, д. 4, г. Краснодар, 350063, Россия. E-mail: ilya.bh@mail.ru.

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INTRODUCTION

Modern medicine considers the feasibility and efficacy of using hepatoprotectors in various clinical situations. One such situation involves alcoholic damage to the liver parenchyma, as this organ is the main target of ethanol, where it is metabolized predominantly by alcohol dehydrogenase or microsomal ethanol-oxidizing system of hepatocytes [1, 2]. A wide range of substances, including several thiol-containing and thioether compounds, are considered to be drugs with the ability to protect and restore the liver after damage of different etiopathogenesis. Among SH-containing compounds, N-acetylcysteine, glutathione, and lipoic acid are considered to be potential hepatoprotectors; among thioether derivatives, methionine and adenosylmethionine [3, 4]. In some situations, the efficacy of other sulfur-containing compounds is analyzed: taurine, sodium thiosulfate, thiotriazoline, etc. In spite of significant advances in these drugs demonstrated in experimental studies on laboratory animals, randomized clinical trials do not clearly indicate their efficacy in liver damage. One of the potentially promising areas in pharmacotherapy is the combined use of known drugs or even the creation of combination drugs, such as Remaxol (Polysan, Russia) [5].

Some evidence exists regarding the efficacy of lipoic acid as a hepatoprotective agent. The main effect of lipoic acid consists in pronounced antioxidant action due to the presence of two reduced -SH groups. Lipoic acid is shown to be able to inhibit free radical processes and, by participating in the activity of the pyruvate dehydrogenase complex, to regulate energy exchange, as well as glucose and lipid oxidation. Literature contains data on the use of this drug in people with nonalcoholic fatty liver disease, as well as data on its use in people with chronic alcohol intoxication [6, 7]. According to our data, despite significant metabolic support for the antioxidant system, the use of lipoic acid in the experiment is accompanied by an insignificant decrease in the severity of cytolytic syndrome associated with the alcoholization of animals [8]. Most likely, no direct relationship exists between antioxidant action and cytoprotective activity toward liver cells.

Better known are the hepatoprotective properties of ademetionine, whose efficacy was proven in experimental studies and which was used successfully according to clinical trial data [9]. The main effect of ademetionine consists in supporting liver detoxification by supplying the active form of methionine, the methyl donor in detoxification reactions. The synthesis of ademetionine is also known to be reduced in chronic liver diseases [10, 11]. This also determines the potential efficacy of correction with the administration of an exogenous metabolite.

Of particular interest are attempts to use a combination therapy with ademetionine and lipoic acid. Given the difference in the mechanism of action and the experimentally demonstrated hepatoprotective effect of both drugs, ademetionine and lipoic acid could be expected to potentiate each other's effects. This was the main hypothesis of the study and determined its aim.

The study **aims** to determine the effect of the combined use of ademetionine and lipoic acid on cytolytic syndrome and oxidative metabolism in the blood of alcoholic hepatitis patients.

METHODS

Study design

The study adopted the design of a controlled randomized prospective study involving 30 patients diagnosed with alcoholic liver disease and 15 healthy individuals.

Eligibility criteria

Inclusion criteria

Male individuals aged from 20 to 40 years; for the control group: absence of exacerbated somatic and psychiatric disorders; for patients: diagnosis of alcoholic liver disease (alcoholic hepatitis) established by specialists of the Drug Abuse

Clinic of the Krasnodar Krai Ministry of Health; voluntary informed consent in writing.

Exclusion criteria

Presence of malignant neoplasms, pulmonary and cardiovascular diseases, infectious diseases, nervous and mental disorders; aggravated history of allergies; uncontrolled use of drugs and parapharmaceuticals.

Removal criteria

Voluntary refusal of the patient to receive medical care or participate in this study; development of complications not directly related to this study, including the development of an acute respiratory infectious disease.

Study conditions

The clinical study was conducted at the inpatient facility of the Drug Abuse Clinic of the Krasnodar Krai Ministry of Health, where patients underwent a course of detoxification and stabilization treatment. The control group consisted of volunteers undergoing a preventive medical examination at the Clinic of the Kuban State Medical University (Ministry of Health of the Russian Federation). The laboratory stage of this study was conducted at clinical diagnostic laboratories of the university clinic and Drug Abuse Clinic, as well as the Laboratory of the Department for Basic and Clinical Biochemistry of the Kuban State Medical University.

Duration of the study

The clinical study, including the laboratory stage, was conducted in May 2022 – February 2024.

Medical interventions

All people in the control group donated biomaterial (blood) for the laboratory stage of the study. The results of studying the control group, or Group 1, served as a reference for comparing the same markers in experimental group patients. As indicated above, the patients were randomized into three experimental groups differing in the hepatoprotective agent received as part of a comprehensive detoxification-stabilization regimen. Group 2 patients received ademetionine (400 mg intravenously per day, Hepcifol, Pharmasyntez, Russia). Group 3 patients were administered lipoic acid (600 mg intravenously per day, Octolipen, Ufa Vitamin Plant, Russia). Group 4 patients received combination therapy with the administration of ademetionine and lipoic acid in the specified dosages and forms. The observation, as well as detoxification and stabilization treatment, at the inpatient facility lasted 15–18 days. Biomaterial (blood) was collected twice: on admission of patients (prior to treatment) and prior to their discharge.

Study outcomes

Main study outcome

The main outcome of analyzing the efficacy of the combined use of sulfur-containing hepatoprotectors consists in a clinically significant decrease (by 40% or more) in the level of hepatocyte cytolysis markers (ALT, AST, and LDH) relative to the initial level established on admission of the patient [12]. Taking into account the indirect character of the specified cytolytic syndrome markers, we considered the possibility of decreasing the bilirubin concentration and increasing the concentration of total protein and human serum albumin, which also reflect the functional state of the liver parenchyma, to be the main outcomes of the study. Since the efficacy analysis of hepatoprotective therapy included the use of sulfur-containing compounds showing a pronounced antioxidant activity, the normalization of prooxidant-antioxidant imbalance was considered the main outcome of the study.

Additional study outcomes

No additional outcomes are intended.

Methods for recording outcomes

Standard serum biochemistry parameters were determined using a Super Z chemistry analyzer (Rayto Life and Analytical Sciences Co., Ltd., China) and reagents manufactured by Randox (UK). These laboratory biochemical parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyltransferase (GGT), total protein (TP), and human serum albumin, as well as total and direct bilirubin.

In order to assess the prooxidant-antioxidant balance, total antioxidant activity (TAA) and total level of sulfhydryl groups were determined in blood serum; the concentrations of reduced glutathione and thiobarbituric acid reactive substances (TBA reactive substances) were determined in erythrocyte suspension.

In order to determine the ferric reducing ability (FRAP assay)—one of the analogues of the total antioxidant activity determined via the chemical colorimetric method—a biofluid (blood serum) was incubated with a solution of Fe^{3+} ions and a chromogenic reagent (2,2'-dipyridyl) yielding a red complex with Fe^{2+} ions. The optical density of the solution of the obtained complex of dipyridyl with ferrous iron is directly proportional to the antioxidant activity, which is expressed in mM of the ascorbic acid solution adopted as the standard solution [13].

The antiradical activity, which can also be considered a variant of total antioxidant activity determined via the chemical colorimetric method, was analyzed in the ABTS (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) colored cation radical test system. The rate of neutralization of this radical and, therefore, the rate of its bleaching or optical density decrease of the solution was considered a measure of serum antiradical activity, which was also expressed in mM of the ascorbic acid solution adopted as the standard solution [14].

The level of serum thiol groups, as well as glutathione in erythrocyte suspension, was established through the reaction of -SH groups with Ellman's reagent, which releases a colored thionitrophenyl anion, with the optical density of the solution being directly proportional to the concentration of thiol groups or thiol-containing compounds in the biofluid [15].

The concentration of TBA reactive substances was determined using the well-known property of lipid peroxidation products, such as malondialdehyde, to react with 2-thiobarbituric acid at high temperatures to form a brick-red compound. The optical density of the resulting solution is directly proportional to the concentration of products resulting from free radical damage to biomolecules; therefore, this laboratory parameter is considered to be one of the key markers of oxidative stress [16].

Randomization

The study involved 30 patients with alcoholic hepatitis, who were distributed into three groups via simple randomization with the use of random number tables and later received different hepatoprotective agents as part of comprehensive medical treatment.

Data anonymity assurance

Patients were randomized into groups with the use of the BIA. The samples of biological material coming to the laboratory were encrypted to exclude personal information. All stages of the research work were completed without the involvement of outside researchers.

Statistical procedures

Principles behind sample size determination

The sample size was not determined in advance.

Statistical methods

In order to assess the distribution normality for the numerical samples of parameters, the Shapiro-Wilk test was calculated, which in most cases indicated a deviation from the normal distribution. In this connection, the results were presented in median and quartile format (Me (Q1/Q3)), and the sampled groups were subsequently compared using nonparametric criteria. Several samples were compared using the Kruskal–Wallis test; subsequent, if necessary, pairwise comparisons, were conducted using the Mann-Whitney U test. Pre- and post-treatment results were compared using the Wilcoxon test. The differences between the parameters of the groups were considered statistically significant at $p < 0.05$. The data were statistically processed using STATISTICA 10 (StatSoft, Inc., 2011).

RESULTS

Sampling

The sampling principle and the overall study design are presented in the block diagram (Figure). The control group included 15 healthy men undergoing a preventive medical examination at the outpatient dispensary. The people in Group 1 donated blood for laboratory studies and to establish the conditional baseline for biomarkers. The general group of patients was formed to include patients with alcoholic liver disease (alcoholic hepatitis). During randomization, these patients were divided into three groups, in which different sulfur-containing hepatoprotectors were used in the comprehensive treatment regimen. Group 2 patients (n = 10) received ademetionine; Group 3 patients (n = 10) were administered lipoic acid; Group 4 patients (n = 10) received combination hepatoprotective therapy with ademetionine and lipoic acid.

Characteristics of the study sample (groups)

All study participants were male and comparable in age. All of them were Russian citizens (Krasnodar Krai residents) and belonged to the Caucasian race. The age of 20–40 years was one of the inclusion criteria. Thus, healthy volunteers aged 29 (25/32) years were recruited; the age of patients in Groups 2–4 was 28 (25/32), 30 (27/33), and 28 (26/33) years, respectively. No statistically significant age differences were found between the four groups (p = 0.74). The body mass index of the subjects ranged from 20 to 30, which corresponded to the normal or overweight body mass index. Also, no statistically significant body mass index differences were found between the four groups (p = 0.30). The key initial parameters of patients adopted for comparing hepatoprotective activity were the activity of aminotransferases and GGT. These criteria were selected due to the need to recruit a sufficient number of participants and to form sufficiently homogeneous study samples in terms of the cytolytic syndrome level. The comparison of the activity of aminotransferases and GGT in the three groups of patients in the initial phase of observation revealed the comparability of samples (p = 0.665 for ALT, p = 0.169 for AST, and p = 0.658 for GGT) (Table 1).

Main study results

In Group 2–4 patients with alcoholic hepatitis, the initial ALT, AST, and GGT activity was 6.7–8.7 times higher (Table 1). An activity analysis of enzymes (hepatocyte cytolysis markers) showed a statistically significant tendency to their decrease in the course of alcoholic hepatitis treatment. The use of ademetionine as a hepatoprotective agent as part of the comprehensive mental health and detoxification regimen allowed ALT and AST activity to be reduced by 2.5 and 5.3 times, respectively. The use of lipoic acid under similar conditions helped to reduce the activity of serum aminotransferases in alcoholic hepatitis patients by 2.0–4.4 times relative to the initial level established on admission. GGT activity decreased to a lesser extent in the blood of Group 2 and 3 patients, reaching levels that exceeded the baseline values by 4.5 times.

The main study hypothesis assumed the possibility of achieving a better reduction in the level of hepatocyte cytolysis in the context of a combination hepatoprotective therapy with the simultaneous use of ademetionine and lipoic acid. The conducted studies suggest the possibility of a 4.5-fold decrease in serum ALT activity in Group 4 patients after a course of treatment lasting 15–18 days (Table 2). After the combination hepatoprotective therapy, AST activity decreased by 5.9 times. Under the same conditions, GGT activity decreased by 2.7 times relative to the initial level established on admission to the inpatient facility of the Drug Abuse Clinic. The best evidence of the efficacy of combination hepatoprotective therapy was a 1.7-fold reduction in serum GGT activity in the blood of Group 4 patients as compared to the same laboratory parameter of Group 2 and 3 patients. Thus, the combination therapy with sulfur-containing hepatoprotectors helped to achieve the lowest possible enzyme activity (cytolytic syndrome markers) in alcoholic hepatitis patients.

An analysis of changes in the concentration of total protein showed the maintenance of this parameter in alcoholic hepatitis patients within the values typical for healthy individuals (72.6 (68.3/75.5) g/L). Hepatoprotective therapy (with any of the used regimens) had no significant effect on this parameter of serum protein metabolism. An analysis of human serum albumin concentration in the blood of alcoholic hepatitis patients revealed its 11% reduction relative to the control group. The median serum albumin concentration in Group 2–4 patients was 38.5 (36.7/41.0) g/L, whereas in the control group, this parameter amounted to 43.2 (40.4/45.0) g/L. The therapy

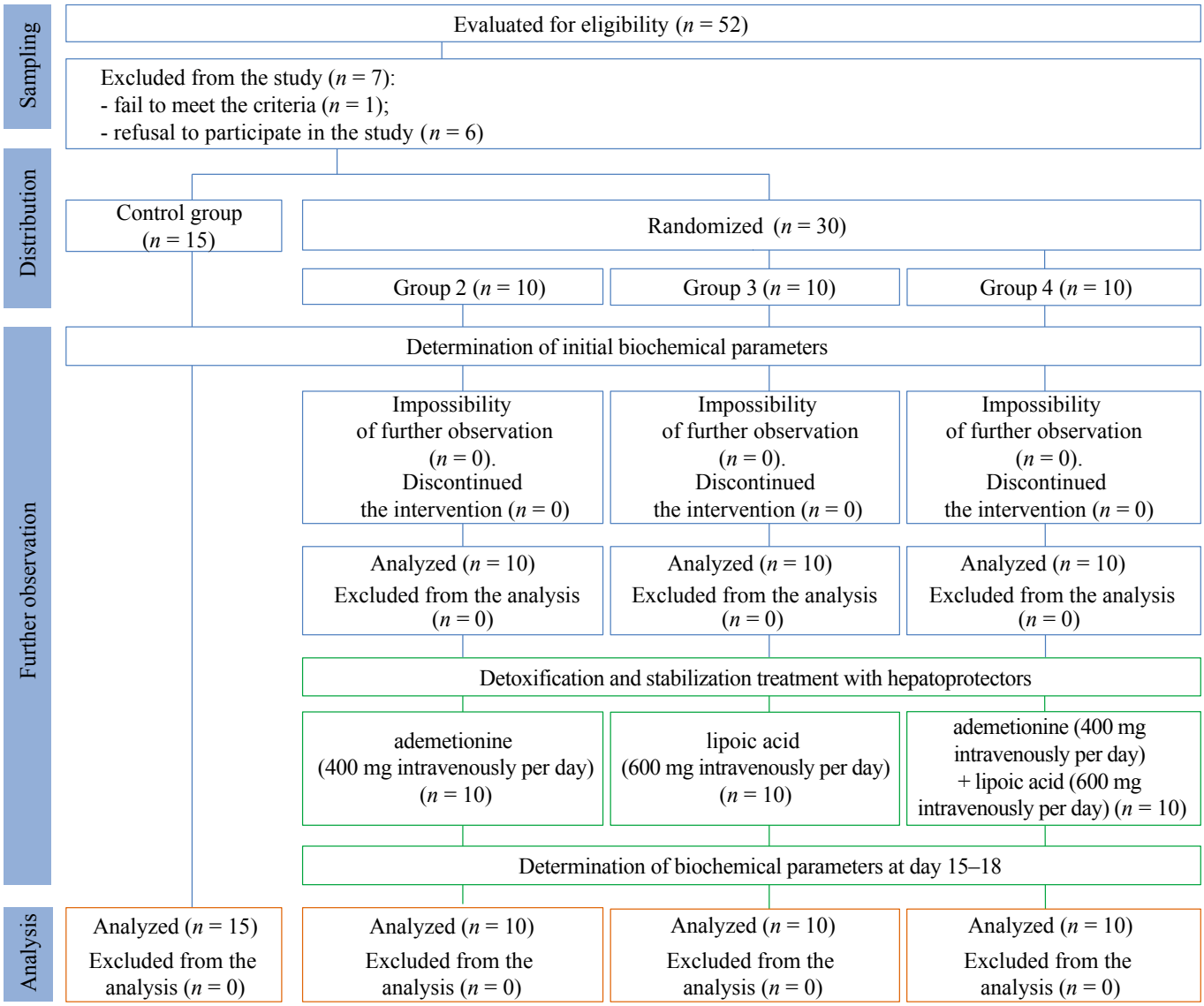

Fig. Block diagram of the conducted study

Note. The block diagram was created by the authors (as per CONSORT recommendations).

Рис. Блок-схема проведенного исследования

Примечание: Блок-схема выполнена авторами (согласно рекомендациям CONSORT). Сокращения: в/в — внутривенно; сут — сутки.

with sulfur-containing drugs was accompanied by tendencies toward the growth of serum albumin concentration, which after 15-–18 days of inpatient therapy returned to normal values typical for the people in Group 1. Although the protein synthesis function of the liver is disturbed in alcoholic hepatitis patients, the relatively low severity of the disease, as well as the high functional reserves and regenerative ability of the organ, allow serum albumin deficiency to be quickly supplemented. The concentration of total serum bilirubin amounted to 21.7 (17.5/27.4) μmol/L in Group 2–4 patients on admission to the inpatient facility, which exceeded the corresponding marker in the group of healthy individuals by 2.4 times (8.9 (5.3/13.5) μmol/L). An analysis of direct bilirubin concentration revealed a similar pattern of changes. The level of conjugated bilirubin amounted to 7.7 (5.6/8.7) μmol/L in the presence of alcoholic hepatitis, which was 2.9 times higher than the parameter of the control group (2.7 (2.3/3.3) μmol/L). After therapy, total bilirubin concentration decreased equally to 8.4 (6.2/8.8) μmol/L regardless of the type of used hepatoprotector. Under the same conditions, the concentration of direct bilirubin amounted to 2.9 (2.1/3.3) μmol/L, which corresponded to the values of corresponding parameters in the control group. Thus, no statistically significant differences were revealed in the dynamics of changes in the concentration of total protein, albumin, and bilirubin depending on the used sulfur-containing hepatoprotector.

The assessment of changes in the prooxidant-antioxidant balance involved determining the concentration of TBA reactive substances, as well as ferric reducing antioxidant power and antiradical activity. The initial oxidative homeostasis imbalance established on admission to the inpatient facility was characterized by an 87–95% elevation in the level of

Table 1. Median values of some parameters with quartiles (Me (Q1/Q3)) in the compared groups of patients prior to treatment and in the group of healthy individuals
Таблица 1. Медианные значения с квартилями (Me (Q1/Q3)) некоторых показателей в сравниваемых группах больных пациентов до лечения и в группе здоровых индивидуумов

Parameters	Control group (*n* = 15)	Group 2 (*n* = 10)	Group 3 (*n* = 10)	Group 4 (*n* = 10)	Significance level, *p*
ALT, U/L	22.3 (17.3/25.2)	178.2 (160.8/188.2)*	176.0 (157.8/190.2)*	183.5 (162.3/201.0)*	0.665#
AST, U/L	24.5 (18.0/28.7)	213.7 (182.1/228.5)*	210.1 (183.5/230.0)*	217.8 (181.4/229.5)*	0.169#
GGT, U/L	34.2 (28.6/38.5)	246.1 (215.5/266.4)*	230.2 (211.5/246.3)*	235.7 (214.7/251.2)*	0.658#
TAA (FRAP assay), mM of vitamin C	0.55 (0.52/0.60)	0.41 (0.35/0.45)*	0.39 (0.34/0.44)*	0.39 (0.34/0.44)*	0.600#
TAA (ABTS assay), mM of vitamin C	0.60 (0.55/0.64)	0.48 (0.43/0.51)*	0.45 (0.41/0.50)*	0.46 (0.43/0.51)*	0.753#
TBA reactive substances, arb. unit	0.38 (0.33/0.42)	0.71 (0.63/0.80)*	0.74 (0.67/0.80)*	0.72 (0.68/0.79)*	0.314#

*Notes: * statistically significant differences as compared to the parameter of Group 1; # significance level according to the Kruskal–Wallis test only for Groups 2, 3, and 4. Abbreviations: AST — aspartate aminotransferase; ALT — alanine aminotransferase; GGT — gamma-glutamyltransferase; TAA — total antioxidant activity; FRAP — ferric reducing antioxidant power; ABTS — 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; TBA — thiobarbituric acid.*
*Примечания: * статистически значимые различия при сравнении с показателем группы № 1; # уровень значимости по критерию Краскела — Уоллиса только для групп №2, 3, 4. Сокращения: AST — аспартатаминотрансфераза; ALT — аланинаминотрансфераза; GGT — гамма-глутамилтрансфераза; TAA — общая антиоксидантная активность; FRAP — железо-восстанавливающая способность плазмы крови; ABTS — 2,2'-азино-бис-(3-этилбензтиозолин-6-сульфокислоты) диаммониевая соль (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)); TBA — тиобарбитуровая кислота.*

Table 2. Median values with quartiles (Me (Q1/Q3)) of such parameters as aspartate aminotransferase, alanine aminotransferase, and gamma-glutamyltransferase in the compared groups of patients prior to and following treatment and in the group of healthy individuals
Таблица 2. Медианные значения с квартилями (Me (Q1/Q3)) таких показателей, как аспартатаминотрансфераза, аланинаминотрансфераза, гамма-глутамилтрансфераза в сравниваемых группах больных пациентов до и после лечения и в группе здоровых индивидуумов

Study groups	Stage of observation	Analyzed parameters		
		ALT, U/L	AST, U/L	GGT, U/L
Control group (healthy volunteers, *n* = 15)		22.3 (17.3/25.2)	24.5 (18.0/28.7)	34.2 (28.6/38.5)
2 (Ademetionine) *n* = 10	prior to treatment	178.2* (160.8/188.2)	213.7* (182.1/228.5)	246.1* (215.5/266.4)
	following treatment	71.0*^ (61.3/77.4)	40.2*^ (35.7/45.1)	150.6*^ (127.4/174.5)
3 (LA) *n* = 10	prior to treatment	176.0* (157.8/190.2)	210.1* (183.5/230.0)	230.2* (211.5/246.3)
	following treatment	86,2*^ (75.7/101.4)	48,4*^ (43.1/57.4)	152,9*^ (134.5/168.3)
4 (Ademetionine + LA) *n* = 10	prior to treatment	183.5 * (162.3/201.0)	217.8* (181.4/229.5)	235.7* (214.7/251.2)
	following treatment	41.6*^ (34.2/45.8)	37.5*^ (30.3/42.2)	88.2*^ (58.7/102.5)

*Notes: * statistically significant differences as compared to the parameter of Group 1; ^ statistically significant differences from the baseline parameter. Abbreviations: AST — aspartate aminotransferase; ALT — alanine aminotransferase; GGT — gamma-glutamyltransferase; LA — lipoic acid.*
*Примечания: * статистически значимые различия при сравнении с показателем 1 группы; ^ статистически значимые различия от исходного значения показателя. Сокращения: AST — аспартатаминотрансфераза; ALT — аланинаминотрансфераза; GGT — гамма-глутамилтрансфераза; LA — липоевая кислота.*

products of peroxide damage to biomolecules and a 20–29% decrease in the integral markers of antioxidant capacity (Table 3). The therapy with sulfur-containing drugs used due to their antioxidant capacity (which is particularly characteristic for lipoic acid) was accompanied by tendencies to normalization of free radical homeostasis. The total antioxidant activity in blood plasma determined using the ferric reducing method increased by 12% in the context of therapy with ademetionine. After treatment with ademetionine, the total antiradical activity in blood plasma increased by 21% in alcoholic hepatitis patients. The use of lipoic acid to treat Group 3 and 4 patients contributed to a 52–64% increase in the analyzed markers relative to the initial value. Thus, lipoic acid exhibited the most pronounced antioxidant effect; however, no additional potentiation was observed when it was combined with ademetionine.

Unlike antioxidant activity, the level of TBA reactive substances in the erythrocyte suspension of Group 2–4 patients decreased in the course of hepatoprotective therapy. The use of ademetionine or lipoic acid resulted in a 21–28% decrease in this laboratory parameter. The combination therapy with the administration of ademetionine and lipoic acid contributed to a 36% reduction in the considered oxidative stress marker in the blood (Table 3).

An analysis of the thiol link of the antioxidant support network revealed a downward trend in the concentration of thiol-containing components in the blood plasma and erythrocytes of patients with alcoholic liver damage (Table 4). Prior to the therapy, the concentration of protein SH groups and the level of glutathione in the blood of Group 2–4 patients were 23–25% and 17–19% lower, respectively, than the corresponding parameters of the control group. Therapy with ademetionine was accompanied by a rather modest 12% increase in glutathione concentration, whereas the level of thiol groups underwent no statistically significant changes. The use of lipoic acid yielded slightly better results in restoring laboratory markers of thiol homeostasis: glutathione concentration increased by 17%, and the level of plasma SH groups rose by 18%. The best dynamics of analyzed markers was achieved with combination hepatoprotective therapy. In this case, the level of glutathione in erythrocyte suspension increased by 25% and the level of SH groups rose by 33%. After a three-week course of treatment, both blood parameters in Group 4 patients corresponded to the conditional baseline characteristic for the control group (Table 4). Thus, an analysis of changes in the integral parameters of prooxidant-antioxidant balance revealed no advantages of the combined use of sulfur-containing compounds. However, a more detailed analysis of the thiol link of the antioxidant support network revealed better rates of normalization of oxidative homeostasis with the combined use of ademetionine and lipoic acid.

Additional study results

No additional results were obtained during the study.

Adverse events

No adverse events were observed during the study.

DISCUSSION

Research limitations

Factors potentially limiting the clinical relevance of the data presented in the study are as follows: 1) relatively small sample size; 2) short period of observation. Hepatoprotective agents are usually expected to be effective if they are used in the longest course of treatment possible. However, in this study, the treatment was limited to the stay of patients at the Drug Abuse Clinic. Follow-up was limited by the potentially low compliance of alcohol-abusing patients. An additional limitation of the study consists in the absence of a comparison group comprising patients that do not receive hepatoprotective or other detoxification therapy. However, the formation of such a group does not comply with the current legislation on standards related to healthcare delivery in the Russian Federation.

Generalizability/extrapolation

The results of this study can be extended to other clinical and experimental settings. Taking into account similar pathobiochemical processes in liver parenchyma damage, the combined use of sulfur-containing hepatoprotectors (ademetionine and lipoic acid) can effectively reduce the severity of cytolytic syndrome in liver damage caused by different etiopathogenetic factors. In particular, similar positive effects of therapy can be observed in other forms of toxic and viral hepatitis and ischemic liver injury.

Summary of the main study result

The combination therapy with sulfur-containing hepatoprotectors helped to achieve the lowest possible enzyme activity (cytolytic syndrome markers) in patients with alcoholic hepatitis.

Discussion of the main study result

The hepatoprotective efficacy assessment of sulfur-containing drugs was based on the dynamics analysis of changes in the level of hepatocyte cytolysis marker since in clinical and laboratory practice, these are the simplest but quite accurate indicators of liver parenchyma damage. An activity analysis of aminotransferases and GGT in the context of separate administration of ademetionine or lipoic acid set a downward trend in the severity of the cytolytic syndrome, though not to the control group level; in the absence of placebo control, it is difficult to judge the presence of a true hepatoprotective effect. In this case, the key factor in restoring the liver structure and function can be alcohol cessation under inpatient treatment, as well as infusion detoxification, with infusion of large fluid volumes. Nevertheless, the present study suggests the possibility of enhancing the cytoprotective effect of used drugs through their combined administration. The use of ademetionine together with lipoic acid helped to achieve lower ALT, AST, and GGT activity in the blood plasma of Group 4 patients than in the case of separate use of sulfur-containing drugs. Thus, after the treatment, the plasma activity of ALT and GGT in Group 4 patients was 1.7–2.1 times lower than the corresponding markers in Group 2 and 3 patients at the same stage of observation. These results indicate a synergistic effect with the combined use of ademetionine and lipoic acid and demonstrate the fea-

Table 3: Median values with quartiles (Me (Q1/Q3)) of such parameters as total antioxidant activity (FRAP assay), total antioxidant activity (ABTS assay), and TBA reactive substances in the compared groups of patients prior to and following treatment and in the group of healthy individuals

Таблица 3. Медианные значения с квартилями (Me (Q1/Q3)) таких показателей, как общая антиоксидантная активность (FRAP-метод), общая антиоксидантная активность (ABTS-метод) и ТБК-реактивные продукты, в сравниваемых группах больных пациентов до и после лечения и в группе здоровых индивидуумов

Study groups	Stage of observation	Analyzed parameters		
		TAA (FRAP assay), mM of vitamin C	TAA (ABTS assay), mM of vitamin C	TBA reactive substances, arb. unit
Control group (healthy volunteers, *n* = 15)		0.55 (0.52/0.60)	0.60 (0.55/0.64)	0.38 (0.33/0.42)
2 (Ademetionine) *n* = 10	prior to treatment	0.41* (0.35/0.45)	0.48* (0.43/0.51)	0.71* (0.63/0.80)
	following treatment	0.46* (0.37/0.49)	0.58^ (0.54/0.63)	0.56*^ (0.50/0.64)
3 (LA) *n* = 10	prior to treatment	0.39* (0.34/0.44)	0.45* (0.41/0.50)	0.74* (0.67/0.80)
	following treatment	0.63^ (0.56/0.68)	0.74^ (0.67/0.78)	0.53*^ (0.48/0.58)
4 (Ademetionine + LA) *n* = 10	prior to treatment	0.39* (0.34/0.44)	0.46* (0.43/0.51)	0.72* (0.68/0.79)
	following treatment	0.62^ (0.57/0.69)	0.70^ (0.64/0.75)	0.46*^ (0.41/0.50)

*Notes: * statistically significant differences as compared to the parameter of Group 1; ^ statistically significant differences from the baseline parameter. Abbreviations: TAA — total antioxidant capacity; FRAP — ferric reducing antioxidant power; ABTS — 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; TBA — thiobarbituric acid; LA — lipoic acid.*

*Примечания: * статистически значимые различия при сравнении с показателем 1 группы; ^ статистически значимые различия от исходного значения показателя. Сокращения: ТАА — общая антиоксидантная активность; FRAP — железо-восстанавливающая способность плазмы крови (Fluorescence Recovery After Photobleaching); ABTS — 2,2'-азино-бис-(3-этилбензтиозолин-6-сульфокислоты) диаммониевая соль (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)); ТВА — тиобарбитуровая кислота; LA — липоевая кислота.*

Table 4. Median values with quartiles (Me (*Q*1/*Q*3)) of such parameters as glutathione and SH groups in the compared groups of patients prior to and following treatment and in the group of healthy individuals

Таблица 4. Медианные значения с квартилями (Me (*Q*1/*Q*3)) таких показателей, как глутатион и SH-группы, в сравниваемых группах больных пациентов до и после лечения и в группе здоровых индивидуумов

Study groups	Stage of observation	Analyzed parameters	
		Glutathione, μmol/L	SH groups, 100*ODU/g of protein
Control group (healthy volunteers, *n* = 15)		2.56 (2.43/2.70)	0.57 (0.54/0.61)
2 (Ademetionine) *n* = 10	prior to treatment	2.12 (1.95/2.20)*	0.44 (0.41/0.46)*
	following treatment	2.38 (2.21/2.47)*^	0.47 (0.44/0.50)*
3 (LA) *n* = 10	prior to treatment	2.08 (1.90/2.16)*	0.44 (0.41/0.47)*
	following treatment	2.44 (2.35/2.53)^	0.52 (0.49/0.53)*^
4 (Ademetionine + LA) *n* = 10	prior to treatment	2.08 (1.94/2.18)*	0.43 (0.41/0.46)*
	following treatment	2.60 (2.46/2.65)^	0.57 (0.53/0.59)^

*Notes: * statistically significant differences as compared to the parameter of Group 1; ^ statistically significant differences from the baseline parameter. Abbreviation: LA — lipoic acid.*

*Примечания: * статистически значимые различия при сравнении с показателем 1 группы; ^ статистически значимые различия от исходного значения показателя. Сокращение: LA — липоевая кислота.*

sibility of using these hepatoprotectors as part of the comprehensive treatment of alcoholic hepatitis patients.

Changes in the prooxidant-antioxidant balance indicate the development of oxidative stress in patients with alcoholic liver damage. The analysis of oxidative homeostasis usually constitutes a nontrivial task due to the complex multilevel organization of the nonspecific resistance system. This task can be somewhat simplified for the purposes of clinical and laboratory practice by reducing the range of markers to total antioxidant activity and one of the parameters characterizing the accumulation of products of free radical damage to biomolecules. A comparison of our data with the results of other authors, obtained when studying other nosologic forms [17, 18], suggests a low intensity of oxidative stress. The validity of the obtained data is confirmed by the comparable values of oxidative stress markers found in alcoholic patients by other authors [19, 20]. In spite of a relatively small decrease in antioxidant activity and an increase in the level of TBA reactive substances in the blood of Group 2–4 patients, a three-week therapy with ademetionine failed to achieve complete normalization of oxidative homeostasis. The administration of lipoic acid had a more pronounced antioxidant effect, which was accompanied by a significant increase in the ferric reducing ability and antiradical activity of plasma to the level exceeding the control group values. The combined use of ademetionine and lipoic acid was characterized by similar tendencies toward normalization of prooxidant-antioxidant imbalance, which was largely expected given the properties of this vitamin-like compound. Due to the presence of two SH groups, reduced lipoic acid exhibits strong antioxidant properties. The therapeutic efficacy of this thiol-containing drug is relatively low due to the peculiarities of its pharmacokinetic profile: short half-life and low bioavailability (about 30%), which can be attributed to hepatic degradation, poor solubility, and disintegration in the digestive tract [21]. Nevertheless, the ways to improve the therapeutic efficacy are related to using different innovative forms or to the parenteral administration, which was used in this work.

The high antioxidant activity of lipoic acid, which was associated with SH groups, was also confirmed by the changes in thiol homeostasis in the blood of Group 3 and 4 patients. In this case, the combination hepatoprotective therapy was found to be associated with the increase in the concentration of glutathione in erythrocytes and protein sulfhydryl groups in the blood plasma. It is likely that the separate use of antioxidant agents is not enough to achieve a cytoprotective effect in the presence of toxic damage to liver parenchyma; however, through combination with ademetionine, it is possible to achieve a synergistic effect.

Of interest are data described in the article [22] showing that alpha-lipoic acid elevates the levels of *S*-adenosylhomocysteine and depletes the levels of *S*-adenosylmethionine. In this setting, the administration of exogenous ademetionine can be one of the ways to enhance the combined action of the drugs, although the data presented by the present authors were obtained by administering a dosage of lipoic acid exceeding the therapeutic concentration for humans by 4–10 times. Another basis for the synergistic effect of the combined use of lipoic acid and ademetionine lies in the different molecular targets of the drugs. While ademetionine supports liver detoxification by supplying the active form of methionine, the methyl donor, lipoic acid is a cofactor of the pyruvate dehydrogenase complex, which ensures the transition from anaerobic to aerobic energy processes, and a powerful antioxidant.

CONCLUSION

The study revealed a synergistic protective effect of ademetionine and lipoic acid in alcoholic hepatitis. Following a three-week course of treatment, the combination therapy with two sulfur-containing hepatoprotectors helped to achieve 1.7–2.1 times lower plasma activity of ALT and GGT as compared to the corresponding markers in the groups of patients receiving only one of the drugs. The combined use of ademetionine and lipoic acid can have an effect on different components of nonspecific resistance, specifically the antioxidant system and functional detoxification system. This was confirmed by better rates of normalization of metabolic parameters of thiol-containing antioxidants (glutathione and SH groups), which, unlike in the case of monotherapy, increased to the normal level characteristic for healthy individuals.

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