ORIGINAL ARTICLE

Increasing the efficiency of hypolipidemic therapy with the combined use of quercetin in patients with non-alcoholic fatty liver disease on the background of the metabolic syndrome

Zvýšení účinnosti hypolypidemické léčby kombinovaným užíváním kvercetinu u pacientů s nealkoholickou steatózou jater a souvisejícím metabolickým syndromem

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Received October 4, 2023 /Accepted November 12, 2023

Summary

The article analyzes the results of a set of biochemical indicators in the course of treatment with the combined use of rosuvastatin with quercetin in patients with metabolic syndrome (MS) combined with non-alcoholic fatty liver disease.

Changes in blood biochemical parameters have been identified and presented with MS, essential for revealing general biological mechanisms development and interrelationship between the components of MS and non-alcoholic fatty liver. The effect of an increase in free cholesterol and triglycerides and activation of free-radical oxidation of lipids followed by the accumulation of oxidative stress products was noted.

In the course of long-term hypolipidemic therapy (90 days), 86 patients with non-alcoholic fatty liver disease on the background of metabolic syndrome were previously divided into 2 groups: a comparison group (45 patients), who were treated with basic therapy – only rosuvastatin, the main group (41 patients) received quercetin together with rosuvastatin – 40 mg 3 times a day, clinical and laboratory-instrumental examinations were carried out.

On the 90th day of treatment, positive results in the functional state of the liver and lipid spectrum of the blood were registered in all patients. A more significant advantage of the therapeutic combination of rosuvastatin with quercetin was proved. The inclusion of quercetin contributed to reducing the intensity of oxidative stress and enhancing antioxidant protection activity, resulting in a decrease in apoptosis of hepatocytes (cytokeratin-18 level was 1.27 times decreased). The studies have shown the feasibility of combined use of quercetin with rosuvastatin for the prevention of the development and progression of metabolic disorders associated with non-alcoholic fatty liver disease.

Key words: metabolic syndrome • non-alcoholic fatty liver disease • oxidative stress • quercetin • apoptosis of hepatocytes

Souhrn

Článek analyzuje výsledky souboru biochemických ukazatelů v průběhu léčby současným užíváním rosuvastatinu a kvercetinu u pacientů s metabolickým syndromem (MS) v kombinaci s nealkoholickou steatózou jater.

Byly zjištěny změny biochemických ukazatelů v krvi, které byly prezentovány u MS a které jsou důležité pro odhalení obecných biologických mechanismů vývoje a vzájemných vztahů mezi složkami MS a nealkoholickou steatózou jater. Byl zaznamenán vliv zvýšení hladiny volného cholesterolu, triglyceridů a aktivace volných radikálů oxidace lipidů s následnou akumulací produktů oxidačního stresu.

V průběhu dlouhodobé hypolipidemické léčby (90 dní) bylo 86 pacientů s nealkoholickou steatózou jater a souvisejícím metabolickým syndromem předem rozděleno do dvou skupin: srovnávací skupina (45 pacientů), která byla léčena základní terapií – pouze rosuvastatinem, hlavní skupina (41 pacientů) dostávala

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kvercetin spolu s rosuvastatinem – 40 mg 3krát denně, byla provedena klinická a laboratorně-instrumentální vyšetření.
Devadesátý den léčby byly u všech pacientů zaznamenány pozitivní výsledky funkčního stavu jater a lipidového spektra krve. Byla prokázána významnější výhoda léčebné kombinace rosuvastatinu s kvercetinem. Zařazení kvercetinu přispělo ke snížení intenzity oxidačního stresu, posílení antioxidační ochranné aktivity, v důsledku čehož došlo ke snížení apoptózy hepatocytů (hladina cytokeratinu-18 se snížila 1,27krát). Studie prokázaly možnost kombinovaného užívání kvercetinu s rosuvastatinem v prevenci rozvoje a progrese metabolických poruch spojených s nealkoholickou jaterní steatózou.
Klíčová slova: metabolický syndrom • nealkoholická steatóza jater • oxidační stres • kvercetin • apoptóza hepatocytů

Introduction

Research of last years indicates an increased prevalence of non-alcoholic fatty liver disease (NAFLD), which occurs in parallel with the spread of obesity and metabolic syndrome (MS). At the same time, hypertriglyceridemia and MS are defined as independent factors associated with the development of NAFLD[1]. There is no clear understanding of the pathophysiological mechanisms of the relationship between MS and NAFLD due to the complex biological interactions between them. There is increasing evidence for a bidirectional relationship between NAFLD and various chains of MS pathogenesis. Insulin resistance (IR) is considered a central pathophysiological process, which is general for these conditions[2, 3]. IR leads to lipid, carbohydrate, and fat metabolism disorders, resulting in the overproduction of free fatty acids (FFA). Under the conditions of FFA accumulation in hepatocytes, mitochondrial oxidation decreases, which creates a deficit of ATP in cells, and the increase in the microsomal oxidation intensity is accompanied by an excess of reactive oxygen species and the activation of lipid peroxidation (LPO)[4, 5]. LPO leads to inflammation, activation of cytokines, and damage to hepatocytes. So, special attention is drawn to the possibility of using antioxidants of plant origin, capable of correcting indicators of oxidative stress and inflammation, particularly flavonoid compounds, such as quercetin, in the treatment schemes of patients with NAFLD against the background of MS.

The clinical course and prognosis in patients with MS, to a great extent depending on the presence of comorbid pathological conditions, have a negative impact on the development of complications. The high prevalence of comorbid diseases in the set of symptoms structure over the past 10 years in Ukraine necessitates a comprehensive approach to the assessment of patients with MS, taking into account the accompanying pathological conditions to prevent complications and implement optimal strategies for treatment and prevention[6].

Our study aimed to assess the efficiency of hypolipidemic therapy with the combined use of quercetin in patients with non-alcoholic fatty liver disease on the background of the metabolic syndrome.

Experimental part

Material and methods

The group of 86 patients (average age 46 + 8 years) with metabolic syndrome was examined. The metabolic syndrome was diagnosed according to the Metabolic Syndrome Consensus[7].

All the examined patients underwent a general clinical examination, which included the anamnestic data collection, physical examination, laboratory (clinical blood and urine analysis, biochemical tests of the blood serum: liver enzymes (alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGTP), lipid profile (total cholesterol (TC), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), high-density lipoproteins (HDL), triglycerides (TG), LPO indicators, AOS[8] enzyme immunoassay with determination of IL-6 and CK-18 concentration[9].

The malondialdehyde (MDA) level was determined by the reaction with thiobarbituric acid, which, at high temperatures in an acidic environment, proceeds with the formation of a dyed trimethyl complex. The activity of superoxide dismutase (SOD) was determined by the level of inhibition by the nitrotetrazolium blue reduction enzyme with the participation of reduced nicotinamide-adenine dinucleotide (NAD) and phenazine methosulfate[8].

The liver ultrasound, liver elastography, and ECG were carried out.

The diagnosis «Non-alcoholic fatty liver disease» was established according to the current guideline «Non-alcoholic fatty liver disease» (2014), the recommendations «Diagnosis and treatment of non-alcoholic fatty liver disease: a practical guide of the American Association for the Study of Liver Diseases, College of Gastroenterology and the American Gastroenterological Association (2012) and EASL – EASD – EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease (2021)[10, 11].

For the diagnosis of non-alcoholic steatosis, ultrasound was used, which allows not only the assessment of the state of the liver (both the morphological component and acoustic properties) but also the detection of gallbladder and liver vessel abnormalities. The presence of steatohepatitis was diagnosed when ultrasound signs of fatty liver dystrophy were combined with increased levels of liver transaminases.

The patients were divided into 4 groups by «simple randomization»: the first group – 41 patients with NAFLD with MS, who received a standard hypolipidemic therapy, HMG-CoA reductase inhibitor rosuvastatin «Rosuvastatin IS» (Interchim, Ukraine) per os 20 mg/day and the quercetin preparation (Quertin chewable tablets

produced by the Borshchahivka Chemical Plant) by 40 mg three times a day for a long time, for 3 months; the group 2 – 45 patients with NAFLD with MS used the same term (90 days), the rosuvastatin course consisted of 20 mg/day; the group 3 included 20 patients with MS without liver complications, who were not subject to hypolipidemic therapy; the group 4 (the control group) consisted of 14 practically healthy people.

After registration of initial data, the basic therapy and quercetin were prescribed to all the patients of the studied groups.

The comprehensive laboratory-instrumental examination was conducted at the beginning of treatment, on the 40th and 90th day.

The mathematical computer processing of the research results was done using the software package «Statistica 8.0" – (Stat Soft Inc, USA). The differences were considered reliable at the level of statistical significance $p < 0.05$.

Results of the study and their discussion

At the beginning of the study, the patients with NAFLD and MS complained of rapid fatigue, general weakness, heaviness in the right hypochondrium, lack of appetite, flatulence, and a tendency to constipation. During the objective examination, moderate enlargement of the liver was noted, sometimes pain during palpation. Ultrasound data confirmed the liver enlargement and an uneven increase in the echogenicity of the liver tissue was observed. The patients of group 1 complained of heaviness and discomfort in the right hypochondrium – 37 patients (90.2%). Appetite was decreased in 34 patients (82.9%). The patients of group 2 had heaviness and discomfort in the right hypochondrium – 40 patients (88.8%). Appetite was decreased in 37 patients (82%). Fatigue took place in 98.6% of patients. In group 1, according to ultrasound, 2nd-degree liver steatosis (mostly diffused) was detected in 25 patients (60.9%), and 37 patients (90.2%) had the diaphragmatic margin U-sound attenuation, according to elastography, liver density up to 5.8 kPa was in 32 patients (78%). In group 2, 26 patients (57.7%) had the 2nd level of fatty infiltration (diffused), 38 patients (84.4%) had U-sound attenuation, and 35 patients (77.7%) had the liver density up to 5.8 kPa according to the results of elastography.

Biochemical parameters of the blood serum compared to patients of the control group were elevated: liver enzymes ALT, AST, triglycerides, total cholesterol, LDL, MDA level, the concentration of IL-6, CK-18 with a decrease in the SOD activity, and the level of HDL. GGTP indicators in all groups ranged within the reference values (Table 1).

After 40 days of treatment, a tendency to decrease in the liver enzyme activity and lipidogram indicators was determined in all the patients. Still, there was no statistical reliability between the groups.

After 90 days of the hypolipidemic therapy with rosuvastatin alone, the patients experienced a decrease in complaints: 33 patients (73.3%) felt heaviness and discomfort, 29 patients (64.4%) had a decrease in appetite, 15 patients (33.3%) had flatulence. Physical examination revealed a swollen abdomen in 8 patients (17.7%) and an enlarged liver in 32 (71.1%). 23 patients (51.1%) had 2nd-degree hepatic steatosis, and 32 patients (71.1%) had U-sound attenuation.

On the 90th day, the patients of group 2 had a significant decrease in the LDL level – by 21.2% ($p < 0.001$) compared to the beginning of treatment, but the LDL level did not reach the values of the control group – it was higher by 47.4%. The HDL level exceeded the values before treatment by 43.3% ($p < 0.001$). TG decreased by 19.1%. The activity of the liver enzymes, in particular ALT, was reduced by an average of 15%, AST – by 7.5% compared to before treatment, while their values did not reach a statistically significant difference. HGTP activity was noted at the level of the initial data. The IL-6 level on the 90th day was lower by an average of 10% and the CK-18 level – by 8.2% without statistical reliability of the indicators.

During the rosuvastatin treatment, changes in lipid peroxidation (LPO) and the antioxidant system condition were determined in the blood serum of MS patients with NAFLD. A decrease in the end product of free radical oxidation – MDA level by 35% ($p < 0.05$) was revealed compared to before treatment. The activity of the critical antioxidant enzyme – SOD increased by an average of 27.3 % ($p > 0.05$).

A comparative analysis of the clinical data of the group 1 patients, who were administered quercetin together with rosuvastatin, determined a more pronounced tendency to reduce complaints, in particular, heaviness and discomfort in the right hypochondrium – 17 (41.4%), decreased appetite – 12 (29.2%), flatulence – 10 (24.3%) patients. Physical examination revealed abdominal distension in 3 patients (7.3%) and liver enlargement in 21 (51.2%). According to ultrasound, 18 patients (43.9%) had 2nd-degree steatosis and U-sound attenuation – in 27 patients (65.8%).

The blood serum analysis of these patients on the 90th day of treatment revealed a significant decrease in the LDL level – by 39.5% ($p < 0.05$) compared to before treatment, and was significantly lower – by 22% compared to the group 2 ($p < 0,01$). The TG level was determined reliably ($p < 0.05$) 2 times lower compared to before treatment, being on average (1.06 ± 0.08) mmol/l, also reliably 1.6 times lower than the indicator in group 2. The HDL level was significantly higher on average by 72% ($p < 0.05$) compared to before treatment and 12% higher than on average in group 2. AST activity was significantly lower – by 27% ($p < 0.001$) compared to before treatment and by 16% compared to the second group. The GGTP activity ranged within the limits of the reference values. A significant decrease in IL-6 concentration on average by 23% ($p < 0.05$) and CK-18 level by 22% ($p < 0.001$) compared to before treatment was noted.

In the patients of group 1, the MDA dynamics was more pronounced: the decrease occurred on average

by 62% compared to the indicators before treatment ($p < 0.05$), and it was almost twice as significant as in the patients of group 2. An increase in SOD activity was noted on average 2.25 times compared to before treatment. Activation of the antioxidant enzyme was 66.4% higher in patients of this group than in patients of the basic therapy only.

So, the main complaint of MS patients with NAFLD was heaviness in the right hypochondrium. When assessing the objective status, the liver enlargement was revealed by 2–3 cm below the costal arch level. An increase in the echogenicity of the liver, mainly diffused, was found in all patients during ultrasound. When evaluating the biochemical parameters of blood in patients with MS and NAFLD, an increase in the transaminase activity was found in 42% of cases and GGTP – in 32% of the examined, with a probable difference from the control group, which indicates the presence of the cytolysis and cholestasis laboratory syndromes in patients, which confirm the development of steatohepatitis and can be compared with literature data (Table 2).

Studies of indicators of lipid metabolism showed an increase in the TC level in 8 % of patients compared to the control group. An increase in the LDL level was

Table 1. The studied serum blood indicators in patients with metabolic syndrome and NAFLD before the treatment

Indicators	Groups							
	Group 1					Group 2		
	Control group, n = 14	MS n = 20	NAFLD n = 11	Steatosis n = 22	NASH n = 8	NAFLD n = 12	Steatosis n = 23	NASH n = 10
TG, mmol/l, p p_1	1.15 ± 0.07	1.90 ± 0.07 < 0.05	1.96 ± 0.08 < 0.05 > 0.05	2.08 ± 0.08 < 0.05 > 0.05	2.26 ± 0.09 < 0.05 < 0.05	1.9 ± 0.09 < 0.05 > 0.05	2.10 ± 0.08 < 0.05 > 0.05	2.32 ± 0.09 < 0.05 < 0.05
VLDL, mmol/l, p p_1	0.38 ± 0.12	0.68 ± 0.11 < 0.05	0.72 ± 0.10 < 0.05 > 0.05	0.76 ± 0.13 < 0.05 > 0.05	0.80 ± 0.14 < 0.05 > 0.05	0.74 ± 0.12 < 0.05 > 0.05	0.78 ± 0.13 < 0.05 > 0.05	0.82 ± 0.16 < 0.05 > 0.05
LDL, mmol/l p p_1	2.78 ± 0.11	4.40 ± 0.20 < 0.05	5.18 ± 0.18 < 0.05 < 0.05	5.20 ± 0.20 < 0.05 < 0.05	5.22 ± 0.19 < 0.05 < 0.05	5.15 ± 0.16 < 0.05 < 0.05	5.18 ± 0.19 < 0.05 < 0.05	5.20 ± 0.22 < 0.05 < 0.05
HDL, mmol/l p p_1	1.25 ± 0.08	1.10 ± 0.09 > 0.05	1.10 ± 0.09 > 0.05 > 0.05	1.30 ± 0.08 > 0.05 > 0.05	1.10 ± 0.07 > 0.05 > 0.05	1.00 ± 0.07 < 0.05 > 0.05	1.20 ± 0.09 > 0.05 > 0.05	1.18 ± 0.09 > 0.05 > 0.05
TC, mmol/l p p_1	4.25 ± 0.06	8.00 ± 0.60 < 0.05	8.28 ± 0.54 < 0.05 > 0.05	8.34 ± 0.60 < 0.05 > 0.05	8.40 ± 0.64 < 0.05 > 0.05	8.16 ± 0.58 < 0.05 > 0.05	8.26 ± 0.60 < 0.05 > 0.05	8.30 ± 0.58 < 0.05 > 0.05
ALT, units/l p p_1	24.20 ± 2.2	105.80 ± 7.60 < 0.05	102.60 ± 5.8 < 0.05 > 0.05	106.80 ± 6.4 < 0.05 > 0.05	107.4 ± 6.2 < 0.05 > 0.05	106.80 ± 5.8 < 0.05 > 0.05	108.00 ± 6.50 < 0.05 > 0.05	107.60 ± 6.68 < 0.05 > 0.05
AST, units/l p p_1	27.10 ± 1.60	94.00 ± 8.20 < 0.05	92.50 ± 6.80 < 0.05 > 0.05	93.00 ± 7.00 < 0.05 > 0.05	94.00 ± 8.00 < 0.05 > 0.05	94.00 ± 7.60 < 0.05 > 0.05	93.80 ± 8.00 < 0.05 > 0.05	94.00 ± 8.20 < 0.05 > 0.05
MDA, mmol/l p p_1	2.10 ± 1.04	8.04 ± 1.10 < 0.05	7.98 ± 0.90 < 0.05 > 0.05	8.14 ± 1.08 < 0.05 > 0.05	8.00 ± 1.06 < 0.05 > 0.05	7.84 ± 1.20 < 0.05 > 0.05	8.08 ± 1.12 < 0.05 > 0.05	8.16 ± 0.98 < 0.05 > 0.05
SOD, units/l p p_1	26.60 ± 3.80	12.08 ± 4.20 < 0.05	11.98 ± 3.60 < 0.05 > 0.05	12.20 ± 4.00 < 0.05 > 0.05	10.84 ± 2.80 < 0.05 > 0.05	12.24 ± 3.60 < 0.05 > 0.05	11.46 ± 2.80 < 0.05 > 0.05	10.96 ± 2.40 < 0.05 > 0.05
GGTP units/l p p_1	48.34 ± 1.80	51.4 ± 3.00 > 0.05	47.20 ± 2.8 > 0.05 > 0.05	46.80 ± 3.00 > 0.05 > 0.05	48.40 ± 4.20 > 0.05 > 0.05	48.60 ± 3.80 > 0.05 > 0.05	48.00 ± 4.20 > 0.05 > 0.05	52.60 ± 4.80 > 0.05 > 0.05
IL-6 p p_1	1.18 ± 0.20	5.00 ± 0.18 < 0.05	4.86 ± 0.22 < 0.05 > 0.05	4.92 ± 0.23 < 0.05 > 0.05	4.95 ± 0.22 < 0.05 > 0.05	4.95 ± 0.22 < 0.05 > 0.05	4.98 ± 0.23 < 0.05 > 0.05	5.00 ± 0.23 < 0.05 > 0.05
CK-18 p p_1	40.60 ± 6.80	365.80 ± 14.00 < 0.05	356.60 ± 14.00 < 0.05 > 0.05	360.00 ± 15.00 < 0.05 > 0.05	364.00 ± 16.00 < 0.05 > 0.05	366.80 ± 16.00 < 0.05 > 0.05	371.40 ± 14.80 < 0.05 > 0.05	368.00 ± 16.00 < 0.05 > 0.05

p – the difference in reliability relative to the control group indicators

p_1 – the difference in reliability relative to indicators of the group with metabolic syndrome

noted in 68% of patients and reliably differed from the healthy group. However, the LDL level was reduced in 72% of patients. The TG level was 1.8 times higher in 76% of patients compared to the control group. The TG/HDL ratio can indirectly assess insulin resistance, which underlies MS development. In our studies, the TG/HDL ratio was, on average, 0.92 ± 0.14 in healthy people, while in the NAFLD patients with MS, it was 1.78 ± 0.22, which indicated the presence of insulin resistance (IR).

IR is considered the main connecting link of all components of MS and liver damage[13, 14]. The initial effect of IR is the accumulation of free fatty acids in hepatocytes, which leads to liver steatosis. Against the background of steatosis and the formation of reactive oxygen species due to complex interactions between cytokines, endotoxins, macrophages, and hepatocytes, lipolysis in adipose tissue increases, and fatty dystrophy of hepatocytes occurs[15, 16]. At the same time, the oxidative stress develops with the formation of the inflammatory reaction and its transformation into steatohepatitis and further into non-alcoholic cirrhosis of the liver. Free fatty acids released into the portal circulation enter the

Table 2. Changes in blood serum indicators in patients with non-alcoholic fatty liver disease on the background metabolic syndrome after treatment

Indicators	Groups						
	Group 1				Group 2		
	Control group n = 14	NAFLD n = 11	Steatosis n = 22	NASH n = 8	NAFLD n = 12	Steatosis n = 23	NASH n = 10
TG, mmol/l, p p_1	1.15 ± 0.07	1.00 ± 0.06 > 0.05	1.08 ± 0.08 > 0.05	1.12 ± 0.10 > 0.05	1.58 ± 0.08 < 0.05 < 0.05	1.64 ± 0.10 < 0.05 < 0.05	1.80 ± 0.08 < 0.05 < 0.05
VLDL, mmol/l, p p_1	0.38 ± 0.12	0.60 ± 0.11 < 0.05	0.68 ± 0.10 < 0.05	0.70 ± 0.11 < 0.05	0.72 ± 0.12 < 0.05 > 0.05	0.78 ± 0.10 < 0.05 > 0.05	0.80 ± 0.08 < 0.05 > 0.05
LDL, mmol/l p p_1	2.78 ± 0.11	3.10 ± 0.13 > 0.05	3.19 ± 0.15 < 0.05	3.24 ± 0.14 < 0.05	4.10 ± 0.12 < 0.05 < 0.05	4.10 ± 0.13 < 0.05 < 0.05	4.28 ± 0.15 < 0.05 < 0.05
HDL, mmol/l p p_1	1.25 ± 0.08	1.96 ± 0.15 < 0.05	1.92 ± 0.12 < 0.05	1.94 ± 0.20 < 0.05	1.68 ± 0.12 < 0.05 > 0.05	1.72 ± 0.13 < 0.05 > 0.05	1.76 ± 0.15 < 0.05 > 0.05
TC, mmol/l p p_1	4.25 ± 0.06	6.46 ± 0.56 < 0.05	6.50 ± 0.62 < 0.05	6.55 ± 0.58 < 0.05	7.48 ± 0.48 < 0.05 < 0.05	7.64 ± 0.54 < 0.05 > 0.05	7.86 ± 0.50 < 0.05 > 0.05
ALT, units/l p p_1	24.20 ± 2.2	76.10 ± 6.70 < 0.05	79.00 ± 7.20 < 0.05	78.00 ± 6.40 < 0.05	92.50 ± 7.10 < 0.05 > 0.05	90.40 ± 6.70 < 0.05 > 0.05	92.30 ± 5.80 < 0.05 > 0.05
AST, units/l p p_1	27.10 ± 1.60	66.40 ± 4.20 < 0.05	69.80 ± 4.00 < 0.05	70.80 ± 3.60 < 0.05	78.40 ± 3.80 < 0.05 > 0.05	82.60 ± 5.00 < 0.05 > 0.05	85.90 ± 3.60 < 0.05 < 0.05
MDA, mmol/l p p_1	2.10 ± 1.04	2.80 ± 0.80 > 0.05	3.20 ± 0.52 > 0.05	3.00 ± 0.60 > 0.05	5.80 ± 0.65 < 0.05 < 0.05	4.80 ± 0.80 < 0.05 > 0.05	5.20 ± 0.75 < 0.05 < 0.05
SOD, units/l p p_1	26.60 ± 6.75	24.80 ± 4.60 > 0.05	25.60 ± 3.60 > 0.05	28.40 ± 5.20 > 0.05	16.20 ± 3.60 > 0.05 > 0.05	15.90 ± 4.40 > 0.05 > 0.05	12.20 ± 4.00 > 0.05 > 0.05
GGTP, units/l p p_1	48.34 ± 1.80	41.40 ± 3.80 > 0.05	42.40 ± 4.20 > 0.05	41.80 ± 4.30 > 0.05 > 0.05	58.00 ± 5.20 > 0.05 > 0.05	56.40 ± 5.00 > 0.05 > 0.05	55.60 ± 5.12 > 0.05 > 0.05
IL-6 p p_1	1.18 ± 0.20	3.75 ± 0.22 < 0.05	3.80 ± 0.20 < 0.05	3.85 ± 0.28 < 0.05	4.54 ± 0.30 < 0.05 > 0.05	4.42 ± 0.36 < 0.05 > 0.05	4.38 ± 0.80 < 0.05 > 0.05
CK-18 p p_1	40.60 ± 6.80	280.00 ± 20.00 < 0.05	286.00 ± 22.00 < 0.05	288.00 ± 22.00 < 0.05	340.00 ± 20.00 < 0.05 < 0.05	339.00 ± 21.00 < 0.05 > 0.05	335.00 ± 22.00 < 0.05 > 0.05

p – the difference reliability relative to the indicators of the control group

p_1 – the difference reliability in indicators between the group 1 and group 2

liver and become a source of LDL formation, serving as transport forms of cholesterol[17, 18]. NAFLD often leads to the development of highly atherogenic dyslipidemia with high titers of triglycerides, LDL, and low HDL, maintaining the subclinical inflammation condition due to an increase in the concentration of pro-inflammatory cytokines, leukocytes, C-reactive protein (CRP), LPO processes enhancement[14, 19].

In our research, in patients with MS and NAFLD, LPO activation was determined, which manifested itself by a reliable 3.8 times increase in MDA level compared to the control group. During the antioxidant protection study, according to the SOD activity in the blood serum, a reliable 2.2 times decrease was found compared to healthy people. The obtained results indicate a reduction in the functional capacity of the antioxidant system in NAFLD against the MS background. Suppression of the antioxidant system activity is one of the reasons for the oxidative stress development in MS patients because, in this case, SOD is responsible for the removal of the primary active forms of oxygen – the superoxide anion radical. Our results are confirmed by the data of researchers[12, 17], who found direct and reliable relationships between MDA and, ALT, LDL, indicating the parallelism of LPO activation and the severity of cytolysis syndrome, dyslipidemia, and an increase in adipose tissue in the liver during MS. The SOD activity demonstrates reliable inverse relationships with transaminases[17].

The analysis of the effect of treatment on lipid peroxidation and the state of antioxidant status showed that the group of patients with NAFLD and MS who received quercetin in addition to the basic therapy had a significant decrease in the level of the secondary product of free radical oxidation – MDA in the blood plasma compared to the group receiving the basic therapy only. At the same time, an increase in the SOD activity was noted, which indicates the activation of the organism's antioxidant protection. It can be connected with the fact that quercetin is one of the most common flavonoids with a multimodal effect due to antioxidant activity, anti-inflammatory, anti-hypoxic, membrane-stabilizing, immunomodulating properties, the addition of which to the hypolipidemic therapy has a positive effect on both the oxidant and the antioxidant system manifested in the LPO inhibition and the compensatory processes activation, which provides the maintenance of free radicals at the level necessary for the normal course of metabolic processes in the cell[20, 21].

The research data indicate that the development of chronic subclinical inflammation in MS patients with NAFLD is characterized by an increase in the concentration of markers of systemic inflammation – pro-inflammatory cytokines IL-6, which were determined to be four times higher than the indicators of the control group, which is probably caused by the metabolic disorders severity and was compared with changes in liver ultrasound and elastography data. The patients with rosuvastatin monotherapy had decreased concentrations of IL-6 and CK-18. However, patients who were prescribed quercetin in addition to rosuvastatin had more pronounced anti-inflammatory and hypolipidemic effects and significantly lower levels of IL-6 and CK-18[15, 21, 22].

Therefore, the combined hypolipidemic therapy with quercetin corrects metabolic processes in the liver tissue cells, the organism, reduces chronic systemic inflammation, realizing an additional pathogenetically necessary effect on lipid metabolism (decrease of LDL, TG, TC, increase of HDL), on structural and functional state of the liver (ALT, AST, CK-18).

The results obtained in the course of the study open up new perspectives for the correction of metabolic shifts, elimination of the oxidative stress in patients with NAFLD with MS by administration of the complex hypolipidemic therapy with quercetin, which significantly reduces the risk of the metabolic syndrome complications and improves their treatment.

Conclusions

MS patients have a high risk of developing NAFLD (72.8% according to ultrasound data) with corresponding shifts of clinical and laboratory parameters compared to healthy patients. The relationship between laboratory indicators (hypercholesterolemia, hypertriglyceridemia, cytolysis of hepatocytes, systemic inflammation) and clinical characteristics, results of instrumental research methods, which indicate the liver damage severity in MS, was revealed. Hypolipidemic therapy with rosuvastatin in patients with NAFLD for 3 months led to a decrease in the TG level by 21.1% ($p < 0.05$), LDL – by 21.2% ($p < 0.05$), CK – by 9% ($p > 0.05$). A decrease in IL-6 by 10.0 % and CK-18 – by 8.2% was found, but these data did not have a statistical significance. The liver enzyme activity and ultrasound results did not change significantly after treatment.

Against the background of the combined therapy with rosuvastatin and quercetin, a clear regression of clinical manifestations of the disease was observed in patients with NAFLD and MS than in patients with rosuvastatin only: there were almost two times fewer complaints, namely, periodic pain in the right subcostal region, symptoms of asthenovegetative and dyspeptic syndromes. The positive dynamics of clinical symptoms were accompanied by a decrease in the activity of the inflammatory process in the liver tissue: the level of serum transaminases, markers of POL and lipid metabolism, and concentration of IL-6 and CK-18. The obtained biochemical and immunological indicators corresponded to the changes according to the elastography data: a decrease in the degree of liver steatosis (n = 9; 23.8%), a decrease in the size of the liver (n = 8; 18.6%), the U-sound attenuation to the diaphragmatic margin (n = 6; 15.6%). A comparative analysis of the comprehensive examination of patients with NAFLD with MS determined quercetin effectiveness during a long-term combined hypolipidemic therapy and the necessity of

preventing the development and progression of metabolic disorders associated with the disease.

Further studies will be aimed at studying indicators of the systemic inflammation metabolic shifts of lipid and carbohydrate metabolism in patients with comorbid conditions of MS to assess the applied therapy and the duration of its effects, with the determination of the remote treatment results.

The study is a fragment of the research project «Comorbid conditions in patients with metabolic syndrome: familial hypercholesterolemia, fatty hepatosis, periodontopathies (pathogenesis, diagnosis, correction)», state registration number 0121U00263.

Conflicts of interest: none.

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