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Acute Destructive Pancreatitis: Immune and Oxidant Disorders, Their Correction.

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ABSTRACT

The article is devoted to the study of peculiarities of immune and metabolic disorders in patients with acute destructive pancreatitis of biliary and nonbiliary etiology. All the surveyed had a distinct reduction of components of the complement system, in blood, phagocytic function of neutrophils, the raise of the concentration of pro-inflammatory cytokines (TNF, IL-1β, IL-6) and metabolic activity of neutrophils, activating the processes of lipid peroxidation, increasing the activity of antioxidant enzymes. The established changes turned out to be more pronounced in cases with nonbiliary etiology of the disease. Applying the combination of Refortan, Mexicor and Heptral we achieved the best results of immunorehabilitation in patients with biliary etiology of acute destructive pancreatitis, and using the combination of Ferrovir, Mexidol, Phosphogliv – in patients with nonbiliary etiology of the disease.

Keywords: acute destructive pancreatitis, immune and oxidant changes, correction of disorders.

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INTRODUCTION

The value of changes of the immune indicators and the balance of oxidant-reduction reactions for most of inflammatory diseases, including acute pancreatitis remains insufficiently studied. On the one hand, these changes are protective-adaptive mechanisms, and on the other hand – the pathogenetic basis of disease [1-4].

Relatively low clinical and laboratory efficiency of standard methods of treatment of acute destructive pancreatitis, causes the necessity to clarify the nature of the immune and oxidant disorders having various forms and etiology options that can not only shed light on the unexplored questions of pathogenesis of this disease, but also become the starting point of the optimization of conservative treatment [5-7].

In this case a comprehensive approach in the choice of pharmacological correction means of immunometabolic disorders is required. Drugs with immunomodulatory, lipoic and antioxidant orientation, which allow to influence different pathogenetic links of the disease [8].

The purpose is to establish the immune and oxidant disorders and possibilities of their correction in patients with destructive forms of acute pancreatitis depending on the etiology of the disease.

MATERIAL AND METHODS

Since 2006 up to 2012 65 patients with acute destructive pancreatitis were under the supervision in the surgical department of the Kursk city clinical hospital №4. Among them there were 25 people with acute biliary pancreatitis (ABP) and 40 people with acute nonbiliary pancreatitis (ANBP), with the expected mild disease (less than 6 points on the scale of APACHE II). In the complex treatment of these patients, the initial and final method of surgical treatment were minimally invasive procedures.

The criteria for the inclusion in the study were: people aged from 24 to 70; verified diagnosis of acute pancreatitis; the sum of scores on the scale of APACHEII not more than 6; people coming to hospital within 3 days after appearing of the first symptoms of the disease; the presence of comorbidities in remission; the tolerability of all drugs investigated in the work; written informed consent to participate in the research.

The criteria for the exclusion from the study were: patients over 70 years of age; the general severe and very severe condition of health; those with concomitant somatic pathology at the stage of incomplete remission and exacerbation stage; people with cancer or allergic reaction to treatment; patients who refused this research.

Randomization of patients with acute pancreatitis was held according to sex, age, method of treatment, concomitant pathology, complications, duration and the etiology of the disease.

Patients with ABP were divided into 3 groups: 1st group (8 people) received standard

treatment (antifermentny, antisecretory, detoxification and antibacterial therapy, infusion with antispasmodics and analgetics, drugs, improving the flow properties of blood, minimally invasive surgical methods of treatment); group 2 (9 patients) in the combined treatment were receiving the combination of Ferrovir (1,5% to 5.0, intramuscularly, every 12 hours, #20), Mexidol (200 mg intravenously every 24 hours, #10), Phosphogliv (2 caps. inside, every 6 hours, #30); group 3 (8 patients) – the combination of Refortan (6 per cent - 500, intravenous, every 24 hours, #5), Mexicor (200 mg intravenously every 24 hours, #10), Heptral (400 mg intravenously every 24 hours, #10).

Patients with ANBP were divided into 3 sub-groups: 1 group (10 people) received standard treatment (antifermentny, antisecretory, detoxification and antibacterial therapy, infusion with antispasmodics and analgetics, drugs, improving the flow properties of blood, minimally invasive surgical methods of treatment); group 2 (14 patients) in the complex treatment received the combination of Ferrovir, Mexidol and Phosphogliv; 3 group (16 patients) – a combination of Refortan, Mexicor and Heptral in the same dosage.

All drugs were injected according to the recommendations given in the attached guidelines and annotations.

Laboratory tests of blood were carried out by standard methods. The contents of TNF, IL-1β, IL-2, IL-6, IL-8, IL-18, G-CSF, IFα, IFγ, IL-4, IL-10 receptor antagonist for the IL-1 (Ra IL-1), C_3, C_4-components of the complement factor H and C_1-inhibitor, was determined in blood plasma with the help of sets of reagents JSC “Vector-best” (Novosibirsk) and LLC “Protein contour” (Sankt-Petersburg).

The activity and the intensity of neutrophil phagocytosis, isolated from peripheral blood centrifugation, was assessed by phagocytic index, phagocytic number and activity index phagocytosis (PhI, PhN, IPh) [9]. The activity of blood oxygen level-dependent systems of neutrophils was evaluated by the reaction of recovery of nitroxinil tetrazole, spontaneous and stimulated by zymosan, with the calculation of functional reserve (RRNT-sp., RRNT-st., PhRN, IStN) [10].

The intensity of the processes of lipid peroxidation (PLPO) was evaluated by the content of allhydrocodone (AGP) and malonic dialdehyde (MDA) in the blood plasma [11]. In addition, the activity of catalase [12] and superoxide dismutase (SOD) [13] was detected. The total antioxidant activity (TAA) in blood plasma was determined by the method based on the degree of inhibition ascorbate – and terraincerebral oxidation of the TWIN-80 up to MDA. The optical density was measured at 532 nm over 48 hours incubation at 40ºc.

Statistical analysis of the results of the study in patients with acute destructive pancreatitis, the significance of differences was measure by U-criterion, the criterion χ^2, as well as the coefficient of the Spearman's rank correlation was calculated. Differences with p=0,05 were believed statistically significant.

RESULTS

Patients with ABP before treatment in comparison with healthy donors identified the

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increase in plasma concentration of pro-inflammatory cytokines (TNF, IL-1β), IL-2, IL-4, C_4-component of complement, Oxygen dependent activity of neutrophils in the peripheral blood, peroxidation (MDA and AGP), catalase activity, SOD, TAA and lower – Ra IL-1, phagocytic activity of granulocytes, C_1-inhibitor system of complement factor H (tab. 1).

Standard drug therapy in the post surgery period allowed patients with ABP fully normalize PhN and partially concentrations of TNF, IL-2, C_4-component of the complement and MDA (tab. 1).

Using a combination of Ferrovir, Mexidol and Phosphoglive in cases with ABP also fully normalizes TAA of blood plasma and increases the concentration of IL-1β and IL-2 (tab. 1).

The inclusion of the combination of Refortan, Mexicor, Heptral in treatment of patients with ABP compared to standard treatment additionally normalizes oxygen dependent activity of neutrophils, the concentration of MDA and C_1-inhibitor and increases the concentration of IL-4, H-factor, the activity of catalase and SOD (tab. 1).

Table 1: The state of immune and metabolic parameters in patients with ABP before and after different methods of treatment

Indicators	Units of measure	1	2	3	4	5
		Healthy	Before treatment	Standard treatment	Standard treatment + Refortan + Mexicor + Heptral	Standard treatment + Ferrovir + Mexidol + Phosphogliv
TNF	pcg/ml	61,5±9,4	135,8±15,1^{*1}	92,1±7,1^{*1,2}	89,7±8,2^{*1,2}	94,9±7,7^{*1,2}
IL-1β	pcg/ml	1268,6±127,1	1734,2±191,3^{*1}	430,1±81,0^{*1,2}	1407,3±91,2^{*1,2}	1578,2±101,4^{*1}
IL -6	pcg/ml	88,1±7,8	93,4±13,5	92,7±10,0	94,1±9,5	91,8±9,8
IL -8	pcg/ml	172,8±12,0	171,3±19,8	161,2±15,2	166,1±16,1	158,1±14,6
IL -2	pcg/ml	0,08±0,02	25,8±2,2^{*1}	10,3±1,2^{*1,2}	12,4±1,1^{*1,2}	11,2±1,9^{*1,2}
IL -4	pcg/ml	0,27±0,03	1,31±0,07^{*1}	24,8±3,3^{*1,2}	31,8±4,0^{*1-3}	23,8±2,7^{*1,2,4}
IL -10	pcg/ml	39,6±4,2	41,8±5,1	44,2±5,1	40,1±6,8	47,2±8,3
Ra IL-1	pcg/ml	444,7±32,1	347,8±35,1^{*1}	421,3±30,8^{*2}	400,1±48,3^{*2}	448,5±41,3^{*2}
C_3	mg/l	92,8±8,8	93,1±9,6	94,1±8,3	96,1±8,1	96,8±8,8
C_4	mg/l	231,8±26,1	404,1±47,8^{*1}	333,1±24,2^{*1,2}	289,9±17,7^{*1,2}	356,1±20,7^{*1,4}
Factor H	mg/l	112,3±13,1	89,1±9,2^{*1}	107,8±10,1	134,8±10,5^{*2,3}	121,9±9,1^{*2}
C_1-ing.	mg/l	222,1±28,2	164,7±28,3^{*1}	183,3±19,1^{*1}	231,3±21,5^{*2,3}	202,1±19,4^{*2}
PhI	%	60,2±3,7	51,1±4,1^{*1}	59,6±4,2	60,2±4,8	58,1±3,9
PhN	abs.	9,1±0,8	5,5±0,9^{*1}	8,8±0,95^{*2}	8,4±0,87^{*2}	8,5±0,92^{*2}
RRNT-sp.	%	15,1±1,5	19,7±1,2^{*1}	20,4±1,9^{*1}	14,0±1,1^{*2,3}	21,4±1,8^{*1,4}
RRNT-st.	%	48,7±2,8	57,3±4,1^{*1}	58,7±5,1^{*1}	49,3±3,7^{*2,3}	55,8±3,3^{*1,4}
MDA	mkmol/l	4,1±0,11	7,1±0,51^{*1}	6,0±0,44^{*1,2}	4,31±0,91^{*2,3}	6,1±0,52^{*1,4}
AGP	mkmol/l	0,42±0,04	2,0±0,5^{*1}	1,89±0,51^{*1,2}	0,91±0,04^{*1-3}	1,68±0,09^{*1-4}
Catalase	kat/l	81,4±6,1	611,8±49,7^{*1}	403,7±48,1^{*1,2}	527,3±51,2^{*1,3}	399,1±37,8^{*1,2,4}
SOD	units/ml	48,2±4,0	100,4±8,9^{*1}	107,1±12,9^{*1}	131,2±8,8^{*1-3}	99,8±9,7^{*1,4}
TAA	%	40,0±3,9	51,3±5,9^{*1}	58,1±4,9^{*1}	60,7±5,1^{*1}	41,3±4,8^{*2-4}

Note: significant differences in the average (p = 0,05) are marked with an asterisk in tables 1 and 2; the numbers next to the asterisk show the indicators of which group these differences are given to.

In patients with ANBP the level of pro- (TNF, IL-1β) and anti-inflammatory cytokines

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(IL-4, IL-10), of complement components, products of lipid peroxidation, oxygen dependent activity of neutrophils to a greater extent was increased , but levels of IL-2, RA IL-1, C_1-inhibitor, factor H, TAA, phagocytic activity of granulocytes were decreased (tab. 2).
The standard treatment of patients with ANBP corrected PhI, PhN, indicators of oxygen dependent activity of neutrophils in the peripheral blood, the level of PLPO products. In addition we could state the increase of all studied pro- and antiinflammatory cytokines, components of the complement system and its regulators, catalase activity in the absence of dynamics of activity of SOD and TAA (tab. 2).

Table 2: The state of immune and oxidant parameters in patients with ANBP before and after different methods of treatment

Indicators	Units of measure	1	2	3	4	5
		Healthy	Before treatment	Standard treatment	Standard treatment + Refortan + Mexicor + Heptral	Standard treatment + Ferrovir + Mexidol + Phosphogliv
TNF	pcg/ml	61,5±9,4	192,1±7,1^{*1}	232,1±2,8^{*1,2}	100,3±21,4^{*1-3}	156,2±27,2^{*1,2,4}
IL-1β	pcg/ml	1268,6±127,1	2430,1±81,0^{*1}	4430,1±42,0^{*1,2}	1723,7±99,3^{*1,2}	1823,8±104,7^{*1,2}
IL -6	pcg/ml	88,1±7,8	192,7±12,0^{*1}	331,7±22,0^{*1,2}	115,7±10,2^{*1,2}	113,6±13,8^{*1,2}
IL -8	pcg/ml	172,8±12,0	161,2±15,2^{*1}	542,1±17,2^{*1,2}	166,8±17,1^{*2,3}	202,2±13,9^{*1,4}
IL -2	pcg/ml	0,08±0,02	0,02±0,01^{*1}	1,2±0,02^{*1,2}	13,4±1,2^{*1-3}	28,4±2,4^{*1-4}
IL -4	pcg/ml	0,27±0,03	24,8±3,3^{*1}	44,8±3,3^{*1,2}	186,8±18,3^{*1-3}	99,9±8,4^{*1-4}
IL -10	pcg/ml	39,6±4,2	144,2±5,1^{*1}	244,2±5,1^{*1,2}	62,9±7,1^{*1-3}	33,2±5,1^{*2,4}
Ra IL-1	pcg/ml	444,7±32,1	221,3±30,8^{*1}	521,3±30,8^{*1,2}	424,1±42,8^{*2,3}	360,9±19,6^{*2-4}
C_3	mg/l	92,8±8,8	122,1±8,3^{*1}	168,2±3,3^{*1,2}	99,7±9,8^{*2}	95,8±11,3^{*2}
C_4	mg/l	231,8±26,1	633,1±4,2^{*1}	733,7±7,2^{*1,2}	362,2±27,1^{*1-3}	510,7±58,2^{*1,2,4}
Factor H	mg/l	112,3±13,1	47,1±10,1^{*1}	87,2±9,1^{*1,2}	105,6±8,1^{*2,3}	102,5±7,9^{*2,3}
C_1-ing.	mg/l	222,1±28,2	83,3±19,1^{*1}	93,3±29,1^{*1,2}	221,3±18,1^{*2}	156,2±14,9^{*1,3,4}
PhI	%	60,2±3,7	39,6±4,2^{*1}	49,1±2,2^{*1,2}	52,1±5,2^{*2}	51,1±4,1^{*1,2}
PhN	abs.	9,1±0,8	3,8±0,95^{*1}	4,8±0,55^{*1,2}	9,9±0,7^{*2,3}	8,5±0,9^{*2,3}
RRNT-sp.	%	15,1±1,5	26,2±1,9^{*1}	46,2±3,9^{*1,2}	15,6±1,1^{*2,3}	11,8±1,1^{*1,3,4}
RRNT-st.	%	48,7±2,8	68,7±1,1^{*1}	88,7±2,1^{*1,2}	43,3±3,8	42,1±3,5
MDA	mkmol/l	4,1±0,11	16,0±0,44*1	11,0±0,14*1,2	4,31±0,91*2,3	6,1±0,52*1,4
AGP	mkmol/l	0,42±0,04	13,89±0,51^{*1}	6,8±0,16^{*1,2}	0,91±0,04^{*1-3}	1,68±0,09^{*1-4}
Catalase	kat/l	81,4±6,1	403,7±48,1^{*1}	703,7±48,1^{*1,2}	527,3±51,2^{*1,3}	399,1±37,8^{*1,2,4}
SOD	units/ml	48,2±4,0	67,1±12,9^{*1}	77,2±12,9^{*1}	131,2±8,8^{*1-3}	99,8±9,7^{*1,4}
TAA	%	40,0±3,9	28,1±4,9^{*1}	32,2±2,9^{*1}	60,7±5,1^{*1}	41,3±4,8^{*2-4}

The use of the combination of Refortan, Mexicor and Heptral in complex treatment of patients with ANBP, compared to standard treatment, normalizes the concentration of IL-8, Ra IL-1, C_3-component of complement factor H and C_1-inhibitor, functional activity of neutrophils, MDA, correcting the concentration of other cytokines, enzymes of antioxidant system more than standard treatment (tab. 2).

The inclusion of the combination of Ferrovir, Mexidol and Phosphogliv in complex treatment normalizes the concentration of IL-10, Ra IL-1, C_4-component of the complement factor H, PhN, TAA in blood plasma (tab. 2).

The calculation of indicators, changed before treatment and corrected by various

methods of treatment in patients with acute destructive pancreatitis depending on the etiology, has allowed to establish the following. In patients with ABP 77,8% of indicators were broken, and in patients with ANBP 100 % of indicators were broken before treatment (tab. 3).

Standard treatment in cases of ABP normalized and corrected up to 14,8% while in ANBP – 0 and 29,6% correspondingly (tab. 3).

In patients with ABP the combination of Refortan, Mexicor and Heptral turned out to be more effective, as this combination normalized 47,6% and corrected 28,6% indicators. The combination of Ferrovir, Mexidol and Phosphogliv normalized and corrected 42,8 and 33,3% of the indicators (tab. 3).

In patients with ANBP the most effective was the combination of Ferrovir, Mexidol and Phosphogliv as it normalized and corrected 40,7% and 51,5% of indicators that in total is more than in the application of Refortan, Mexicor and Heptral, as this combination normalized and corrected 55,5% and 25,9% correspondingly (tab. 3).

Table 3: Laboratory efficiency of different methods of treatment of patients with acute destructive pancreatitis depending on the etiology of the disease

No	Group of sick people,	Changed laboratory indicators before treatment	After treatment (%):		
			Normalized	Corrected	Remained without any changes
Patients with ABP					
1.	Standard treatment	77,8	14,8	14,8	70,4
2.	Standard treatment + Refortan + Mexicor + Heptral		47,6	28,6	23,8
3.	Standard treatment + Ferrovir + Mexidol + Phosphogliv		42,8	33,3	23,9
Patients with ANBP					
4.	Standard treatment	100	0	29,6	66,7
5.	Standard treatment + Refortan + Mexicor + Heptral		55,5	25,9	18,6
6.	Standard treatment + Ferrovir + Mexidol + Phosphogliv		40,7	51,8	7,5

CONCLUSION

Thus, it was established that in patients with the destructive ANBP there are more distinct changes of the studied rates compared to the destructive ABP. The standard treatment is not enough effective in cases of ABP, it is even less effective at ANBP. The combination of immunomodulators, antioxidants and membraneprotectors had a different intensity of effects on the studied immune and antioxidant indicators: in destructive acute pancreatitis of biliary etiology the greatest efficiency has the inclusion of the combination of Refortan, Mexicor, Heptral in the standard algorithm of conservative treatment of diseases, and when we have cases of nonbiliary etiology of the disease it is the combination of Ferrovir, Mexidol and Phosphogliv.

It is known that ABP and ANBP considerably differ by the severity of the disease, the

number of emerging complications, as well as forecasts and outcomes of acute pancreatitis. This is due to the mechanisms of damage of the pancreas, with different etiology of the disease, i.e. premorbid background, and differences in immune homeostasis. In patients with ABP initially there may not be immune status changes, because the cause of development of acute pancreatitis becomes biliary hypertension, causing the ingress of bile, activating pancreatic juice into the pancreatic duct. In case of ANBP pancreas is already compromised by the prolonged abuse of alcohol, in these patients, most often there appears the state of secondary immunodeficiency, the very tissue of the pancreas is greatly fibrously changed. In this regard, disorders of the immune homeostasis in case of ABP are random in nature and they are mostly due to disorders of microcirculation in the area of the pancreas, and in case of ANBP the existing changes of immunity are chronic, caused by prolonged alcohol intoxication [15-17].

Great immunomodulating efficiency of the combination of Refortan, Mexicor and Heptral in cases of ABP can be explained by the fact that the drug, having expressed rheological properties, eliminates the phenomenon of endotoxemia in a shorter time, improves the oxygenation of the tissues, which positively affects the function of immune cells and the process of reparative regeneration. It plays a significant role in the delimitation of pancreatic destruction points, consequently, of their infection, and reduce the effects of autointoxication that becomes a prerequisite for the recovery of the function of immune cells. In turn, Mexicor shows more distinct antioxidant effects dealing with pathology caused by atherosclerotic lesions of vessels that can be observed in patients with cholelithiasis. In addition, the well proven drug Heptral, which has pronounced lipoic effects due to ademetionin, reduces the intensity of PLPO in the membranes of pancreatitus

The combination of Ferrovir, Mexidol and Phosphogliv happened to be more effective in cases of ANBP not by chance. Ferrovir has pronounced immunomodulating effects, reduces the degree of the inflammatory reaction. The proof of it was the normalization of the level of proinflammatory, the raised levels of anti-inflammatory cytokines. The corrective effects of Ferrovir in respect of immunoregulatory cytokine – IL-2, manifested in the reduction of its content, are explained by the suppression of hyperactively humoral immune mechanisms which can damage unchanged tissues of the pancreas. The ability of the drug to increase the body's resistance to infection is apparent, in our view, in increasing the level of IFα and IFγ. Mexidol in its chemical structure does not differ from Mexicor, however, it is more effective in cases of pathology of the central nervous system, which can be seen in patients who abuse alcohol. In turn, Phosphogliv has a wide range of pharmacological effects, because it includes not only essential phospholipids, but glycyrrhizin acid, which probably increases the immunomodulating effects of the whole scheme as a whole.

The results of the research do not only justify the use of combinations of immunomodulators, antioxidants and membraneprotectors in cases of destructive forms of acute pancreatitis of different etiology, but also open certain prospects for finding more effective schemes of immunorehabilitation.

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