DOI 10.11603/2414-4533.2023.3.14150
UDC 616.381-002.1-031.81:616-056.52:616-099]-092.9

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Dynamics of changes in markers of endogenous intoxication in rats with simulating acute generalized peritonitis on a background of obesity

The aim of the work: to study the state of indicators of endogenous intoxication in the body of experimental animals with simulated AGP on the background of obesity and to compare them with groups of animals with obesity and with animals with AGP.
Materials and Methods. The experiment used 64 white Wistar rats, which were divided into three groups: the main group – 24 animals with AGP modeling against the background of obesity; the comparison group – 8 animals with modeling of obesity only; another comparison group – 24 animals with only AGP simulation; the control group consisted of 8 intact animals kept in standard vivarium conditions. APP was modeled by injecting 10 % filtered fecal suspension into the abdominal cavity. Obesity was simulated using a high-calorie diet. The content of indicators of endogenous intoxication in the blood of animals was determined using the photospectrometric method.
Results and Discussion. It was established that in animals with AGP against the background of obesity, the indicators of lipoperoxidation were significantly activated (by 1.8 times the level of TBA-AP compared to intact animals) and the antioxidant defense was depleted (according to a statistically significantly lower index of superoxide dismutase by 2.4 times compared to the control)) ($p < 0.05$).
Conclusions. The development of AGP is accompanied by the EIS complex, which indicates an increase in catabolic processes in the dynamics of pathology modeling, and is laboratory-detected by a probable increase in the levels of MSM, EII. The depth of endotoxemia in rats increases during all stages of development of acute peritonitis and depends on the presence of accompanying obesity, which is confirmed by significantly higher levels of MM_{254} and MM_{280}, EII during all observed terms of animals with combined pathology.

Key words: acute generalized peritonitis; obesity; endogenous intoxication.

Statement of the problem and analysis of the latest research and publications. Worldwide, acute generalized peritonitis (AGP) is a common medical and surgical emergency that is a major cause of mortality, despite improvements in diagnosis and surgical and intensive care [1, 2]. Despite the obvious successes of diagnostics, the use of various antibacterial drugs of the new generation, the introduction of modern methods of minimally invasive treatment, the mortality rate of AGP ranges from 12.5 % to 39.2 % [3, 4]. The basis of the severe course and high mortality rates in acute peritonitis is often concomitant pathology, among which obesity is present in 7.5 % to 14.0 % [5, 6].

The effectiveness of using different criteria to detect obesity is not equal, this requires further discussion and comparative analysis of existing diagnostic criteria and requires further in-depth study of biochemical indicators under conditions of obesity. It is known that the endogenous intoxication syndrome (EIS) is considered by most clinical, biochemical and immunological manifestations as a non-specific process caused by a discrepancy between the synthesis and elimination of the products of "normal" and pathological metabolism [7, 8]. One of the main causes of EIS in acute peritonitis is bacterial toxins, which initiate the release of a large number of endogenous inflammatory mediators with the formation of systemic pathological reactions [9].

There are quite a lot of works dedicated to the study of EIS in various pathological conditions [10]. However, the question of the role of the activity of endogenous intoxication processes in the pathogenesis of systemic abnormalities in AGP against the background of obesity remains to be fully elucidated.

The aim of the work: to study the state of indicators of endogenous intoxication in the body of experimental animals with simulated AGP on the background of obesity and to compare them with groups of animals with obesity and with animals with AGP.

Materials and Methods. The experiments were carried out in the Central Research Laboratory of I. Horbachevsky Ternopil National Medical University. The experiment used 64 white Wistar rats, which were divided into three groups: the main group – 24 animals with AGP modeling against the background of obesity; the comparison group – 8 animals with modeling of obesity only; another comparison group – 24 animals with only AGP simulation; the control group consisted of 8 intact animals kept in standard vivarium conditions. All compared groups of animals were of the same weight, sex, and age.

Male rats of the control group were fed a normal control diet. Male rats of the main group (n=24) were fed a high-fat diet (more than 60 % of energy from fat) for 16 weeks, thus simulating the development of obesity in them.

AGP was modeled by injecting 10 % of filtered fecal suspension into the abdominal cavity at a dose of 0.5 ml per 100 g of animal weight according to

V. A. Lazarenko and co-authors (2008) [11]. Fecal suspension was obtained by mixing the isotonic solution and cecal contents of three intact animals and filtering it twice through a double layer of gauze. Fecal suspension was administered no later than 20 minutes after its preparation. In order to prevent damage to internal organs, the animals were kept in a vertical position, with the caudal end up. Using the method of ventral wall puncture in the center of the midline of the anterior abdominal wall, directing the end of the needle alternately into the area of the right and left hypochondrium, into the right and left pubic areas, the required amount of fecal suspension was injected.

In terms of etiological factors, clinical manifestations and phasic course, this model is close to a similar process in humans. Experimental animals were removed from the experiment by overdose of sodium thiopental (at the rate of 100 mg/kg of weight).

Statistical processing of digital data was carried out using Excel and STATISTICA software using parametric and non-parametric methods of evaluating the obtained data. For all indicators, the value of the arithmetic mean of the sample (M), its variance and the error of the mean (m) were calculated. The reliability of the difference in values between independent quantitative values was determined in the case of a normal distribution using the Student's t-test, in other cases – using the Mann–Whitney U-test (differences at $p<0.05$ were considered reliable).

The assessment of the state of endogenous intoxication was carried out by determining the content of medium-mass molecules (MM) in the blood of rats by the method of N. I. Gabrielyan and co-authors (1981) [12]. The method is based on the precipitation of high-molecular-weight peptides and proteins of biological fluids using trichloroacetic acid and quantitative determination by centrifugation in the obtained supernatant of medium-molecular peptides by absorption in a monochrome stream of light at a wavelength of 254 nm and 280 nm. Accordingly, MSM_{254} is an identified nucleoprotein component and MM_{280} are products of protein proteolysis.

The erythrocyte intoxication index (EII) was determined according to the method of A. A. Togaibaev. [13]. This method is based on the concept of erythrocytes as a universal adsorbent, which allows you to estimate the level of EI by changing the sorption capacity of erythrocytes, before the penetration of methylene blue through their membrane.

Statistical processing of digital data was carried out using Excel and STATISTICA software using parametric and non-parametric methods of evaluating the obtained data. For all indicators, the value of the arithmetic mean of the sample (M), its variance and the error of the mean (m) were calculated. The reliability of the difference in values between independent quantitative values was determined in the case of a normal distribution using the Student's t-test, in other cases – using the Mann–Whitney U-test (differences at $p<0.05$ were considered reliable).

Results and Discussion. MM_{254}, MM_{280} and EII are considered to be the most universal markers that reflect the intensity of EI processes. During the study of EII in animals with experimental AGP, an increase in the content of MM_{254} for 1st day by 81.8 % compared to the indicator of the control group was found (table 1). On the 3rd day after AGP modeling, the level of MM_{254} exceeded the control by 78.8 %. On the 7th day, the blood content of this indicator was statistically significantly lower than on the 1st and 3rd days, but remained 72.7 % higher than the value of the control group.

Table 1. Indicators of the content of MM_{254} in the blood serum of rats with simulated AGP and obesity (M±m)

Groups of animals	The term of observation		
	1st day (n=8)	3rd day (n=8)	7th day (n=8)
AGP (n=24)	0.60±0.07*#	0.59±0.02*#	0.57±0.03*#
AGP + obesity (n=24)	0.64±0.05*# $p_1<0.05$	0.65±0.01*# $p_2<0.05$	0.67±0.04*# $p_3<0.05$
Obesity (n=8)	0.51±1.07*		
Control (n=8)	0.33±0.01		

Notes: * - reliability of the difference of indicators with the control;
- reliability of the difference in indicators with the obesity group;
p_1 - reliability of the difference in indicators with the AGP group for 1st day;
p_2 - reliability of the difference in indicators with the AGP group for 3rd day;
p_3 - reliability of the difference in indicators with the AGP group for 7th day.

In the group of experimental animals with AGP on the background of obesity, an increase in the content of MSM_{254} by 97.0 % compared to the values in the control group was found. The highest level of MM_{254} was observed on the 7th day after AGP modeling, it was 0.67±0.04 units, which was 103.0 % higher than the value in the control group. As for the group of obese animals, the level of MM_{254} in the blood of these animals also exceeded the control, but was lower than the groups of animals with simulated AGP and animals with combined pathology. When studying the level of MM_{254} in the group of animals with AGP against the background of obesity, an increase of this indicator by 93.9 % was found for 1 day compared to the control group.

When assessing the level of MM_{280} for 1 day in animals with AGP, the level of MM_{280} was 140.0 % higher than the corresponding indicator of endogenous intoxication in the control group. When compared with the control group, MM_{280} increased by 120 % on the 3rd day and by 100% on the 7th day (Table 2).

Accordingly, the concentration of MM_{280} on the 1st day of animals with AGP under conditions of pre-simulated obesity increased 3.8 times compared to the value in the control group. The levels of MM_{280} in animals on day 3 and day 7 with AGP on the background of obesity did not differ among themselves and were 4.4 times higher than the values in the control group.

We found an increase in EII for 1 day in the group of rats with fecal peritonitis by 124.2 % compared to the data in the control group. On day 3 and day 7, EII was higher by 113.0 % and 107.1 %, respectively, than the values in the control group of animals (Table 3).

Table 2. Indicators of the content of MM_{280} in the blood serum of rats with simulated AGP and obesity (M±m)

Groups of animals	The term of observation		
	1st day (n=8)	3rd day (n=8)	7th day (n=8)
AGP (n=24)	0.12±0.01*#	0.11±0.01*#	0.10±0.01*#
AGP + obesity (n=24)	0.19±0.02*# $p_1<0.05$	0.22±0.01*# $p_2<0.05$	0.22±0.02*# $p_3<0.05$
Obesity (n=8)	0.08±0.01*		
Control (n=8)	0.05±0.01		

Notes: * - reliability of the difference of indicators with the control;
- reliability of the difference in indicators with the obesity group;
p_1 - reliability of the difference in indicators with the AGP group for 1st day;
p_2 - reliability of the difference in indicators with the AGP group for 3rd day;
p_3 - reliability of the difference in indicators with the AGP group for 7th day.

Table 3. Indicators of the content of EII in the blood serum of rats with simulated AGP and obesity (M±m)

Groups of animals	The term of observation		
	1st day (n=8)	3rd day (n=8)	7th day (n=8)
AGP (n=24)	72.2±1.6*#	68.6±2.4*#	66.7±2.3*#
AGP + obesity (n=24)	73.2±1.7*# $p_1<0.05$	78.8±2.6*# $p_2<0.05$	80.1±3.4*# $p_3<0.05$
Obesity (n=8)	60.18±1.07*		
Control (n=8)	32.2±1.5		

Notes: * - reliability of the difference of indicators with the control;
- reliability of the difference in indicators with the obesity group;
p_1 - reliability of the difference in indicators with the AGP group for 1st day;
p_2 - reliability of the difference in indicators with the AGP group for 3rd day;
p_3 - reliability of the difference in indicators with the AGP group for 7th day.

In the group of animals with combined pathology, for 1 day, the level of EII increased by 127.3 % compared to the value in the control group of animals. On the 3rd day, in rats with combined pathology, the EII level exceeded the control indicator by 144.7 %. The maximum value of EII was observed on the 7th day in the group of animals with GPP combined with obesity, it was 148.8 % greater than the control value.

Thus, when modeling AGP against the background of obesity, a significant increase in water-soluble toxic products is observed, confirmed by high concentrations of MM and EII fractions.

Conclusions. The development of AGP is accompanied by the EIS complex, which indicates an increase in catabolic processes in the dynamics of pathology modeling, and is laboratory-detected by a probable increase in the levels of MSM, EII. The depth of endotoxemia in rats increases during all stages of development of acute peritonitis and depends on the presence of accompanying obesity, which is confirmed by significantly higher levels of MM_{254} and MM_{280}, EII during all observed terms of animals with combined pathology.

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Отримано 27.06.2023

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ДИНАМІКА ЗМІН МАРКЕРІВ ЕНДОГЕННОЇ ІНТОКСИКАЦІЇ У ЩУРІВ ІЗ СИМУЛЯЦІЄЮ ГОСТРОГО ГЕНЕРАЛІЗОВАНОГО ПЕРИТОНІТУ НА ТЛІ ОЖИРІННЯ

Мета роботи: вивчити стан показників ендогенної інтоксикації в організмі піддослідних тварин із симульованим ГГП на тлі ожиріння та порівняти їх із групами тварин із ожирінням та тваринами із ГГП.
Матеріали і методи. В експерименті використано 64 білих щурів лінії Вістар, яких було поділено на три групи: основна група – 24 тварини з моделюванням ГГП на тлі ожиріння; група порівняння – 8 тварин лише з моделюванням ожиріння, інша група порівняння – 24 тварини лише з моделюванням ГГП; контрольну групу склали 8 інтактних тварин, які утримувалися в стандартних умовах віварію. ГГП моделювали шляхом введення в черевну порожнину 10 % фільтрованої суспензії калу. Ожиріння моделювали за допомогою висококалорійної дієти. Вміст показників ендогенної інтоксикації в крові тварин визначали фотоспектрометричним методом.
Результати досліджень та їх обговорення. Встановлено, що у тварин із ГГП на тлі ожиріннння показники ендогенної інтоксикації достовірно активізувалися за всі терміни спостереження порівняно з групою тварин із ізольованим ГГП та групою з ожирінням за всіма дослідними показниками, зокрема $ММ_{254}$, $ММ_{280}$, ЕІІ ($p<0,05$).
Розвиток ГГП супроводжується комплексом ЕІС, що свідчить про посилення катаболічних процесів у динаміці моделювання патології та лабораторно виявляється за вірогідним підвищенням рівнів ММ, ЕІІ. Глибина ендотоксемії у щурів зростає на всіх етапах розвитку гострого перитоніту і залежить від наявності супутнього ожиріння, що підтверджується достовірно вищими рівнями $ММ_{254}$, $ММ_{280,}$ ЕІІ протягом усіх спостережуваних термінів тварин із поєднаною патологією.

Ключові слова: гострий генералізований перитоніт; ожиріння; ендогенна інтоксикація.