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Original article

Reprint

Experimental study of organ dysfunction and inflammatory response in acute post-manipulation pancreatitis

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Abstract:
Objective: to examine the possibility of reducing the severity of organ dysfunction and inflammatory response with the L-17 compound in a model of acute post-manipulation pancreatitis (APMP).
Materials and Methods. APMP was modeled on 40 rats. Laboratory rats were distributed among control (n=20, without treatment) and experimental (n=20, with the introduction of the L-17 compound) groups. The daily dose of the compound was 40 mg/kg of rat body weight. Results. APMP developed in all animals. In the control group, persistent organ dysfunction and inflammatory response corresponded to a severe course of acute pancreatitis with a mortality rate of 70%. In the experimental group, there was a decrease in the severity of organ dysfunction and inflammatory response, with a decrease in mortality down to 30%. Conclusion.Administration of the L-17 compound reduced the severity of organ dysfunction and inflammatory response in experimentally modeled APMP.

Keywords: acute post-manipulation pancreatitis, experimental pancreatitis, post-ERCP pancreatitis, substituted thiadiazines, L-17 compound.

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Introduction

Endoscopic retrograde cholangiopancreatography/pancreatectomy (ERCP/PE) as a therapeutic and diagnostic surgical procedure has been known since 1968. Acute post-manipulation pancreatitis (APMP) is considered a common and severe complication associated with both diagnostic and therapeutic ERCP/PE [1-7]. According to various authors, the incidence of APMP reaches 3.5–15% or more [1, 3-5, 8, 9]. Some patients (5–15%) develop a severe course of APMP leading to extended hospitalization in the intensive care unit using substantialhospital resources [3, 8, 9]. Besides, patients with severe APMP have a high mortality rate (up to 30%) [10]. It is important to note that each case of APMP development carries the potential risk of a criminal investigation, particularly in the event of death.

Although technology and equipment for ERCP/PE continue to improve, reducing the APMP incidence remains a major clinical challenge. There is still much debate regarding the significance of individual risk factors causing the APMP development [6, 8, 7, 11]. Consequently, to date, an effective contemporary means of conservative treatment that could significantly alleviate the course of developed ARMP and increase patient survival has not yet been proposed.

In 2010, we proposed a technique for the formation of diffuse pancreatic necrosis [12], which effectively reproduces severe APMP. The study used the L-17 compound (2-morpholino-5-phenyl-6H-1,3,4-thiadiazine hydrobromide) from a new group of chemical compounds (5-phenyl-substituted-6H-1,3,4-thiadiazine-2-amines) [13]. The effect of L-17 on the course of organ dysfunction and the inflammatory response severity in APMP has not been sufficiently elucidated.

The goal of our study was to explore the possibility of reducing the severity of organ dysfunction and inflammatory response with L-17 compound in a model of APMP.

Materials and Methods

Modeling of APMP was performed on 40 apparently healthy sexually mature non-linear rats aged 4-6 months. with an average weight of 250 g. The animals without signs of disease were kept in the vivarium of the Institute of Immunology and Physiology, Ural Branch of the Russian Academy of Sciences (Ekaterinburg, Russia). All experimental procedures with animals were approved by the Ethics Committee at the Institute of Immunology and Physiology (Protocol #02/21 of December 1, 2021) and were carried out in accordance with the principles of the European Convention for the Conservation of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, France, 18 March 1986), as well as with regulatory documents recommended by the European Science Foundation (ESF) and the Declaration of Helsinki on the Humane Treatment of Animals (2000) and in accordance with the Rules for

Laboratory Research (Order of the Russian Ministry of Healthcare No. 464n of May 18, 2021).

To simulate experimental APMP, we employed a persistent increase in pressure in the main pancreatic duct and common bile duct of animals by precision ligation of the main pancreatic duct at the junction with the duodenum [12]. The developed experimental model of APMP according to the mechanism of acute pancreatitis development corresponded to the main pathogenetic elements of APMP after ERCP/PE. After animals were removed from the experiment (Day 1 and Day 7 after surgery), laboratory parameters were examined that reflected the severity of the inflammatory response and the severity of organ dysfunction.

Statistical data processing was carried out using the statistical STATISTICA 12 software package and the R language statistical software. Data are presented in the form of M±m. Data were tested normality by the Shapiro–Wilk test, and its results indicated the normality of the distribution of most indicators (p-value>0.05). The critical level of significance when testing statistical hypotheses was taken equal to 0.05. When comparing laboratory data on Day 1 vs. Day 7, the Mann–Whitney U test was used, which allowed comparing independent samples with respect to the sampling distribution, since Levene's test for the equality of variances indicated the heterogeneity of the compared data (p-value<0.05).

The control group included 20 animals with experimental APMP without medicamentous exposure, while the experimental group included 20 animals with experimental APMP caused by administration of the L-17compound. Withdrawal from the experiment was carried out on Day 1 and Day 7. The study design is presented in *Figure*.

Results

Summary results of laboratory tests are presented in the *Table*. The formation of acute pancreatitis was observed in all animals from the first hours after surgery. On Day 1, 20 animals were removed from the experiment (10 from each group).

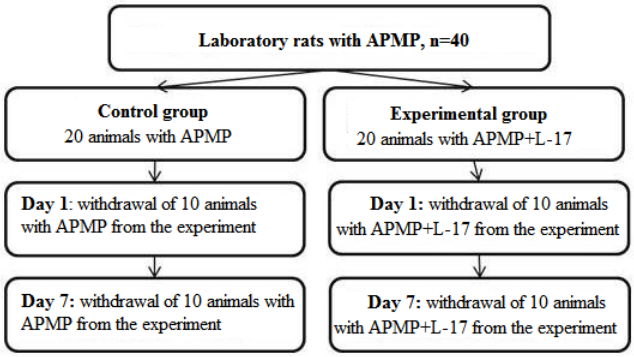

Figure. Experimental design

Table. Summary of laboratory test results

Indicator	Group	
	control	experimental
α-amylase,IU/L		
Day 1*	13,300±1,618	6,164±659
Day 7	1,738±102.6	1,836±262.5
Alanine aminotransferase*/aspartate aminotransferase *, U/L		
Day 1	507.0±47.3/558.3±47.5	305.0±41.6/453.8±42.9
Day 7	106.0±28.3/251.7±4.4	56.2±9.1/162.3±21.0
Albuminbinding capacity, %		
Day 1*	88±3.1	96±2.0
Day 7*	86±4.4	98±2.4
Coefficient *K*		
Day 1	0.45±0.04	0.41±0.07
Day 7	0.54±0.11	0.45±0.02
D-dimers,pg/mL		
Day 1*	918.6±170	175.6±25.3
Day 7*	716.1±196	60.7±5.5
Coefficient *E*		
Day 1*	360±55.4	213.4±18.1
Day 7*	213.7±35.0	166.5±24.9
Leucocytes, $\times 10^9$/L		
Day 1*	2.3±0.1	3.65±0.37
Day 7*	9.75±0.88	4.63±1.05
Granulocytes, $\times 10^9$/L		
Day 1*	1.85±0.2	2.57±0.2
Day 7*	7.45±0.84	3.22±0.62
Monocytes, $\times 10^9$/L		
Day 1	0.09±0.01	0.13±0.03
Day 7*	0.44±0.06	0.28±0.03
IL-1,pg/mL		
Day 1*	545.5±139.5	54.45±1.3
Day 7	77.23±40.7	48.03±5.0
IL-6,pg/mL		
Day 1*	221.7±43.6	58.48±0.7
Day 7	58.36±18.1	55.95±13.3
IL-10,pg/mL		
Day 1*	746.3±136	61.05±18.1
Day 7*	66.24±9.6	50.65±4.9

*, p<0.05 for comparison groups; IL, interleukin

In all animals, weobserved the development of organ dysfunctions andinflammatory response with the formation of extensive zones of pancreatic necrosis and serous peritonitis. In the control group with APMP, the general condition of rats, changes in laboratory parameters and the severity of morphological changes were more noteworthy. In case of APMP during the administration of L-17, the concentration of serum α-amylase on Day 1 was twice lower vs. the control group without drug exposure (13,300±1,618 and 6,164±659 IU/L). This implied the presence of a protective effect of compound L-17 on the exocrine function of the pancreas during APMP. By Day 7, the level of amylase in both groups was within normal limits and did not reflect the severity of the animals' condition. The severity of liver dysfunction in ARMP was assessed by the concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The ALT content on Day 1 was significantly higher in the control group (507.0±47.3 vs. 305.0±41.6 U/L), while the AST level was lowerwith

administration of L-17 (453.8 ±42.9 vs. 558.3±47.5 U/L). In the control group, the ALT concentration by Day 7 was 1.5 times higher than the values in the experimental group (106.0±28.3 and 56.2±9.1 U/L, respectively). The AST concentration changed in a similar way but was lower in animals of the experimental group (251.7±4.4 and 162.3±21.0 U/L). Accordingly, we suggested that L-17 compound had some protective effect on liver function in patients with APMP. The level of endogenous intoxication, which indirectly reflects renal dysfunction, was assessed by the albuminbinding capacity and the distribution coefficient,K (the ratio of the concentration of substances with low and medium molecular weight in plasma and erythrocytes). On Day 1, in the control group, the albumin binding capacity was significantly lower than in the experimental group with exposure to L-17 (88±3.1 and 96±2.0%, respectively). By Day 7, albumin binding capacity in the control group started decreasing (86±4.4%), while in the experimental group, against the background of L-17 compound, on the contrary, it recovered to normal values (98±2.4%). On Day 1, the coefficient K was 0.45±0.04 in the control group vs.only 0.41±0.07 in the experimental group. By Day 7,K value in the control group continued increasing (up to 0.54±0.11), whileit remained close to the value of intact animals in the experimental group (0.45±0.02), which confirmed a reduction in endogenous intoxication in APMP due to the administration of the L-17 compound.

When studying the indicators of the inflammatory response on Day 1 in the control group with APMP, we observed signs of severe leukopenia (2.3±0.1×109/L) with a small number of granulocytes (1.85±0.2×109/L) and monocytes (0.09±0.01×109/L) and a simultaneous surge of IL concentration (IL-1 up to 545.5±139.5 pg/mL, IL-6 up to 221.7±43.6 pg/mL, and IL-10 up to 746.3±136 pg/mL). Meanwhile, in the experimental group with APMP against the background of L-17 administration, the number of leukocytes corresponded to the level in intact animals (3.65±0.37×109/L) with a large number of granulocytes (2.57±0.2×109/L) and monocytes (0.13±0.03×109/L). At the same time, a lower concentration of IL was detected (IL-1 up to 54.45±1.3 pg/mL, IL-6 up to 58.48±0.7 pg/mL, and IL-10 up to 61.05±18.1 pg/mL). By Day 7, progression of the inflammatory response was noted in the control group (leukocytes: 9.75±0.88×109/L; granulocytes: 7.45±0.84×109/L; monocytes: 0.44±0.06×109/L). In the experimental group, on the contrary, the severity of changes in the inflammatory response did not differ significantly from Day 1 (leukocytes: 4.63±1.05×109/L; granulocytes: 3.22±0.62×109/L; monocytes: 0.28±0.03×109/L). IL concentration by Day 7 naturally declined and was similar in the comparison groups.

Taking into account the natural connection between the inflammatory response and coagulopathic changes, we examined the indicators reflecting the severity of blood clotting disorders. In the control group with APMP on Day 1, we revealed a sharp increase in the concentration of D-dimers (918.6±170 pg/mL), which reflected the severity of inflammatory response and blood clotting disorders. Thromboelastogram data were as follows: R, 3.38±0.3 min;K, 0.7±0.2 min; maximum amplitude [MA], 74.8±3.5 mm.The elasticity coefficient Esignificantly increased to 360±55.4 (while the reference value is 134.5±6.2). Our dataimplied the presence of pronounced hypercoagulation in the control group. In the experimental group on Day 1, the concentration of D-dimers (175.6±25.3 pg/mL) was an order of magnitude lower vs. the control group. The thromboelastogram parameters (R, 4.78±0.5 min; K, 1.3±0.2 min; MA, 67.8±1.3 mm) and values of E coefficient (213.4±18.1) suggested reduced severity of hypercoagulation vs.the control group with APMP. By Day 7, the concentration of D-dimers in the control group remained high (716.1±196 pg/mL). Thromboelastogram data (R, 4.21±0.3 min;K, 0.8±0.1 min; MA, 67.2±2.7 mm) and high values of elasticity coefficient E (213.7±35.0), together with D-dimers, showed the persistence of hypercoagulation, which can be regarded as the formation of disseminated intravascular coagulation (DIC) syndrome. In the experimental group by Day 7, we observed a twofold decrease in the concentration of D-dimers (60.7±5.5 pg/mL). Thromboelastogram indices (R, 2.9±0.7 min; K, 1.2±0.1 min; MA, 61.8±2.9 mm) and the value of the elasticity coefficient E (166.5±24.9), together with the values of D-dimers, suggested normalization of the hemostatic system and a decrease of hypercoagulation in the experimental group with APMP.

Discussion

The results of our experimental study confirmed the clinical data of many authors [3, 8, 9, 14] that acute pancreatitis characterized by a severe course can develop after trauma to the major duodenal papilla. In our experiment, its course by Day 7 was accompanied by diffusepancreatic necrosis, progression of organ dysfunction and inflammatory response, along with the development of purulent diffuse peritonitis, phlegmon of peripancreatic tissue and bilateral confluent lobar pneumonia.

We revealed that the use of L-17 compound from the group of substituted thiadiazines leads to a decrease in the severity of experimental APMP in 70% of cases with a decrease in the severity of organ dysfunction and inflammatory response. In the pathogenesis of organ dysfunction in severe acute pancreatitis, microcirculation and hypercoagulation disorders play an important role leading to a decrease in tissue perfusion, which is a component of pancreatogenic aggression and subsequent inflammatory coagulation disorders [15, 16, 17]. The initially determined properties of compound L-17 [13] and the results of the study suggest that a decrease in the severity of organ dysfunction and the severity of the inflammatory response during the administration of compound L-17 is likely due to improved tissue perfusion, accompanied by a decrease in the severity of the inflammatory response.

Conclusion

The results of our experiment implied the fundamental possibility of reducing the severity of major organ dysfunctions and the inflammatory response with L-17compound, which requires further investigation.

Author contributions: All authors contributed equally to the manuscript preparation.

Conflict of interest: None declared.

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