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Research Article Rus

Pharmacological correction of metabolic disorders in experimental acute pancreatitis on the background of chronic alcohol intoxication

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Abstract

Introduction. Acute pancreatitis and pre-existing diseases (chronic alcohol intoxication) are challenging issues of modern surgery in terms of frequency of deaths and the number of complications.

Objective. To identify the best combination of immunomodulators, antioxidants and hepatoprotectors to correct immunometabolic disorders in acute destructive pancreatitis on the background of chronic alcohol intoxication (CAI).

Materials and methods. Studies were conducted on 377 healthy adult Wistar rats weighing 150-200 g. Alcohol intoxication was modeled by forced intragastric administration of a 20% ethanol solution at a dose of 3 ml/kg or 2.92 g/kg at 24 hours for 5 days, 30 days (CAI-30) or 60 days (CAI-60). Acute destructive pancreatitis (ADP) was modeled after R.N. Wang et al. (1995), modified by S.A. Alekhin et al. (2006). The efficacy of combining Hepon (5 mg/kg, orally, at 24 hours, No. 14), Hypoxen (750 mg/kg, orally, at 24 hours, No. 14), Phosphogliv (800 mg/kg, orally, at 24 hours, No. 14), Glutoxim (20 mg/kg, intramuscularly, at 24 hours, No. 5), Mexidol (50 mg/kg, intraperitoneally, at 24 hours, No. 14) and Heptral (760 mg/kg, intraperitoneally, at 24 hours , No. 5) was examined in animals with ADP on the background of CAI-30 and CAI-60.

Results and discussion. Different degrees of ethanol intoxication depending on time leads to the development of an impaired capacity of hepatocytes, as well as immune and metabolic disorders. The administration of Phosphogliv, Hypoxen and Hepon in case of CAI-30+ADP normalizes 12.1% and corrects 60.6% of the changed values. The combination of Heptral, Glutoxim and Mexidol normalizes 57.8% and corrects 42.4% of the values. In case of CAI-60+ADP, Phosphogliv, Hypoxen and Hepon normalizes 11.8% and corrects 38.2% of the values. The combination of Heptral, Glutoxim and Mexidol normalizes 17.6% and corrects 67.6% of the values, respectively.

Conclusion. In case of ADP along with CAI-30 and CAI-60, the combination of Heptral, Glutoxim and Mexidol is more preferable than that of Phosphogliv, Hypoxen and Hepon.

Keywords

alcohol intoxication, metabolic disorders, pharmacological correction.

Introduction

Acute or chronic intoxication with alcohol and its substitutes, as well as alcohol-related diseases, is among the most important medical and social problems. The organs of the pancreatobiliary system are the most sensitive to the effects of alcohol, which is the main etiological factor in the development of diseases of this system (Dunaevskaya and Antyufrieva 2013, Khalyutin et al. 2015, Zhao et al. 2018).

Some Russian and foreign authors state that the alcohol abuse and the development of acute pancreatitis (AP) causes a decrease in the protective factors at the organism level and prevents the formation of resistance to microbial flora, which increases the risk of suppurative complications of destructive pancreatitis and reduces the processes of reparative regeneration (Vinnik et al. 2012, Mkhitarov et al. 2015, Minkov et al. 2015, Seifert et al. 2017).

Oxidative stress (OS) is the most common pathogenetic mechanism of most diseases, its essence being the imbalance of lipid peroxidation (LPO) products and the activity of antioxidant systems. In complicated AP, due to OS, oxidized products get formed that affect the state of regulatory systems in an ambiguous way (Vinnik et al. 2011, Sunyaykina et al. 2015, Goyal et al. 2017, Schmidt et al. 2017).

A number of works prove that, besides the gas-transport function, red blood cells can regulate the state of immune homeostasis, which is vital in various hepatic and pancreatic disorders (Konoplya et al. 2013, Gorsky et al. 2014, Sunyaykina et al. 2015, Chen et al. 2017).

The development of AP on the background of such diseases as chronic alcohol intoxication, diabetes, systemic inflammatory diseases is certain to have an adverse effect on the course and outcome of the underlying disease. This fact is important both in surgical treatment and and when choosing a multi-component pharmacotherapy, which allows influencing several links of the disease pathogenesis (Karaulov et al. 2012, Atayoğlu et al. 2017).

Target organs in AP are pancreatic, brain, liver, immune system tissues and red blood cells. The negative impact of ethanol and its metabolites includes a significant violation of homeostasis. The development of AP on the background of CAI, not independently, but jointly, is the cause of secondary immunodeficiency, stimulation of LPO processes, changes in the capacity of red blood cells, metabolic disorders, and reparative regeneration processes (Gorsky et al. 2014, Loktionov et al. 2015, Chen et al. 2016).

In this regard, certain aspects of the state of the lipid peroxidation processes, of antioxidant protection systems, of immune resistance in CAI, of metabolic and immune changes in AP in the context of alcohol intoxication require further experimental and clinical studies.

So it is obviously imperative to assess the pathogenetic mechanisms of AP in the context of CAI and to carry out clinical studies of non-biliary (alcoholic) pancreatitis. This will serve as the basis for the development and introduction of new methods of pharmacological rehabilitation, based on immunotropic and membrane-protective drugs (Konoplya et al. 2013, Khalyutin et al. 2015, Mkhitarov et al. 2015, Jiang et al. 2015, Razumova et al. 2016, Soares et al. 2017).

Objective to determine the specific features of immunometabolic changes in acute pancreatitis in the context of short-term and chronic alcohol intoxication and to develop the ways of pharmacological correction of the detected violations.

Materials and methods

Studies were conducted on 377 healthy adult Wistar rats weighing 150–200 g. All the studies were conducted at the same time of day, from 8 am to 12 pm; animal maintenance and slaughter of animals were conducted in compliance with the principles set forth in the Convention on the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, 1986) and according to the rules of laboratory practice of the Russian Federation (Order of the Ministry of Healthcare of the Russian Federation No. 267 of June 19, 2003).

Experimental models

Alcohol intoxication was modeled by forced intragastric administration of a 20% ethanol solution at a dose of 3 ml/kg or 2.92 g/kg at 24 hours for 5 days. In case of chronic alcohol intoxication (CAI), ethanol was injected for 30 or 60 days (CAI-30, CAI-60).

ADP was modeled after R.N. Wang et al. (1995) modified by S.A. Alekhin et al. (2006) by ligating the duct of the left and right lobes of the pancreas and by subsequent triple stimulating pancreatic secretion. Stimulation was carried out 30 minutes after surgery by administering Proserinum at a dose of 0.2 mg/kg after 60 minutes. As early as on the first day after the simulation, a study through the microscope revealed edema of the pancreas, as well as foci of centrolobular necrosis with moderate neutrophil infiltration of the necrotic foci and pancreatic stroma. ADP was simulated immediately with the short-term administration of ethanol or on the 25th and 55th day after its long-term administration.

To obtain morphological confirmation of the simulated pathogen and to carry out a comprehensive assessment of the efficacy of the drugs, a histological study of the liver and pancreas was performed. For this, pieces of these organs were fixed in 10% neutral formalin on 0.1 M phosphate buffer pH=7.2, embedded in paraffin. Paraffin sections 7–10 μm thick were stained with Regaud's hematoxylin and eosin.

Dosages, methods and frequency of administration of medicines are shown in Table 1. Dosages for administering medicines to the experimental animals were calculated using dose conversion factors (mg/kg per mg/m^2) for rats, depending on body weight, or empirically, basing on $LD_{50.}$

Table 1. Dosages, mode and frequency of drug administration in animals with an experimental model of acute destructive pancreatitis

Drug	Mode of administration	Single dose, mg/kg	Dose regimen	
			Number of injections	Dosing interval, h
Hepon (Thr-Glu-Lys-Lys-Arg-Arg-Glu-Thr-Val-Glu-Arg-Glu-Lys-Glu) (IMMAFARMA LLC, Russia)	orally	5	14	24
Hypoxen (poly-(2,5-dihydroxyphenylene)-4-sodium thiosulfonate) (OLIFEN LLC, Russia)	orally in 1% starch suspension	750	14	24
Phosphogliv (phosphotidin choline+trisodium salt of glycyrrhizinic acid) (Pharmstandard-UFA Vitamin Factory JSC, Russia)	orally in 1% starch suspension	800	14	24
Glutoxim (bis-(gamma-L-glutamyl)-L-cysteinyl-bis-glycine disodium salt) (Pharma V.A.M CJSC, Russia)	intramuscularly	20	5	24
Mexidol (2-ethyl-6-methyl-3-hydroxypyridine succinate) (ALSI-Pharma CJSC, Russia)	intraperitoneally	50	14	24
Heptral (S-Adenosyl-L-methionine 1,4-butanedisulphonate) (Hospira S.p.A, Via Fosse Ardeatine, 2-20060 Liscate (MI), Italy)	intraperitoneally	760	5	24

Note: rats were slaughtered 24 hours after the last injection of ethanol or ethanol and drugs.

Biochemical research methods

Blood sampling in the experimental animals was carried out under anesthesia, by intracardiac injection. Plasma was obtained from heparinized blood by centrifuging for 5 min at 400 g. To assess the functional status of hepatocytes in blood plasma, the enzyme activities were determined: aspartate and alanine aminotransferase (AST, ALT), alkaline phosphatase (ALP), gamma glutamine transpeptidase (GGT), as well as the content of bilirubin by the standard clinical biochemical methods on an automated biochemical analyzer Cobas c311 (Roche Diagnostics, Germany) with Analyticon® **Biotechnologies AG reagents (**Germany). The prothrombin index (PTI) was determined by a clotting method on a semi-automated hemostasis analyzer Start 4 (Stago, France) using Human reagents (Germany). The concentration of fibrinogen was determined by the Clauss clotting method, using a semi-automated hemostasis analyzer Start 4 (Stago, France) with Diagnostica Stago reagents (France).

The intensity of the LPO processes was assessed by the content of acyl hydroperoxides (AHP) and malonic dialdehyde (MDA) in blood plasma. To assess the state of the antioxidant system, the activity of superoxide dismutase (SOD) was determined using ready commercial kits from Bender Medsystems (Austria) and a Cayman Chemical Catalase Assay Kit (USA). The total antioxidant activity (TAA) was determined by a method based on the inhibition degree of ascorbate-induced and iron-induced oxidation of tween-80 to MDA. The level of stable metabolites of nitric oxide (SMNO) was determined by the Griess reaction, using a solid-phase ELISA kit from R&D Systems (UK). All ELISA results were recorded using a Tecan-Sunrise microplate photometer (Austria).

Immunological research methods

To develop a humoral immune response (HIR), the sheep red blood cells antigen was intraperitoneally injected at the rate of $2x10^9$ cells per 1 kg of body weight. The HIR intensity was assessed on the 5th day after immunization by determining the number of antibody-forming cells in the spleen (Malberg and Sigl 1987). Delayed-type hypersensitivity (TDTH) in rats was induced by intraperitoneal administration of 108 sheep erythrocytes (SE) in 0.5 ml of a 0.15 M solution of sodium chloride (sensitizing dose). After 4 days, 106 sheep erythrocytes in 0.1 ml of a 0.15 M solution of sodium chloride (resolving dose) were injected into the right foot pad. After 24 hours, the regional (at the site of injecting sheep erythrocytes) and contralateral popliteal lymph nodes were harvested. The DTH intensity was assessed by the difference in the masses of the regional and contralateral lymph nodes and the difference in the number of karyocytes in them.

Neutrophils were isolated from the obtained blood by the Ficoll-Urografin density gradient method (ρ=1.078). Their phagocytic activity was assessed by the generally accepted method, determining the phagocytic index (PI), phagocytic number (PN) and phagocytosis activity index (PAI). The activity of oxygen-dependent neutrophil systems was assessed on a PD 303 Apel spectrophotometer (Japan) by the reduction of nitro-blue tetrazolium (NBT-test), spontaneous (NBT-sp.) and induced by zymosan (NBT-ind. n/z), estimating the functional reserve (Zinkin and Godkov 2004).

The study of the metabolic activity of red blood cells

The automated red blood cells count and the calculation of the hemoglobin content in the obtained blood were performed on a Sysmex XP 300 hematology analyzer (Germany). Red blood cells were obtained from heparinized blood, after being centrifuged for 5 min at 400 g and the plasma being separated. The erythrocyte mass was clarified twice in 20 ml of 10 mM Na-phosphate buffer (pH=7.4) containing 0.9% sodium chloride and 3% dextran T-500 for 30 minutes at 37° C. After centrifugation, the supernatant was removed by aspiration, and the ery-

throcyte mass was additionally purified on a chromatographic column of HBS-cellulose.

The total sorption capacity of erythrocytes (SCE) due to the external architectonics of the cell membrane and the sorption capacity of the erythrocyte glycocalyx (SCG) were determined[1].

The statistical processing of the obtained study results was carried out according to the criteria of statistical analysis of variance with calculating mean values (M), arithmetic mean errors (m), by means of using the Microsoft Excel computer software package 2010. The significance of differences was assessed by the t-criterion. Differences with $p<0.05$ were considered statistically significant. The confusion degree of laboratory parameters, their changes under the influence of pharmacological agents, the rating algorithm of laboratory parameters, and the sum of the correction degrees of immunometabolic parameters were determined by the formulas by A.M. Zemskova (2008, 2013).

Results and discussion

After 5-fold administration of ethanol, only an increase in AST activity and the AST/ALT ratio due to this enzyme was detected in the animals. In rats with ADP on the background of 5-day alcohol intoxication, there was observed an increase in bilirubin concentration, the activity of AST, ALT, ALP, GGT and the AST/ALT ratio, a decrease in fibrinogen level, and an increase in PTI (Table 2).

[1] Note. The immunological and biochemical parameters were determined in the Laboratory of Immunofermental Analysis of the Research Institute of Ecological Medicine of Kursk State Medical University and the Clinical Laboratory of City Clinical Hospital No. 4 (Kursk), for which we extend our deep gratitude to their employees.

The introduction of ethanol for 30 days increased PTI, the activity of AST, ALT, GGT, bilirubin content, but decreased fibrinogen concentration and AST/ALT ratio (Fig. 1).

In rats with ADP with CAI-30, there was a more pronounced increase in ALT activity, bilirubin concentration, ALP and GGT activity, a decrease in the AST/ALT ratio below 1, an increase in the GGT/AST ratio, while keeping the syndrome of insufficiency of synthetic processes (decrease in fibrinogen level) at the same level, and activation of blood coagulation system (increased PTI) (Fig. 1).

The 60-day ethanol intoxication, when compared with CAI-30, more significantly increased the AST, ALT, GGT activity, the AST/ALT and GGT/AST ratios, and the content of bilirubin. At the same time, with CAI-60, the concentration of fibrinogen normalized and PTI decreased, but not of the control values (Fig. 1). In rats with ADP on the background of CAI-60, compared with administration of only ethanol for the same period of time or with ADP on the background of CAI-30, the activity of ALT, AST, ALP increased, whereas the rates of enzymatic activity and fibrinogen content decreased (Fig. 1).

With ADP in the context of CAI-60, the combination of Hepon, Hypoxen and Phosphogliv normalized PTI and the fibrinogen level and corrected the activity of AST, ALT, ALP, GGT and bilirubin concentration, but not enough to match the indicators of healthy animals (Fig. 1).

The composition of Glutoxim, Mexidol and Heptral, compared with the previous combination of drugs, additionally corrected the activity of ALP, GGT and the GGT/AST ratio (Fig. 1). The obtained data make it possible to conclude that in animals with ADP on the background of alcohol intoxication, the development of the main biochemical syndromes of liver damage is observed: cytolytic, intracellular cholestasis, toxic inflammatory-type damage and insufficiency of synthetic processes, and the intensity

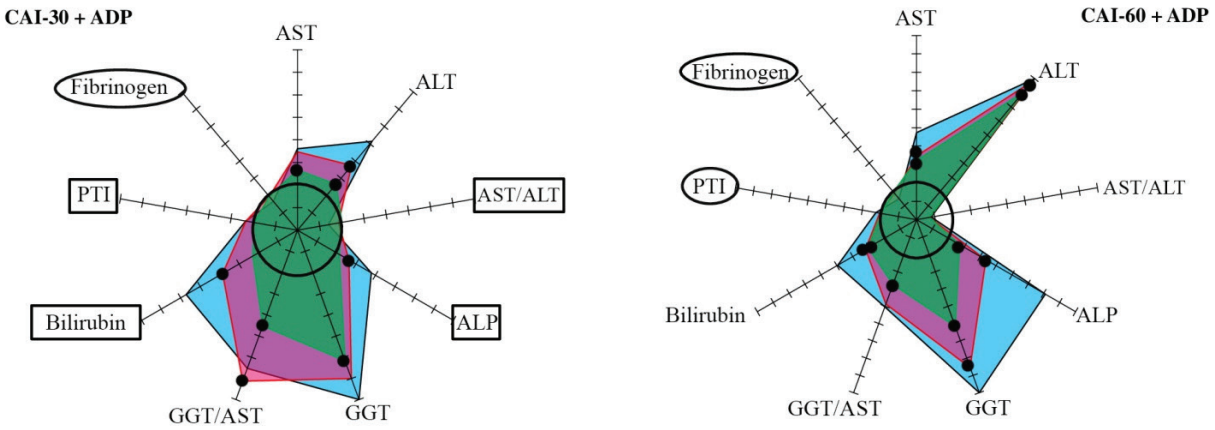

Figure 1. Correction of impaired capacity of hepatocytes in experimental ADP on the background of 30- and 60-day administration of ethanol.Key: line – radius of the indicator circle – of intact animals; blue – indicators of rats with 30- and 60-day CAI+ADP; red – indicators of rats with 30- and 60-day CAI+ADP+Hepon, Hypoxen and Phosphogliv; green – indicators of rats with 30- and 60-day CAI+ADP+Glutoxim, Mexidol and Heptral; black dot – corrected indicator ($p<0.05$); oval – the indicator normalized by the combination of Hepon, Hypoxen, Phosphogliv ($p>0.05$); square – indicator normalized by the combination of Glutoxim, Mexidol, Heptral ($p>0.05$).

Table 2. Functional Activity of Hepatocytes in ADP on the Background of Short-term Administration of Ethanol (M+m)

No	Indicators	Unit of measure	1	2	3
			Control	Ethanol – 5 days	ADP+ethanol 5 days
1	AST	U/L	21.3±1.6	26.1±2.1*1	33.8±3.2*1.2
2	ALT	U/L	20.1±2.4	19.6±1.4	27.7±2.2*1.2
3	AST/ALT ratio	AST/ALT	1.06±0.02	1.3±0.05*1	1.2±0.06*1
4	ALP	U/L	249.2±18.8	261.4±12.5	345.8±10.4*1.2
5	GGT	U/L	4.8±0.22	5.1±1.0	7.1±1.4*1.2
6	GGT/AST ratio	GGT/AST	0.23±0.01	0.2±0.02	0.21±0.01
7	Bilirubin	μmol/L	5.74±1.2	5.9±0.9	7.7±1.4*1.2
8	PTI	%	60.1±1.6	62.7±2.4	70.1±3.1*1.2
9	Fibrinogen	g/L	3.12±0.09	3.3±0.2	2.84±0.1*1.2

Note: in this and subsequent tables, * indicates significant differences in arithmetic means (p=0.05); the numbers next to * show which group of indicators these differences refer to.

of these syndromes increases along with an increase in ethanol intoxication from 30 to 60 days.

Under these conditions, the use of pharmacological correction by means of drugs with immunomodulatory, antioxidant and membrane-protective properties proved to be effective.

Further pharmacological efficacy of immunomodulators, antioxidants and hepatoprotectors to correct disorders of innate and adaptive immunity was studied in case of destructive pancreatitis in combination with alcohol intoxication. A short-term intake of ethanol reduces phagocytic activity (a decrease in phagocytic index (PI), phagocytic number (PN), index of phagocytic activity (IPA)) along with a simultaneous increase in the oxygen-dependent metabolism of peripheral blood neutrophils in NBT-tests, spontaneous and induced by non-opsonized zymosan. However, when compared with intact animals, the functional activity reserves decreased (activity ratio for non-opsonized zymosan (ARn) and activity ratio for opsonized zymosan (ARo)).

In rats with ADP on the background of short-term ethanol intoxication, phagocytic metabolic activity of granulocytes decreased even more, whereas oxygen-dependent one increased. At the same time, all functional activity reserves of cells (ARn, ARo and opsonization index (OI)) decreased.

Modelling chronic alcohol intoxication by means of 30-day intragastric administration of ethanol, in the same was as in animals given a toxicant for 60 days, inhibited phagocytic (phagocytic rate (PR), PN, IPA) and stimulated oxygen-dependent activity of peripheral blood neutrophils. The level NBT-ind. o/z in these groups did not differ from the indicators of the control group of animals (Table 3).

Table 3. Metabolic capacity of circulating neutrophils in acute destructive pancreatitis on the background of 30-day and 60-day chronic alcohol intoxication (M+m)

No	Indicators	Unit of measure	1	2	3	4
			Control	ADP+ Ethanol	Hepon+Hypoxen+ Phosphoglip	Glutoxim+Mexidol+ Heptral
30-day CAI and ADP						
1	PN	abs.	2.8±0.1	2.2±0.08*1	2.48±0.08*1,2	2.9±0.17*2,3
2	PR	%	77.2±1.6	37.3±2.2*1	47.0±4.08*1,2	66.3±3.4*1-3
3	IPA	—	2.2±0.09	0.8±0.08*1	1.57±0.1*1,2	1.91±0.03*1-3
4	NBT-ind.	mOD	0.8±0.02	1.9±0.09*1	1.67±0.09*1,2	1.05±0.05*1-3
5	NBT-ind n/z	mOD	1.3±0.02	2.0±0.15*1	1.72±0.05*1,2	1.32±0.03*2,3
6	NBT-ind o/z	mOD	1.6±0.03	2.2±0.12*1	1.77±0.08*1,2	1.58±0.03*2,3
7	OI	—	1.2±0.04	1.1±0.02*1	1.05±0.02*1,2	1.27±0.04*2,3
8	ARo	—	2.0±0.06	1.07±0.09*1	1.033±0.08*1	1.62±0.04*1-3
9	ARn	—	1.6±0.05	1.15±0.09*1	1.08±0.04*1,2	1.56±0.07*2,3
60- day CAI and ADP						
1	NBT-sp.	mOD	0.8±0.02	1.95±0.03*1	2.0±0.02*1	1.7±0.06*1-3
2	NBT-ind n/z	mOD	1.3±0.02	2.2±0.06*1	2.1±0.05*1	1.8±0.02*1-3
3	NBT-ind o/z	mOD	1.6±0.03	2.1±0.2*1	2.24±0.1*1	1.12±0.05*1-3
4	OI	—	1.2±0.04	0.96±0.07*1	1.07±0.12*1	0.86±0.09*1
5	ARo	—	2.0±0.06	1.14±0.02*1	1.12±0.03*1	1.11±0.02*1
6	ARn	—	1.6±0.05	1.1±0.04*1	1.05±0.03*1	1.1±0.06*1
7	PN	abs.	2.8±0.1	1.9±0.3*1	2.1±0.1*1	2.4±0.2*1
8	PR	%	77.2±1.6	34.3±0.9*1	35.4±2.7*1	45.0±3.4*1-3
9	IPA	—	2.2±0.09	0.7±0.1*1	0.74±0.04*1	1.1±0.02*1-3

Note: in this and subsequent tables, * indicates significant differences in arithmetic means (p=0.05); the numbers next to * show which group of indicators these differences refer to.

In the case of ADP with CAI-30, and to a greater degree with CAI-60, there was a marked decrease in PI, PN, IPA and an increase in the NBT-sp., NBT-ind. o/z and n/z. At the same time, the reserves of oxygen-dependent phagocyte activity (ARo, ARn, and OI in rats chronically exposed to alcohol, including in combination with experimental AP, were reduced (Table 3).

As the most pronounced changes in the metabolic capacity (MC) of circulating neutrophils were found in CAI-30 and CAI-60 with and without the experimental model of ADP, a decision was made to develop methods for pharmacological correction using these very groups of animals.

The combination of Hepon, Hypoxen and Phosphogliv in case of ADP on the background of CAI-30 corrected, but not to the normal level, PI, PN, IPA, NBT-sp., NBT-ind. o/z and n/z, and even more reduced the functional reserves of ARn and OI. The administration of the combination of Glutoxim, Mexidol and Heptral to rats with ADP in the context of CAI-30 normalized PN, NBT-ind. o/z and n/z, ARn, OI and corrected, but not to the normal level, PI, IPA, NBT-sp., ARo (Table 3).

The combination of Hepon, Hypoxen, and Phosphoglip in rats with experimental ADP in combination with CAI-60 did not lead to changes in all neutrophil MC indicators, compared with the group of animals which had not beem treated with the drugs. In experimental ADP on CAI-60, the use of a combination of Glutoxim, Mexidol, and Heptral corrected the phagocytic activity of polymorphonuclear leukocytes, NBT-sp. and NBT-ind. o/z and n/z, but did not affect the already low PN, ARo, OI, ARn (Table 3).

In case of CAI-30, stimulation of TDTH cells was detected, with no changes on the part of humoral immune response (HIR) on SEs. With a 60-day administration of ethanol, suppression of the formation of both cellular and humoral forms of the immune response was found out, as evidenced by a decrease in the immune AFCs in the spleen, as well as karyocyte mass difference (MD) and number difference (ND). In experimental destructive pancreatitis in case of CAI-30, and to a greater extent in case of CAI-60, a pronounced inhibition of HIR and TDTH on SEs was found out, in comparison with the control and the animals that had received only ethanol.

The administration of a combination of Hepon, Hypoxen and Phosphogliv to rats with ADP in the context of a 30-day CAI corrected, but not to the normal level, the formation of adaptive immunity. The use of a combination of Glutoxim, Mexidol and Heptral normalized the number of immune AFCs in the spleen of the experimental animals, and corrected karyocyte MD and ND, though not to reach those of the control group.

The use of Hepon, Hypoxen, and Phosphogliv in the conditions of ADP and CAI-60 partially corrected the HIR value (immune AFCs in the spleen), but did not affect the parameters of TDTH (karyocyte MD and ND of the popliteal lymph nodes). The use of Glutoxim, Mexidol and Heptral corrected both the level of immune AFCs and karyocyte MD and ND, but not to the parameters of healthy animals.

Thus, it can be argued that in acute pancreatitis, developing on the background of long-term exposure to ethanol, the combined pharmacotherapy is more effective, as it makes it possible to affect several components of the pathogenesis of the disease at once.

When pharmacologically correcting oxidative stress and metabolic erythrocyte disorders in the conditions of a long-term administration of ethanol and experimental acute pancreatitis, it was found that in animals after 5-fold administration of ethanol only the content of acylhydroperoxide (AHP) was increased, whereas in rats with ADP in the context of short-term alcohol intoxication, an increase in the content of LPO products (MDA and AHP), as well as a decrease in the SM_{NO} level and SOD activity was found.

Under the conditions of CAI-30, the concentrations of MDA and AGP, as well as catalase activity increased, whereas the SOD and SMNO levels decreased. The simulation of ADP under these conditions led to a more pronounced quantitative increase in the LPO products, a decrease in the SMNO level and antioxidant protection factors (except for catalase activity) (Fig. 2).

The intake of ethanol for 60 days, compared with its 30-day administration, increased the contents of MDA, AHP, $SM_{NO,}$ but reduced the activity of catalase. In case of ADP on the background of 60-day chronic alcohol intoxication, the development of OS, more pronounced according to the laboratory parameters, and a decrease in SM_{NO} level was observed (Fig. 2).

In case of ADP in the context of CAI-30, simultaneous administration of Hypoxen, Gepon and Phosphogliv statistically reliably brought the concentrations of AHP and SMNO, as well as SOD activity nearer to, but not to the point of matching, the indicators of healthy animals, and also normalized TAA together with catalase activity. The use of Mexidol, Glutoxim and Heptral in these conditions corrected TAA, the MDA concentration and catalase activity, normalized the SOD activity, the contents of AHP and SMNO (Fig. 2).

Under the conditions of 60-day ethanol intoxication and ADP, the use of Phosphogliv, Hepon and Hypoxen normalizes catalase activity and corrects, but not to the level of healthy animals, the concentrations of LPO products (MDA, AHP), SMNO and SOD activity. The introduction of Heptral, Glutoxim and Mexidol in the same conditions reduces the indicators of LOP and the content of SMNO towards those of the control animals, but not to the point of matching them, and also normalizes TAA, SOD and catalase activity (Fig. 2).

Further, the indicators of the metabolic activity of circulating red blood cells were studied. It was discovered that short-term introduction of ethanol reduces the total number of red blood cells, SOD activity in them, and sorption parameters (ESC and MCH). Against this background, the simulation of ADC activates the LPO processes (increased AHP and MDA concentrations) and leads

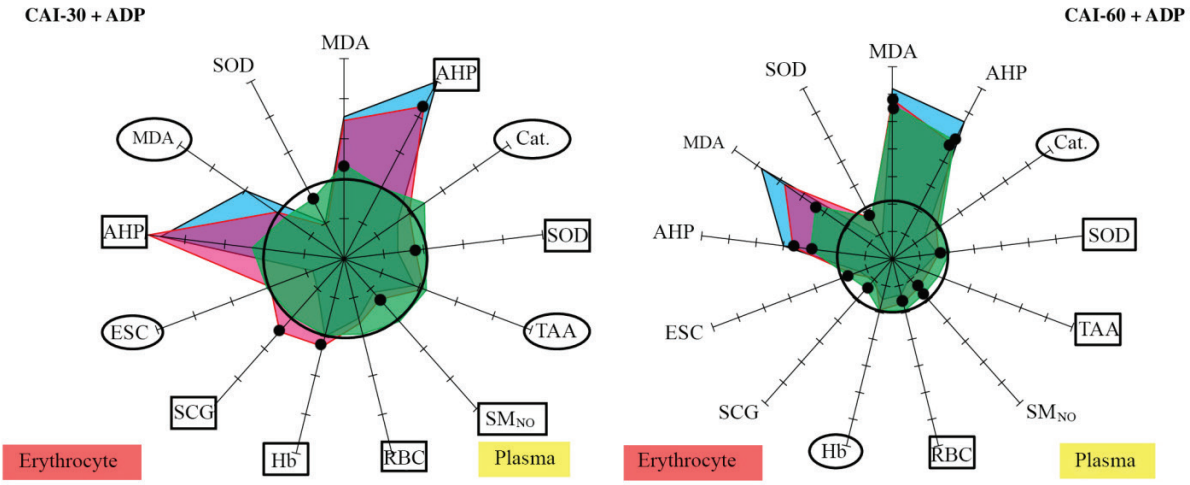

Figure 2. Correction of impaired functional activity of hepatocytes in experimental ADP on the background of 30- and 60-day administration of ethanol.Key: line – radius of the indicator circle - of intact animals; blue – indicators of rats with 30- and 60-day CAI+ADP; red – indicators of rats with 30- and 60-day CAI+ADP+Hepon, Hypoxen and Phosphogliv; green – indicators of rats with 30- and 60-day CAI+ADP+Glutoxim, Mexidol and Heptral; black dot – corrected indicator ($p<0.05$); oval – the indicator normalized by the combination of Hepon, Hypoxen, Phosphogliv ($p>0.05$); square – indicator normalized by the combination of Glutoxim, Mexidol, Heptral ($p>0.05$).

to a more pronounced decrease in SOD activity and ESC parameters (Fig. 2).

In case of CAI-30 and CAI-60, compared with 5-day intoxication, a pronounced development of intra-erythrocyte oxidative stress (increased concentration of LPO products with a simultaneous decrease in antioxidant protection factors) and a decrease in the number of erythrocytes were found, and these developments were more pronounced in the context of CAI-60. It should be noted that a multidirectional change in the erythrocyte sorption properties was revealed: an increase in ESC and MCH in case of CAI-30, and a decrease in these indicators in case of CAI-60, with the ESC values being below the control (Fig. 2).

Simulation of ADP on the background of 30-day alcohol intoxication, compared with the introduction of ethanol alone, was more pronounced in the quantitative terms, the intra-erythrocyte development of OS with multidirectional changes in the erythrocyte membrane sorption parameters. ADP in the context of CAI-60 leads to a more pronounced, when compared with a 2-month only-ethanol exposure, decrease in MCH, the number of erythrocytes, hemoglobin in them, an increase in ESC and the MDA concentration (Fig. 2).

The use of the combination of Phosphogliv, Hypoxen and Hepon in case of ADP and a 30-day administration of ethanol increases the content of hemoglobin (Hb) and MCH, normalizes ECS and the MDA level. Administration of Mexidol, Heptral and Glutoxim in these conditions corrects the SOD activity, but not to the level of healthy rats, normalizes the sorption parameters of the cell membrane, their total number and the intracellular concentration of POL products (AHP and MDA) (Fig. 2).

The administration of the combination of Phosphogliv, Hepon and Hypoxen in ADP against the background of 60-day ethanol intoxication corrects red blood cell count, AHP, SOD activity and normalizes Hb towards the normal level, without no affect on the concentration of MDA in red blood cells and the sorption parameters of the cell membranes. The use of Mexidol, Heptral and Glutoxim in these conditions normalizes the number of erythrocytes and their hemoglobin content, brings the other indicators of the metabolism of circulating erythrocytes nearer, but not to the point of matching, the values of healthy animals (Fig. 2).

In the context of ADP, the development of OS, laboratory signs of which become more evident in case of CAI, is proved by an increase in the concentration of LPO products (AHP and MDA) in blood plasma and erythrocytes, and a decrease in antioxidant protection parameters (TAA, SOD activity and catalase activity).

The use of pharmacological correction by means of drugs with immunomodulatory, antioxidant and hepatoprotective properties has a pronounced corrective effect in partial arrest of OS and disturbance of erythrocytes metabolism.

The use of two different combinations, including various immunomodulators, hepatoprotectors and antioxidants, to correct immunometabolic disorders in ADP against the background of long-term administration of ethanol, made it imperative to compare their effecacy.

First, there were compared the quantity and quality of changes in immunometabolic parameters with administering only ethanol only for 5, 30, and 60 days and under the conditions of ADP against the background of short-term and long-term alcoholization.

In quantitative comparison of the number of the disturbed indicators in different experimental conditions with ranging degrees of disturbance, it was found that with administration of ethanol for 5 days 10 (41.7%) out of the 24

studied laboratory parameters were disturbed, of which only 4 (16.7%) reached degrees 2-3. Ethanol intoxication for 30 or 60 days, respectively, leads to the disturbance of 27 out of 34 (79.4%) and 29 (85.3%) laboratory indicators, with a significant increase in disturbance of degrees II-III: 38.3% and 50%, respectively. The combination of forced intake of ethanol and ADP, compared with the intake of only alcohol, changed most of the indicators towards degrees II-III. For example, in case of 5-day ethanol intoxication and ADP, these changes concerned 87.5% of the parameters, of which 66.7% were of degrees II-III; with ADP against the background of CAI-30 and CAI-60, respectively, the figures were 97.1% and 100%, and considerable disturbances were recorded in 61.8% and 76.4% of the indicators.

Further, efficacy of various combinations of immunomodulators, antioxidants and membrane protectors was compared, in the context of ADP against the background of 30- and 60-day alcohol intoxication.

Analyzing the data obtained in the case of ADP on the background of CAI-30, it can be concluded that 97.1% of the studied laboratory parameters characterizing the functional activity of hepatocytes, cellular and humoral forms of adaptive immunity, LPO, antioxidant protection, metabolic capacity of neutrophils and peripheral blood erythrocytes were disturbed to various degrees. The introduction of Hepon, Hypoxen and Phosphogliv normalized 12.1% of the disturbed indicators, corrected 60.6% and did not affect 27.3% of them. The combination of Glutoxim, Mexidol and Heptral was more effective because it normalized 57.8% and corrected 42.4% of the studied parameters of the immunometabolic status (Table 4).

When qualitatively comparing the number of disturbed immunological parameters under study, it was found that in case of ADP against the background of CAI-30 without administering any drugs, 97.1% of the studied parameters diverged from the normal values; it was also determined that 35.3% of the parameters were of I degree of disturbance, and 26.5% and 35.3% were of II and III degrees of disturbance, respectively. After the correction, which included Hepon, Hypoxen and Phosphogliv, 87.9% of the parameters of the immune status were changed, and 51.5%, 9.1% and 27.3% of the parameters were of I, II and III degrees of disturbance, respectively. After using Glutoxim, Mexidol and Heptral, 42.4% of the indicators remained disturbed (I, II and III degrees of disturbances were recorded in 33.3%, 3% and 6.1% of the indicators, respectively) (Table 4).

In ADP in the context of 60-day ethanol intoxication, 100% of the studied laboratory parameters were disturbed. Administration of Hepon, Hypoxen and Phosphogliv normalized 11.8% of them, corrected 38.2% and did not affect 50%. The combination of Glutoxim, Mexidol and Heptral was more effective because it normalized 17.6% and corrected 67.6% of the parameters, and did not affect 14.7% of the parameters under study.

It should be noted that, in ADP against the background of CAI-30, it was the parameters of metabolic capacity of erythrocytes which were corrected the least, whereas in case of CAI-60, the immune parameters were corrected the least.

When analyzing the qualitative changes, it was found that ADP on the background of CAI-60 without drug administration, 100% of the parameters under study differed from the norm values, with 23.5% of the parameters being of I degree of disturbance, and those being of II and III degrees accounted for 38.2% each. After using Hepon, Hypoxen and Phosphogliv, 88.2% of the parameters of the immune status were changed, and 23.5%, 32.4% and 32.4% of the parameters were of I, II and III degrees of disturbance, respectively. After the use of Glutoxim, Mexidol and Heptral, 79.4% of indicators remained disturbed (those of I and II degrees accounted for 29.4% each, and those of III degree – 18.9% respectively).

The results of evaluating their own corrective pharmacological effects of antioxidants, hepatoprotectors and immunomodulators in case of ADP against the background of CAI are presented in Table 5.

At the same time, the combination of Heptral, Glutoxim, Mexidol (84 points) was more preferable than that of Phosphogliv, Hepon, Hypoxen (46 points) in terms of immunological and metabolic efficacy in case of ADP on the background of CAI-30.

Table 4. Comparative Immunometabolic Efficacy of Various Schemes of Pharmacological Correction in ADP Against the Background of Long-term Alcohol Intoxication

No	Experimental conditions	Indicators different from those of healthy animals	Changed indicators by disturbance degree						
			I		II		III		
		abs.	%	abs.	%	abs.	%	abs.	%
30-day alcohol intoxication									
1	No drug administration	33	97.1	12	35.3	9	26.5	12	35.3
2	Administration of Phosphogliv, Hepon, and Hypoxen	29	87.9	17	51.5	3	9.1	9	27.3
3	Administration of Mexidol, Glutoxim, and Heptral	14	42.4	11	33.3	1	3	2	6.1
60- day alcohol intoxication									
1	No drug administration	34	100	8	23.5	13	38.2	13	38.2
2	Administration of Phosphogliv, Hepon, and Hypoxen	30	88.2	8	23.5	11	32.4	11	32.4
3	Administration of Mexidol, Glutoxim, and Heptral	27	79.4	10	29.4	10	29.4	7	18.9

Table 5. Own corrective effects of various combinations of immunomodulators, antioxidants and hepatoprotectors in case of APD on the background of CAI

No	Pharmacological treatment regimens	Total of correction indicators
1	ADP on the background of CAI-30	
2	Hypoxen, Phosphogliv and Hepon	46
3	Mexidol, Heptral and Glutoxim	84
4	ADP on the background of CAI-60	
5	Hypoxen, Phosphogliv and Hepon	15
6	Mexidol, Heptral and Glutoxim	37

In case of CAI-60, by immunometabolic efficacy, the composition of Mexidol, Heptral and Glutoxim (37 points) was also more effective than the combination of Hypoxen, Phosphogliv and Hepon (15 points).

Thus, the use of various statistical methods makes it possible to assume that under the conditions of ADP against the background of CAI, the combination of Glutoxim, Mexidol and Heptral turned out to be more effective in comparison with that of Hepon, Hypoxen and Phosphogliv.

Conclusion

1. Short-term ethanol intoxication (5 days) causes minor reactive metabolic changes; 30-day, and to a greater extent 60-day, administration of ethanol leads to the development of toxic liver damage, oxidative stress, an imbalance in the formation of an adaptive and innate immune response, and impaired metabolic capacity of circulating red blood cells.
2. In experimental acute destructive pancreatitis on the background of a 30-day, and to a greater extent 60-day, alcohol intoxication, compared with only administration of ethanol, a more pronounced development of cytolytic syndrome (increased activity of alanine aminotransferase in plasma), intracellular cholestasis (increased level of bilirubin, activity of alkaline phosphatase and gammaglutamine transpeptidase), toxic inflammatory liver damage (drop in AST/ALT ratio below 1, increase in the GGT/AST), and activation of the blood coagulation system (increase in prothrombin index).
3. The combination of acute destructive pancreatitis with CAI-30, and, to a greater extent, CAI-60, in comparison with the groups of animals receiving only alcohol, resulted in the reduction of parameters of humoral and cellular adoptive immunity, suppression of phagocytic activity of circulating blood neutrophils along with their increased oxygen-dependent activity and a decrease in phagocytic reserves.
4. Acute destructive pancreatitis, as long as duration of alcohol intoxication increases, leads to the development of systemic and local (erythrocytes) oxidative stress, a decrease in the level of stable metabolites of nitric oxide in blood plasma, in the number of erythrocytes and hemoglobin in them, and multidirectional changes in the sorption parameters of red blood cells.
5. Under conditions of acute destructive pancreatitis, on the background of 30-day alcohol intoxication, 97.1% of the studied laboratory parameters characterizing the functional activity of hepatocytes, cellular and humoral forms of adaptive immunity, lipid peroxidation, antioxidant protection, metabolic capacity of neutrophils and erythrocytes of peripheral blood were disturbed. Administration of Phosphogliv, Hypoxen and Hepon normalized 12.1% of the changed metabolic parameters, corrected 60.6% and did not affect 27.3%. The combination of Heptral, Glutoxim and Mexidol proved to be more effective, since it corrected 42.4% and normalized 57.8% of the studied parameters of the immunometabolic status.
6. In acute destructive pancreatitis under the conditions of ethanol intoxication for 60 days, 100% of the laboratory parameters were disturbed. Administration of Phosphogliv, Hypoxen and Hepon corrected 38.2% of them and normalized 11.8%. The combination of Heptral, Glutoxim and Mexidol corrected 67.6% and normalized 17.6% of the parameters under study.
7. When evaluating their own corrective pharmacological effects in case of acute destructive pancreatitis on the background of 30- and 60-day alcohol intoxication, the combination of Heptral, Glutoxime and Mexidol (84 and 37 points, respectively) was more preferable than that of Phosphogliv, Hypoxen and Hepon (46 and 15 points, respectively).

Recommendations

1. In the educational process of medical universities, to use knowledge of changes in innate and adaptive immunity, metabolic shifts in acute destructive pancreatitis against the background of chronic alcohol intoxication in an animal experiment.
2. To recommend studying the clinical efficacy of combinations of Hepone, Hypoxen, Phosphoglive and Glutoxim, Mexidol, Heptral in patients with acute destructive pancreatitis against the background of chronic alcohol intoxication.
3. To recommend designing and conducting a preclinical study of the effects of a combined drug, including Hepon, Hypoxen, Phosphogliv or Glutoxim, Mexidol, Heptral.

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