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Evaluation of Oxidative Stress and Endogenous Intoxication in Modeling Destructive Cholecystitis in an Animal Experiment

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Abstract

Introduction. In biological fluids and tissues of the body, metabolic by and end products of normal and impaired metabolism accumulate in excessive concentrations, which have a toxic effect and cause dysfunction of organs and systems.
Purpose. Evaluation of oxidative stress (OS) and endogenous intoxication (EI) in animals in modeling destructive cholecystitis.
Materials and methods. Experimental modeling of destructive cholecystitis on 12 Chinchilla rabbits of both sexes weighing 3–4 kg. Blood plasma and erythrocyte membranes were used as a substrate for research.
Results. Modeling of destructive cholecystitis in animals proceeds against the background of development of OS and EI, and these two pathogenetic aspects of inflammation are interrelated. There is a directly proportional relationship between the indicators of lipid peroxidation (LPO) and middle molecular peptides (MMP), namely, between the value of diene conjugates (DC) and MMP (r=0.67), ketotrienes (KT) and MMP (r=0.73) and malondialdehyde (MDA) and MMP (r=0.63). The same dependence appears between LPO and phospholipase activity (r=0.71–0.78). Under the influence of a large number of LPO end products, phospholipase is significantly activated, which is the main enzyme that physics to split the phospholipid capsule of cell membranes. Excessive manifestation of OS and EI leads to disturbances in the cells membrane structures, due to a decrease in antioxidants in cell membranes.
Conclusions. When modeling inflammation in the gallbladder, the oxidative stress and endogenous intoxication develop, leading to the mechanism of apoptosis and necrosis.
Keywords: gallbladder, destructive cholecystitis, erythrocyte membranes, oxidative stress, endogenous intoxication, free radicals, lipid peroxidation, antioxidant defense, medium-molecular peptides, phospholipase

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Оценка окислительного стресса и эндогенной интоксикации при моделировании деструктивного холецистита в эксперименте на животных

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Резюме

Введение. В биологических жидкостях и тканях организма в избыточных концентрациях накапливаются промежуточные и конечные продукты нормального и нарушенного обмена веществ, которые оказывают токсическое влияние и вызывают дисфункцию органов и систем.
Цель. Оценка окислительного стресса и эндогенной интоксикации у животных при моделировании деструктивного холецистита.
Материалы и методы. Экспериментальное моделирование деструктивного холецистита на 12 кроликов породы шиншилла обоих полов весом 3–4 кг. Материалами для проведения исследований явились плазма крови и мембраны эритроцитов.
Результаты. Моделирование деструктивного холецистита у животных протекает на фоне развития окислительного стресса (ОС) и эндогенной интоксикации (ЭИ), причем эти два патогенетических аспекта воспаления взаимосвязаны. Между показателями процессов перекисного окисления липидов (ПОЛ) и среднемолекулярных пептидов (СМП) существует прямо пропорциональная зависимость, а именно между значением диеновых конъюгатов (ДК) и СМП (r=0,67), кетотриенов (КТ) и СМП (r=0,73) и малонового диальдегида (МДА) и СМП (r=0,63). Такая же зависимость проявляется и между ПОЛ и активностью фосфолипазы (r=0,71–0,78). Под действием большого количества конечных продуктов ПОЛ значительно активируется фосфолипаза, которая является основным ферментом, расщепляющим фосфолипидную оболочку клеточных мембран. Чрезмерная выраженность ОС и ЭИ ведет к нарушениям мембранных структур клеток вследствие уменьшения содержания антиоксидантов в клеточных мембранах.
Выводы. При моделировании воспаления в желчном пузыре развивается окислительный стресс, эндогенная интоксикация, приводящие к механизму апоптоза и некроза.

Ключевые слова: желчный пузырь, деструктивный холецистит, мембраны эритроцитов, окислительный стресс, эндогенная интоксикация, свободные радикалы, перекисное окисление липидов, антиоксидантная защита, среднемолекулярные пептиды, фосфолипаза

■ INTRODUCTION

It is known that the causes of inflammation in the bile ducts (BD) are associated with anatomical and physiological features: bile stasis and the possibility of local infection due to intestinal reflux [7, 15]. The BD infection is accompanied by the multiplication of pathogenic microorganisms in the bile, due to the optimal medium [14]. Further entry of bile into the blood leads to intoxication due to the massive entry of bacterial endotoxins and other biologically active products into the bloodstream [2, 8], and this, in turn, causes morphofunctional changes in the liver and other organs.

Another aspect of inflammation is formation of bile peroxidation products as a result of the intense degradation of bio-substrates during acute and chronic stages of inflammation [10]. Oxidation of biomolecules and generation of free radicals leads to damage to cellular structures [9]. Violation of the BD function, dysbiosis, accumulation of pathogenic microbes and toxins, and a decrease in intoxication systems, including the antioxidant defense system (AOD), result in development of oxidative stress (OS) and the accelerated development of the pathological process in the bile duct [11].

OS in humans and animals develops systemically and includes several stages, and the outcome of OS is an increase in the concentration of free radicals [13, 18]. OS is the dominant factor in damage to cell membranes, which proceeds in two directions. The first way is associated with the activation of the synthesis of free radicals, which cause inhibition of enzymatic activity and activation of the peroxide, antioxidant system, which also leads to the activation of LPO [12, 19].

In turn, the OS is closely related to another process called "endogenous intoxication" (EI). At its core, EI is a link and an integral part of OS in many pathological conditions. At the same time, metabolic by and end products of normal and impaired metabolism accumulate in biological fluids and tissues of the body in excessive concentrations, which have a toxic effect and cause dysfunction of organs and systems [16, 17].

The limited data on the development of OS and EI in destructive cholecystitis determined the need for this study.

■ PURPOSE OF THE STUDY

Evaluation of OS and EI by determining lipid peroxidation products, the AOD system, the concentration of medium-molecular peptides (MMP) and the activity of phospholipase in blood plasma and erythrocyte membranes in the experiment in animals when modeling destructive cholecystitis.

■ MATERIALS AND METHODS

The object of the study were 12 rabbits of the Chinchilla breed of both sexes weighing 3–4 kg. Modeling of destructive cholecystitis was carried out in accordance with the

rules for experimentation on animals complying with the European Convention for the Protection of Vertebrate Animals used for Experimental or Other Scientific Purposes (1986).

After introduction in anesthesia condition and laparotomy, a ureteral stand-catheter was installed through the bottom of the gallbladder (GB) into the cavity, fixed with a suture, and 1 ml (1 billion) of a suspension of Escherichia coli agar culture was introduced into the cavity of the gallbladder via this stand-catheter. In order to control the inflammatory process in the cavity of the gallbladder, bile secreted through the catheter was studied, the number of microbial bodies was determined on days 2, 5, 7, and 10. In 11 rabbits, destructive cholecystitis was detected, in 1 – acute cholecystitis, which indicated a high reproducibility of the method. In parallel, venous blood was taken from an ear vein for further special studies.

Blood plasma and erythrocyte membranes were used as a substrate for research. Erythrocyte membranes, despite the specific features of red blood cells, reflect the properties of the cytoplasmic membranes of the body in general and are a convenient model for studying the properties of bio membranes in physiology and pathology [1, 4].

Membranes (erythrocyte shadows) were obtained by hemolysis in a 20-fold volume of 0.005 mol/l sodium phosphate buffer, pH 8.0. The erythrocyte shadows were washed twice with hemolysate medium and once with buffer, pH 7.4. The assessment of the OS was carried out by determining the peroxide products and the AOD system.

Determination of lipid peroxidation products in erythrocyte membranes was carried out by spectrophotometric method by changes in absorption spectra in ultraviolet light. Determination of the concentration of malondialdehyde (MDA) in erythrocyte membranes was carried out by a biochemical method by reaction with thiobarbituric acid.

The antioxidant activity (AOA) of blood plasma lipids was assessed by the inhibitory effect of plasma on the free radical oxidation of erythrocyte membranes, in which oxidation is initiated by ultraviolet light [9]. Determination of catalase activity in blood plasma was carried out by the method of M.A. Korolyuk et al. [6], based on the ability of hydrogen peroxide to form a stable, colored complex with ammonium molybdate salts in the presence of blood plasma.

Endogenous intoxication was assessed by the concentration of MMP in the blood and the activity of phospholipase. The MMP concentration in blood plasma was determined by the spectrophotometric method [3]. Phospholipase activity in erythrocytes was assessed by the method of toxic hemolysis of RBC during their incubation with a solution of a standard preparation of egg lecithin.

The material was processed by the method of variation statistics on a personal computer in Microsoft Office Windows 95 spreadsheets using the statistical program package Statistik 6.0; the significance of differences was assessed using Student's t-test. Confidence code: at P equal to 95%, or $P<0.05$; at P equal to 99%, or $P<0.01$; with P equal to 99.9%, or $P<0.001$.

■ RESULTS AND DISCUSSION

As shown in Table 1, on the 2nd day of the experiment, there is an increase in the concentration in the erythrocyte membranes of the initial and end products of lipid peroxidation relative to the initial values, which manifests itself in an increase in diene conjugates (DC), ketotrienes (KT) and MDA ($P <0.05 – <0.01$).

If the concentration of DC increased by 27%, then KT – by 100%, and MDA – by 50%. On the 5th day of the experiment, a further increase in the content of peroxide products in the erythrocyte membranes is observed. KC are increased two – fold (P<0,01), KT – three – fold (P<0.001) and MDA remains at the level of the second day (P>0.05). On the 7th day, there is a significant activation of lipid peroxidation in cell membranes, characterized by an increase in the concentration of DC, KT and MDA, both in comparison with the

Table 1
Indicators of LPO products in erythrocyte membranes during the development of destructive cholecystitis in rabbits

Examination periods	Statistics	PLO products in erythrocyte membranes		
		DC, nmol/mg protein	KT, nmol/mg protein	MDA, U/ml
Baseline n=12	M ±m	0.28 0.02	0.10 0.01	0.125 0.031
Day 2 n=12	M ±m P 2–1	0.38 0.04 < 0.05	0.20 0.02 <0.01	0.221 0.05 <0.05
Day 5 n=1	M ±m P 3–1 P 3–2	0.53 0.06 <0.01 <0.05	0.33 0.03 <0.001 <0.05	0.262 0.057 <0.05 >0.05
Day 7 n=12	M ±m P 4–1 P 4–2 P 4–3	0.804 0.071 <0.001 <0.01 <0.05	0.54 0.034 <0.001 <0.001 <0.01	0.461 0.061 <0.001 <0.05 <0.05
Day 10 n=12	M ±m P 5–1 P 5–2 P 5–3 P 5–4	0.841 0.072 <0.001 <0.01 <0.05 >0.05	0.58 0.041 <0.001 <0.01 <0.05 >0.05	0.488 0.062 <0.001 <0.05 <0.05 >0.05

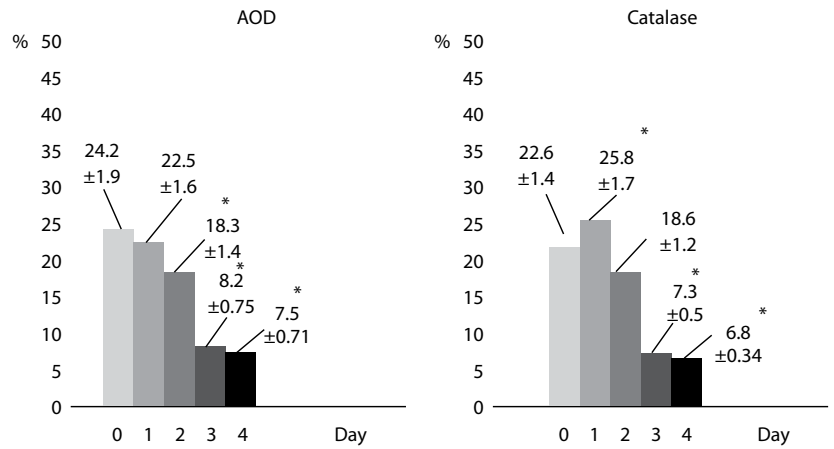

Fig. 1. Indicators of the AOD system in blood plasma in the dynamics of modeling destructive cholecystitis in animals

initial data (P<0.001) and in comparison with the periods on the 2nd and 5th days of the experiment (P <0.05 – <0.01). On the 10th day of the experiment, the trend of activation of lipid peroxidation processes persists.

The study of the AOD system in the experiment showed (Fig. 1) that on the 2nd day of modeling the process in the gallbladder, the level of total AOD in blood plasma practically remains at the level before the start of the experiment (P>0.05), while the catalase activity slightly increases (P<0.05). On the 5th day, there is a decrease in the level of AOD in plasma relative to the baseline and the indicator on the 2nd day (P<0.05). On the 7th day, there is a sharp decrease in both AOD and catalase activity, which are actually 3 times compared with the values before and on the 2nd day of cholecystitis modeling (P<0.001). On the 10th day, this trend persists.

Consequently, during the formation of morphological and functional changes in the gallbladder, associated with obsemination by the bacterial flora of the gallbladder cavity, free radical peroxidation is activated in cell membranes, which is accompanied by the development of destructive cholecystitis. Activation of lipid peroxidation processes is directly related to a change in the functioning of the AOD system and reflects the cellular inflammation mechanisms. It should be noted that MDA is an endogenous aldehyde which is synthesized through processes that include arachidonic acid and other polyunsaturated fatty acids. The accumulation of MDA is one of the signs of the presence of processes associated with the occurrence of oxidative stress situations.

The processes of lipid peroxidation in biological membranes are associated with the activity of EI processes. In our studies, the state of EI was assessed by two indicators, namely, the concentration of MMP in the blood and the activity of phospholipase. It is a well-known fact that the enzyme phospholipase A2, the substrate for which is the phospholipids of cell membranes, is activated in response to the action of LPO end products; after hydrolysis and cleavage of free fatty acids from phospholipids, mediators of a wide range of pro-inflammatory cellular processes are formed [5].

The definition of MMP is justified by the fact that pathological conditions are accompanied by metabolic disorders of varying severity. As a result of the activation of proteolysis, a large amount of protein degradation products is formed – MMP with a molecular weight of 300–5000 Daltons. They affect the vital activity of all organs and systems, since their structure is close to regulatory peptides, they are able to connect and block the receptors of any cell, and inadequately affect its metabolism and functions.

As shown in Figure 2, the concentration of MMP in blood plasma on the 2nd day of the experiment does not reach significant changes compared to the initial value (P>0.05). On the 5th day the MMP indicator exceeds the initial value and the 2nd day indicator (P<0.05). On the 7th day, the concentration of MMP exceeds the initial value by 2 times, as well as the 2nd and 5th days values (P<0<0.001). On the 10th day, its value continues to increase relative to the previous stages of the experiment.

The activity of phospholipase also has an increasing character during the experiment , and on the 7th day it is almost 5 fold higher than the initial value, on the 2nd day – 3 fold and on the 5th day – 2 fold (P<0.001).

Thus, modeling of destructive cholecystitis in animals proceeds against the background of the OS and EI development, and these two pathogenetic inflammation aspects are interrelated. There is a directly proportional relationship between the indicators of LPO and SMP processes, namely, between the value of DC and MMP (r=0.67), KT and MMP (r=0.73) and MDA and MMP (r=0.63).

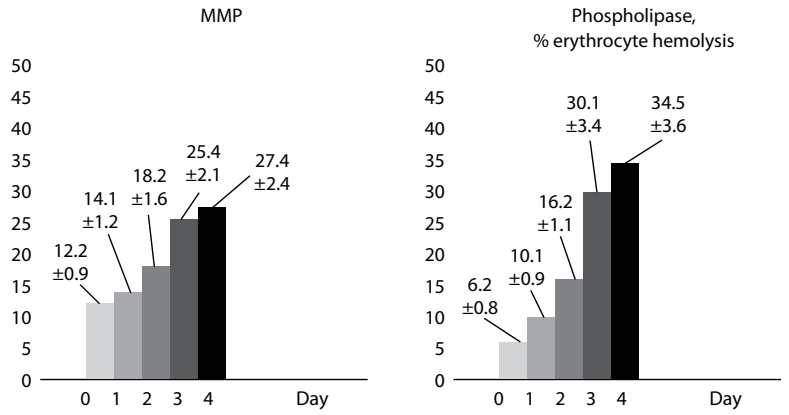

Fig. 2. Indicators of MMP concentration in blood plasma and phospholipase activity in erythrocytes when modeling destructive cholecystitis in animals

The same dependence appears between LPO and phospholipase activity (r=0.71–0.78). Under the influence of a large number of LPO end products, phospholipase is significantly activated, which is the main enzyme that breaks down the phospholipid membrane of cell membranes. Excessive severity of OS and EI leads to disturbances in the membrane structures of cells, due to a decrease in the content of antioxidants in cell membranes.

■ CONCLUSIONS

1. Modeling of destructive cholecystitis in the animal experiment involving oxidative stress and endogenous intoxication reveals the cellular mechanisms of the etiopathogenesis of gallbladder damage.
2. When modeling inflammation in the gallbladder, the activation mechanisms of free radical lipid peroxidation are triggered, which consequently lead to a decrease in the activity of the antioxidant defense system, the accumulation of medium-molecular peptides, and the activation of endogenous phospholipase. Ultimately, oxidative stress and endogenous intoxication develop, leading to the mechanism of apoptosis and necrosis.

■ REFERENCES

1. Aliyeva M.M. *Phospholipid composition of erythrocyte membranes and the intensity of peroxidation in erythrocytes in acute pneumonia in young children. 1st Congress of Children's Doctors of Uzbekistan: Collection of abstracts*. 1980:106–107. (In Russian)
2. Gafurovich N.F., Babajanovich K.Z., Salim D. Modified method of plasmapheresis in the treatment of patients with purulent cholangitis. *European science review.* 2018(7–8):142–7.
3. Gabrielyan N.I. Lipatova VI Experience of using the indicator of average blood molecules for the diagnosis of neurological diseases in children. *Laboratornoe delo,* 1984;3, 138–141.
4. Dobronravov A.V., Novitskaia A.V. Metabolic aspects of pathology of erythrocytic membranes (literature survey). *Vopr Okhr Materin Det.* 1976 Apr;21(4):20–4. (In Russian). Available at: https://pubmed.ncbi.nlm.nih.gov/136800/
5. Ishutina N.A. Phospholipase A2 activity and the state of lipid peroxidation processes in peripheral blood in pregnant women with herpes virus infection. *Advances in current natural sciences.* 2013;2:12–14. Available at: https://natural-sciences.ru/en/article/view?id=31354
6. Koroliuk M.A., Ivanova L.I., Maĭorova I.G., Tokarev V.E. A method of determining catalase activity. *Lab Delo.* 1988;(1):16–9. (In Russian). Available at: https://pubmed.ncbi.nlm.nih.gov/2451064/
7. Leishner U. *A practical guide to diseases of the biliary tract.* M.: GEOTAR-MED. 2001. Available at: https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Leishner+U.+A+practical+guide+to+diseases+of+the+biliary+tract.+M.%3A+publishing+&btnG=

8. Rubtsov G.K. *Methodical aspects of biochemical evaluation of endotoxicosis in patients with cholecystitis. Actual problems of anesthesiology, resuscitation and emergency medical care: diagnostics, intensive care and rehabilitation. Materials of the interregional scientific-practical conference.* Penza: PIFTPh. 2011;100–103. (In Russian). Available at: https://www.piuv.ru/media/fckeditor_storage/Izmeneniya_ustav_09_11_2016.pdf
9. Spektor E.B., Ananenko A.A., Politova L.N. Determination of total antioxidizing activity of blood plasma and cerebrospinal fluid. *Lab Delo.* 1984;(1):26–8. (In Russian). Available at: https://pubmed.ncbi.nlm.nih.gov/6199560/
10. Terekhina N.A., Zarivchanskiĭ M.F., Vladimirov A.A., Khlebnikov V.V. Antioxidative defense parameters in acute and chronic cholecystitis. *Klin Lab Diagn.* 2008 Apr;(4):41–3. (In Russian). Available at: https://pubmed.ncbi.nlm.nih.gov/18623632/
11. Tarasov N.I., Terent'eva N.V., Vorontsova N.L., Barbarash L.S. Dynamics of lipid peroxidation and antioxidant defense in response to dalteparin therapy in acute coronary syndrome. *Klin Med (Mosk).* 2004;82(3):63–6. (In Russian). Available at: https://pubmed.ncbi.nlm.nih.gov/15114779/
12. Bahja J., Dymond M.K. Does membrane curvature elastic energy play a role in mediating oxidative stress in lipid membranes? *Free Radical Biology and Medicine*. 2021 Aug 1;171:191–202. Available at: https://pubmed.ncbi.nlm.nih.gov/34000382/
13. Catalá A. Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. *Chemistry and physics of lipids.* 2009 Jan 1;157(1):1–1.
14. Girgin S., Gedik E., Taçyildiz I.H., Akgün Y., Baç B., Uysal E. Factors affecting morbidity and mortality in gangrenous cholecystitis. *Acta Chirurgica Belgica.* 2006 Jan 1;106(5):545–9.
15. Fagan S.P., Awad S.S., Rahwan K., Hira K., Aoki N., Itani K.M., Berger D.H. Prognostic factors for the development of gangrenous cholecystitis. *The American journal of surgery.* 2003 Nov 1;186(5):481–5.
16. Kujovich J.L. von Willebrand disease and pregnancy. *Journal of Thrombosis and Haemostasis.* 2005 Feb 1;3(2):246–53.
17. Kwek K., Yeo G.S. Current understanding of pre-eclampsia. *Annals of the Academy of Medicine, Singapore.* 2002 May 1;31(3):320–7.
18. Parra-Ortiz E., Browning K.L., Damgaard L.S.E., et al. Effects of oxidation on the physicochemical properties of polyunsaturated lipid membranes. *J Colloid Interface Sci.* 2019;538:404–419. doi:10.1016/j.jcis.2018.12.007. Available at: https://pubmed.ncbi.nlm.nih.gov/30530078/
19. Sies H. Oxidative stress: a concept in redox biology and medicine. *Redox Biol.* 4:180–183.