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CONTENTS

BIOLOGICAL SCIENCES

MEDICAL SCIENCES

EARTH SCIENCES

MEDICAL SCIENCES

Коцюбійчук З.Я.,
Антонів А.А.,
Махрова Є.Г.,
Вівсянник В.В.,
Царюченко Д.С.
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ОКСИДАТИВНЫЙ СТРЕСС КАК УНИВЕРСАЛЬНЫЙ МЕХАНИЗМ ПОВРЕЖДЕНИЯ ТКАНЕЙ ПРИ НЕАЛКОГОЛЬНОМ СТЕАТОГЕПАТИТЕ И ДИАБЕТИЧЕСКОЙ БОЛЕЗНИ ПОЧЕК

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THE OXIDATIVE STRESS WHICH IS A UNIVERSAL MECHANISM OF TISSUE DAMAGE IN NON-ALCOHOLIC STEATOGEPATITIS AND THOSE DIABETIC KIDNEY DISEASES

Анотація.

Установлено, что коморбидность течение неалкогольного стеатогепатита и диабетическая болезнь почек у больных сахарным диабетом типа 2 сопровождается существенным ростом интенсивности оксидативного стресса, сопровождается ростом содержания в крови промежуточных и конечных продуктов перекисного окисления липидов и окислительной модификации белков в пределах 1,9-2, 3 раза (p <0,05). Повреждающее действие оксидативного стресса у больных сахарным диабетом типа 2 приводит к активации процессов апоптоза гепатоцитов с повышением содержания в крови цитокератина-18 (в 7,5 раза, p <0,05), содержание которого коррелирует со степенью оксидативного стресса, интенсивностью повреждения печени и стадией диабетической болезни почек (p <0,05). Оксидативный стресс повышает риск повреждения эндотелия атеросклеротическим процессом через гиперпродукцию гомоцистеина (в 3,9 раза, p <0,05), что способствует прогрессированию диабетической болезни почек. Применение Кверцетина в комплексной терапии неалкогольного стеатогепатита и сахарный диабет типа 2 с диабетической болезнью почек способствует достоверному снижению интенсивности оксидативного стресса, усилению активности факторов антиоксидантной защиты (содержания в эритроцитах восстановленного глутатиона, активности глутатионпероксидазы, каталазы), следствием чего является существенное снижение процессов апоптоза гепатоцитов (снижение содержания цитокератина-18 в 1,7 раза) и повреждения эндотелия (снижение содержания в крови гомоцистеина в 1,9 раза) (p <0,05). Коморбидность течение неалкогольного стеатогепатита и диабетической болезни почек у больных сахарным диабетом типа 2 сопровождается существенным ростом интенсивности оксидативного стресса, сопровождается ростом содержания в крови промежуточных и конечных продуктов перекисного окисления липидов и окислительной модификации белков в пределах 1,9-2,3 раза (p <0,05).

Summary:

It was found that the comorbid course of nonalcoholic steatohepatitis and diabetic kidney disease in patients with type 2 diabetes mellitus is accompanied by a significant increase in the intensity of oxidative stress, accompanied by an increase in blood intermediate and final products of lipid peroxidation, lipid and oxidation. 3 times (p <0.05). The damaging effect of oxidative stress in patients with type 2 diabetes mellitus leads to the activation of apoptosis of hepatocytes with an increase in blood cytokeratin-18 (7.5 times, p <0.05), the content of which correlates with the degree of oxidative stress, the intensity of liver damage and stage of diabetic kidney disease (p <0.05). Oxidative stress increases the risk of endothelial damage by atherosclerotic process due to hyperproduction of homocysteine (3.9 times, p <0.05), which contributes to the progression of diabetic kidney disease. The use of quercetin in the complex therapy of non-alcoholic steatohepatitis and type 2 diabetes mellitus with diabetic kidney disease contributes to the probable reduction of oxidative stress, increased activity of antioxidant defense factors (content of reduced glutathione in erythrocytes, reduction of cytokeratin-18 content by 1.7 times) and endothelial damage (reduction of homocysteine content in blood by 1.9 times) (p <0.05). The comorbid course of nonalcoholic steatohepatitis and diabetic kidney disease in patients with type 2 diabetes mellitus is accompanied by a significant increase in the intensity of oxidative stress, accompanied by an increase in the content of intermediate and final products of lipid peroxidation and oxidative modification. p <0.05).

Ключевые слова*: неалкогольного стеатогепатита, сахарный диабет типа 2, диабетическая болезнь почек, оксидантно-антиоксидантный гомеостаз, апоптоз, атеросклероз, кверцетин.*

Keywords*: non-alcoholic steatohepatitis, type 2 diabetes mellitus, diabetic kidney disease, oxidative-antioxidant homeostasis, apoptosis, atherosclerosis, quercetin.*

Introduction. Every year in Ukraine and the world the incidence of non-alcoholic steatohepatitis (NASH) in patients with obesity and type 2 diabetes mellitus (DM2) increases significantly [1, 2]. The intensity of damage factors increases with the development of diabetic kidney disease (DKD) [1, 2, 3]. Oxidative stress (OS) occupies a leading place in the mechanisms of progression of NASH and DKN in patients with diabetes mellitus [4, 1, 2, 3]. The increase in the intensity of OS under the influence of various inducers underlies the transformation of nonalcoholic hepatic steatosis in NASH, the development and progression of inflammatory-necrotic changes in the liver in NASH, as well as liver fibrosis [1]. Oxidative stress is counteracted by antioxidant defense systems (ADS). The leading system of the natural detoxification system and ADS is the glutathione system. Performing the functions of a universal redox system, glutathione and a number of enzymes that serve it protect cell membranes from the effects of FRO, nitrogen (peroxynitrite), hydroperoxides, as well as binds hydrophilic products of microsomal oxidation (first phase of detoxification) and provides a second phase conjugation) with the excretion of non-toxic compounds from the body [1, 2]. Under conditions of comorbidity of pathological conditions, which are accompanied by a significant degree of OS, it is important to monitor the state of the ADS system, because the glutathione link is constantly depleted and requires its periodic replenishment. In our previous studies, it was proved that appropriate control over the content of reduced glutathione in erythrocytes makes it possible to adequately assess the body's need to restore and stimulate ADS in general in order to counteract OS [4, 1, 2].

In terms of counteracting free radical effects in clinical practice, the drug Quercetin - a flavonoid of plant origin, which inhibits the intensity of the processes of LPO and OMP membranes, stimulates the activity of catalase and superoxide dismutase (SOD) in cells [7, 5, 8]. Quercetin restores the ability of the endothelium to synthesize NO, which explains its cardioprotective effect in ischemic and reperfusion heart disease [7, 8, 9, 10]. The drug has a powerful anti-inflammatory effect, inhibiting 5-lipoxygenase, cyclooxygenase, hyaluronidase, a number of proteases, calcium-dependent ATPase, synthesis of leukotrienes LTC4 and LTB4, has immunomodulatory properties, thus inhibits the production of to reduce the area of necrotized myocardium and enhance reparative processes [11, 6]. There are a number of reports of hypolipidemic, choleretic, anticholestatic, hepatoprotective properties of quercetin, established in an experiment in obesity and in patients with NASH [7, 8, 12, 3, 13-15]. At the same time, the complex effect of Quercetin on the functional state of the LPO-ADS system, the intensity of apoptosis and the factors that regulate them in patients with NASH and DKD on the background of diabetes mellitus 2 has been studied in limited patients or experiment.

The purpose of the study was to determine the intensity of the effect of a complex of metformin, rosuvastatin, essential forte H and quercetin on the state of oxidative-antioxidant homeostasis, as well as the intensity of apoptosis of hepatocytes in the blood cytokeratin-18, which are factors in the progression of NASH and DKD.

Material and methods. Studies in the dynamics of treatment in 75 patients with NASH with type 2 diabetes and stage I-III DKD. According to the prescribed treatment, the examined patients were divided into 2 groups: (1 group - control: 37 patients) received a low-calorie diet with dietary restrictions №9, essential phospholipids (Essentiale forte H (Sanofi-Avensis / Natterman and Cie mg GmbH) 2 caps. 3 times a day) 30 days for the treatment of active NASH, for concomitant type 2 diabetes and hyperlipidemia was prescribed metformin hydrochloride (Metformin-Teva, LLC Teva Operations Poland) 1000 mg per day, rosuvastatin (Rozuvastatin-Teva, LLC Teva Operations Poland) (5 mg 1 time per day) for 1 month. Group 2 consisted of patients (38 people) who, in addition to similar dietary recommendations, Essentiale forte H, similar to hypoglycemic and hypolipidemic therapy for a month, additionally received the drug quercetin and povidone (Corvitin (PC NVC "Borshchahivsky CFP", Ukraine) 500 mg intravenously in 100 ml of isotonic sodium chloride solution) for 10 days. The mean age of patients was (54.7 ± 3.56) years. Groups of patients were randomized by age, sex, duration of the disease. The comparison group for the presentation of the average reference values of homeostasis indicators consisted of 30 healthy individuals (PHP) of the appropriate age.

The diagnosis of NASH was established in accordance with the unified clinical protocol approved by the order of the Ministry of Health of Ukraine № 826 from 06.11.2014, in the presence of criteria for exclusion of chronic diffuse liver disease of viral, hereditary, autoimmune or drug origin as the cause of cytolytic, cholestatic, mesenchymal also the results of ultrasonography (USG) on the ultrasound scanner Ultima PA ("Radmir", Kharkov, Ukraine) with shear wave elastography to determine the stage of liver fibrosis [6], calculation of hepato-renal index (HRI) and biochemical stestotests ("SteatoTest", "ASH" and "NASH-Test" (BioPredictive, France)) - to determine the degree of steatosis of the liver and its nature (alcoholic or non-alcoholic).

Diagnosis of type 2 diabetes was carried out in accordance with the unified clinical protocol approved by the Order of the Ministry of Health of Ukraine № 1118 of 21.12.2012. Diagnosis and treatment of CKD was carried out according to the recommendations of clinical guidelines GA "Institute of Nephrology NAMS of Ukraine" (2012). Calculation of glomerular filtration rate (GFR) was performed using a GFR calculator of the Institute of Nephrology of the National Academy of Medical Sciences of Ukraine on the average value of three calculated indicators: creatinine clearance by

Cocroft-Golt formula, MDRD and CKD EPI [2]. Determination of DKD stages was carried out according to the classification of C.E. Mogensen (1983) [1, 2].

The intensity of oxidative modification of proteins (OMP) in serum was determined by the method of Dubinina O.E. et al. in the modification of I.F. Meshchishen it contains aldehyde and ketondinitrophenylhydrazones (AKDPH) in the blood. The content in the blood of LPO products - isolated double bonds (IDP) in compounds, diene conjugates (DC), ketodienes and conjugated trienes (KCT) - according to I.A. Volchegorsky et al., Malonic aldehyde (MA) in blood plasma and Er - by Yu.A. Vladimirov, A.I Archakov. The content of reduced glutathione (GR) in the blood was determined by the titration method according to O.V. Travina in the modification of I.F. Meshchishena, I.V. Petrova. The activity of enzymes of the ADS system: glutathione peroxidase (GP) was studied by I.F. Meshishenym, glutathione-S-transferase (GT) - by I.F. Meschishenim, catalase - for M.A. Korolyuk et al. Enzyme activity was calculated per 1 g of Hb. The content of cytokeratin-18 (CC-18) in the blood was carried out by enzyme-linked immunosorbent assay (ELISA) using Elisa reagents. The content of homocysteine in the blood was performed by ELISA using a set of reagents Axis® Homocysteine Enzyme Immunoassay.

Before testing the statistical hypotheses, the analysis of the normality of the distribution of values in randomized samples was performed by determining the coefficients of asymmetry and excess using the Khan-Shapiro-Wilkie test. The probability of the difference between the arithmetic mean and its error between the study groups was determined using the bilateral odd Student's t-test. The difference was considered significant at a significance level of $p < 0.05$. Student's t-test was used only in the case of a normal distribution of equal variances of the compared samples, which was checked using Fisher's F-test. In other cases, a nonparametric Mann-Whitney rank test was used to compare the results. The probability of changes in variations in the dynamics of treatment in the case of normal distribution in the samples was determined by Student's paired test, in other cases - by non-parametric paired T-test of Wilcoxon. For statistical analysis of the obtained results we used software packages Statistica for Windows version 8.0 (Stat Soft inc., USA), Microsoft Excel 2007 (Microsoft, USA).

Research results and their discussion. The analysis of the obtained data showed that before treatment in patients of both groups of comparison a significant degree of OS was established, which was accompanied by a significant accumulation in the blood of intermediate and final products of LPO and OMP. Thus, before treatment, the content of MA in blood plasma exceeded the reference values by 2.1 times ($p < 0.05$), the content of FRO - 1.9 times ($p < 0.05$), the content of AKDPH OC - 2.3 times ($p < 0.05$) (Table 1). At the same time, the state of the antioxidant defense system was significantly unbalanced. Thus, the content of GR in the blood was lower than in PHP 1.7 times ($p < 0.05$), the activity of G3 - was inhibited 1.3 times ($p < 0.05$), which explains the high intensity of OS in the subjects patients.

These products of LPO and OMP on the background of significant insufficiency of the ADS system led to the activation of hepatocyte apoptosis. Evidence of this is a significant increase in the content of CC-18 in the blood - 7.5 times ($p < 0.05$) compared with PHP. Intensive OS and metabolic intoxication also resulted in an increase in the blood content of homocysteine in patients with NASH with DKD - 3.9 times ($p < 0.05$), which poses a risk of endothelial damage and progression of DKD.

Table 1

Indicators of oxidative stress intensity, antioxidant protection factors and markers of hepatocyte apoptosis in patients with a combined course of non-alcoholic steatohepatitis, type 2 diabetes mellitus and diabetic kidney disease in the dynamics of treatment (M ± m)

Indexes	PHP, n=30	Groups of examined patients			
		1, control, n=37		2, basic, n=38	
		before	after	before	after
MA plasma, μmol / hourhl	2,22±0,09	4,71±0,09 *	3,18±0,07 */**	4,73±0,07 *	2,35±0,05 **/#
IDP, E220/ml	2,89±0,02	5,53±0,06 *	4,76±0,05 */**	5,52±0,08 *	3,28±0,04 */**/#
AKDPH OC, o.od.g / l of protein	1,37±0,03	3,17±0,08 *	2,75±0,04 */**	3,19±0,05 *	1,70±0,03 */**/#
GR, μkmol / l	0,93±0,04	0,56±0,05 *	0,65±0,04 *	0,55±0,06 *	0,83±0,02 **/#
GP, nmol VG / min × gNb	152,22± 3,46	120,31±5,45 *	131,64±5,14 *	122,18±5,36 *	149,85±3,25 **/#
Cytokeratin-18, Ed / l	57,62 ± 5,37	428,34 ± 17,87 *	385,83±15,83 *	430,52 ± 18,45 *	249,28± 12,19 */**/#
Homocysteine, μkmol / l	9,93 ± 0,42	38,27 ± 1,51 *	32,62±1,37 *	39,23 ± 1,43 *	20,42±1,31 */**/#

Note: * - the difference is probable in comparison with the indicator in PHP ($p < 0,05$);
** - the difference is probable in comparison with the indicator before treatment ($p < 0,05$);
# - the difference is probable in comparison with the indicator in patients of the control group after treatment ($p < 0,05$).

The correlation analysis indicates a strong and medium correlation between the intensity of OS and the content of CC-18 and homocysteine in the blood of patients with NASH with DKD on the background of diabetes mellitus2 (Table 2), as well as weak and medium relationship with markers of liver damage in NASH and stage DKD (p <0,05).

Table 2

Matrix of correlations of morpho-functional parameters of the liver, kidneys, blood cytokeratin-18 and homocysteine with indicators of oxidative-antioxidant homeostasis in patients with NASH and DKD, DM2 (r, p)

Index	MA	IDP	DC	AKDPH	GR	GP	Catalase
Bilirubin	0,32*	0,43*	0,41*	0,38*	-0,45*	-0,21	-0,23
ALT	0,53*	0,57*	0,58*	0,44*	-0,69*	-0,34*	-0,37*
AST	0,51*	0,53*	0,51*	0,39*	-0,64*	-0,33*	-0,38*
GGT	0,49*	0,44*	0,47*	0,32*	-0,57*	-0,20	-0,25
AP	0,41*	0,43*	0,42*	0,33*	-0,43*	-0,28*	-0,12
Thymol test	0,48*	0,49*	0,47*	0,45*	-0,68*	-0,35*	-0,37*
Albumins	-0,34*	-0,41*	-0,42*	-0,34*	0,59*	0,43*	0,45*
Creatinine	0,58*	0,59*	0,60*	0,63*	-0,67*	-0,50*	-0,53*
GFR	-0,61*	-0,63*	-0,65*	-0,62*	0,62*	0,32*	0,33
Steat test	0,60*	0,62*	0,63*	0,51*	-0,65*	-0,49*	-0,50*
NASH- test	0,63*	0,65*	0,66*	0,52*	-0,68*	-0,53*	-0,56*
Fibrotest	0,54*	0,57*	0,59*	0,57*	-0,67*	-0,55*	-0,57*
CC-18	0,63*	0,68*	0,72*	0,70*	-0,75*	-0,64*	-0,65*
Homocysteine	0,51*	0,53*	0,57*	0,44*	-0,61*	-0,43*	-0,48*
Note. * - statistically significant correlation coefficient (p <0,05).							

Analyzing the indicators after treatment should indicate the higher effectiveness of therapy, which additionally contained Quercetin. Thus, significantly increased content of MA in the blood before treatment under the influence of therapy decreased in group 1 by 1.5 times (p <0.05), in group 2 - by 2.0 times (p <0.05). The increased content of the intermediate product LPO IDP decreased by 1.2 and 1.7 times, respectively (p <0.05). The prescribed therapy also had a significant effect on the increased content of AKDPH OC in the blood (2.3 times): yes, the decrease was 1.2 and 1.9 times, respectively (p <0.05). That is, after treatment we found a decrease in the intensity of OS as relative to the oxidation of structural lipids of cell membranes, including endothelium, hepatocytes and podocytes, and relative to structural proteins, due to the established increase in the activity of ADS. This is evidenced by the recovery of more glutathione in erythrocytes: in group 1 - 1.2 times (p> 0.05), in group 2 - 1.5 times (p <0.05) and a probable increase in the activity of GP after treatment - only in patients of group 2 1.2 times (p <0.05) (Table 1).

The obtained research results indicate that a significant decrease in the intensity of apoptosis processes after treatment was registered only in patients of group 2. Thus, the average blood content of CC-18 in patients with NASH with DKD group 2 after treatment probably decreased by 1.7 times (p <0.05), while in patients with group 1 changes were unlikely.

The effect of the proposed therapy with the addition of Quercetin was also more significant on the content of homocysteine in the blood - the decrease was 1.9 times (p <0.05), and in patients of the control group the indicator only tended to decrease (p> 0.05).

Conclusions. Comorbid course of nonalcoholic steatohepatitis and diabetic kidney disease in patients with type 2 diabetes mellitus is accompanied by a significant increase in the intensity of oxidative stress, accompanied by an increase in the content of intermediate and final products of lipid peroxidation and oxidative modification times (p <0.05). The damaging effect of oxidative stress in patients with type 2 diabetes leads to activation of hepatocyte apoptosis (increase in blood cytokeratin-18 by 7.5 times, p <0.05) with the progression of NASH, and an increased risk of endothelial damage due to atherogenesis (hyperproduction of homocysteine 3.9 times, p <0.05) with the progression of DKD. The use of Quercetin in the complex therapy of non-alcoholic steatohepatitis and type 2 diabetes with DHN contributes to a probable reduction in the intensity of oxidative stress, enhancing the activity of antioxidant defense factors (reduced glutathione, glutathione peroxidase), resulting in a significant decrease in the processes of apoptosis of hepatocytes (decrease in the content of CC-18 by 1.7 times) and damage to the endothelium (decrease in the content of homocysteine in the blood by 1.9 times).

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РЕЗУЛЬТАТИ ЗАСТОСУВАННЯ ЛІКУВАЛЬНО-ПРОФІЛАКТИЧНИХ ЗАСОБІВ НА ОСНОВІ ПОДВІЙНОГО ЦИНКУ ТА АРГІНІНУ СЕРЕД СТУДЕНТСЬКОЇ МОЛОДІ М.КИЄВА З ЕРОЗІЯМИ ЗУБІВ НА ТЛІ ЗАХВОРЮВАНЬ ТКАНИН ПАРОДОНТУ З ГЕНЕТИЧНОЮ СХИЛЬНІСТЮ ДО ДАНИХ ЗАХВОРЮВАНЬ

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RESULTS OF THE USE OF THERAPEUTIC AND PREVENTIVE AGENTS BASED ON DOUBLE ZINC AND ARGININE AMONG STUDENTS OF KIEV WITH DENTAL EROSIONS AGAINST THE BACKGROUND OF PERIODONTAL TISSUE DISEASES WITH A GENETIC PREDISPOSITION TO THESE DISEASES

Анотація:

Зміни мікроелементного складу змішаної слини відіграють важливу роль в етіології і в розвитку стоматологічних захворювань, зокрема некаріозних уражень зубів, особливо серед осіб молодого віку. Аналіз літератури дозволяє стверджувати, що практично усі дослідники відмічають серйозні порушення мінералізації і мікроструктури твердих тканин зубів при некаріозних ураженнях, однак показники мінерального обміну не лише у ротовій порожнині, але й в організмі цих пацієнтів залишаються недостатньо вивченими. Тому, актуальним залишається дослідження електролітного балансу слини при захворюваннях твердих тканин зубів, зокрема ерозіях зубів до та після застосування лікувально-профілактичної пасти на основі подвійного цинку та аргініну.

Однак, патологічні зміни, які призводять до порушення формування структури емалі залежить не тільки від впливу факторів зовнішнього середовища та мікроелементного складу слини, а й від індивідуальних особливостей організму, а саме розвитку та будови емалі зубів, які визначаються епігенетичними факторами. Нашими попередніми дослідженнями, виконаними в результаті клінічного і молекулярно-генетичного обстеження осіб молодого віку, була встановлена прогностична значимість в розвитку ерозій зубів поліморфних варіантів генів MMP20, KLK4, ENAM. Тому ми провели спостереження в динаміці через