

Abstract

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**THE EFFECT OF BICYCLOL ON THE STATE OF THE CONNECTIVE TISSUE COMPONENTS OF THE LIVER EXTRACELLULAR MATRIX IN THE COMPLEX THERAPY OF NON-ALCOHOLIC STEATOHEPATITIS WITH LIVER FIBROSIS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS**

**The aim of the research** was to determine the probable effect of metformin, rosuvastatin and bicyclol on markers of hepatocyte cytolysis in patients with nonalcoholic steatohepatitis and diabetes mellitus type 2 with diabetic kidney disease, the degree of hepatocyte steatosis and stage of liver fibrosis, as well as the content of extracellular protein and carbohydrate components in the blood tissues that are markers of the intensity of liver fibrosis and the progression of non-alcoholic steatohepatitis.

**Materials and methods.** Study of changes in the course of treatment of 60 patients with nonalcoholic steatohepatitis with type 2 diabetes mellitus and stage I–IV diabetic kidney disease was conducted, among whom 48 patients were diagnosed with mild non-alcoholic steatohepatitis and 12 – with moderate non-alcoholic steatohepatitis. A comorbid disease, i.e. type 2 diabetes mellitus of moderate severity, was registered in 100% of patients with nonalcoholic steatohepatitis: among them, 15 people had diabetes in the compensatory stage, 45 people – in the subcompensated stage. All patients with nonalcoholic steatohepatitis and type 2 diabetes had comorbid diabetic kidney disease, including 21 cases of stage I–II diabetic kidney disease, 20 cases of stage III diabetic kidney disease, and 19 patients with stage IV diabetic kidney disease.

**Results.** Analysis of the results of extracellular matrix connective tissue metabolism in the blood of patients with non-alcoholic steatohepatitis on the background of type 2 diabetes mellitus and diabetic kidney disease indicated that the inflammatory and dysmetabolic process contributed to a significant imbalance of connective tissue components. In particular, activation of anabolic collagen was shown on the basis of an increase in blood protein-bound oxyproline by 2 times ( $p < 0.05$ ), as well as a decrease in the intensity of collagen catabolism – based on a decrease in blood free oxyproline by 1.4 times ( $p < 0.05$ ), which probably occurred due to inhibition of collagenolytic activity of blood plasma (by 1.4 times,  $p < 0.05$ ). That is, activated processes of collagen synthesis were accompanied by inhibition of its degradation and accumulation in the extracellular matrix. We also found a significant increase in the blood content of hexosamines by 1.5 times ( $p < 0.05$ ) and accelerated degradation of carbohydrate-protein components of the

matrix (with an increase in the content of unbound fucose by 2.6 times, ( $p < 0.05$ )).

**Conclusions.** The combination therapy with metformin, rosuvastatin in combination with Bicyclol in individuals with comorbid nonalcoholic steatohepatitis, type 2 diabetes mellitus and diabetic kidney disease for 3 months helped to eliminate the syndrome of cytolysis of hepatocytes, a significant reduction liver fibrosis intensity due to optimization of spectrum of connective tissue components of the extracellular matrix in the blood with a decrease in the content of markers of collagen anabolism, increase in the content of markers of collagen catabolism due to increased total collagenolytic activity of blood plasma, decrease in the content of hexosamines and carbohydrate protein markers.

**Keywords:** non-alcoholic steatohepatitis, type 2 diabetes mellitus, diabetic kidney disease, liver steatosis, liver fibrosis, Bicyclol.

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#### Резюме

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#### ВПЛИВ БІЦИКЛОЛУ НА СТАН СПОЛУЧНОТКАНИННИХ КОМПОНЕНТІВ ПОЗАКЛІТИННОГО МАТРИКСУ ПЕЧІНКИ У КОМПЛЕКСНІЙ ТЕРАПІЇ НЕАЛКОГОЛЬНОГО СТЕАТОГЕПАТИТУ З ФІБРОЗОМ ПЕЧІНКИ У ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ ТИПУ 2

**Мета роботи:** з'ясувати можливий вплив комплексу засобів метформіну, розувастатину та біциклолу на маркери цитолізу гепатоцитів та стадію фіброзу печінки. білкових компонентів позаклітинного матриксу печінкової тканини, які є маркерами інтенсивності фіброзування печінки та прогресування неалкогольного стеатогепатиту.

**Матеріали та методи.** Проведено дослідження динаміки лікування 60 хворих на неалкогольний стеатогепатит з цукровим діабетом 2 типу та діабетичною хворобою нирок I–IV стадії, з яких у 48 пацієнтів діагностовано неалкогольний стеатогепатит легкого ступеня, а у 12 – неалкогольний стеатогепатит середнього ступеня тяжкості. Коморбідність захворюванням у 100% хворих на неалкогольний стеатогепатит був цукровий діабет типу 2 середнього ступеня тяжкості, серед яких у 15 осіб цукровий діабет був у стадії компенсації, у 45 субкомпенсований. Всі хворі на неалкогольний стеатогепатит і цукровий діабет типу 2 мали коморбідність діабетичної хвороби нирок, зокрема, 21 особа з діабетичною хворобою нирок II стадії, 20 осіб з діабетичною хворобою нирок I–II стадії, 19 осіб з діабетичною хворобою нирок IV стадії.

**Результати.** Аналіз результатів дослідження вмісту продуктів метаболізму сполучної тканини позаклітинного матриксу в крові хворих на неалкогольний стеатогепатит на тлі цукрового діабету типу 2 та діабетичної хвороби нирок вказує на те, що запальний та дисметаболічний процес сприяє суттєвому дисбалансу компонентів сполучної тканини. Зокрема, встановлено активацію анаболічних колагену за зростанням у крові білковозв'язаного оксипроліну в 2,0 рази ( $p < 0,05$ ), а також зниження інтенсивності процесів катаболіза-

му колагену – за зниженням вмісту в крові вільного оксипроліну у 1,4 рази ( $p < 0,05$ ), що виникло, ймовірно, внаслідок гальмування колагенолітичної активності плазми крові (в 1,4 рази,  $p < 0,05$ ). Тобто активовані процеси синтезу колагену супроводжуються гальмуванням його деградації з накопиченням позаклітинного матриксу. Нами також встановлено суттєве підвищення вмісту в крові гексазамінів у 1,5 рази ( $p < 0,05$ ) та прискорена деградація вуглеводно-білкових компонентів матриксу (зі зростанням вмісту в крові фукози, не пов'язаного з білком, у 2,6 рази, ( $p < 0,05$ )).

**Висновки.** Комбінована терапія метформіном, розувастатином у поєднанні з біциклолом у осіб з коморбідним неалкогольним стеатогепатитом, цукровим діабетом 2 типу та діабетичною хворобою нирок протягом 3 місяців дозволила усунути синдром цитолізу гепатоцитів, значно знизити вираженість фіброзу. Сполучнотканинні компоненти позаклітинного матриксу в крові при зниженні вмісту маркерів анаболізму колагену, підвищенні вмісту маркерів катаболізму колагену за рахунок підвищення загальної колагенолітичної активності плазми крові, зниженні вмісту гексазамінів та вуглеводного білка маркери.

**Ключові слова:** неалкогольний стеатогепатит, цукровий діабет типу 2, діабетична хвороба нирок, стеатоз печінки, фіброз печінки, біциклол.

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#### Introduction/Вступ

The urgency of the problem of comorbid non-alcoholic steatohepatitis (NASH), type 2 diabetes mellitus (DM2) and diabetic kidney disease (DKD) have a number of mechanisms of mutual burden, the elimination of which will contribute to the achievement of clinical remission of diseases, compensation of the function of the liver and kidneys. The leading links in the pathogenesis of NASH are hepatocyte steatosis and mesenchymal inflammation, each of which can induce liver tissue fibrosis and NASH progression to liver cirrhosis [1, 2, 3, 4]. Liver tissue fibrosis is based on the activation of perisinusoidal stellate Ito cells, which under the influence of numerous proinflammatory factors, hypoxia and oxidative stress are transformed into myofibroblast-like cells, activated and able to synthesize large amounts of collagen, periin perivenular (centrilobular) and then penetrate

deep into the lobe, forming septal liver fibrosis [5, 4, 6, 7, 8, 9].

One of the probable pathogenetic mechanisms of diabetes and DKD progression is also pancreatic fibrosis in the islets of Langerhans and renal parenchyma as a consequence of microangiopathies and endothelial dysfunction [6, 10].

These processes are opposed by various anti-inflammatory factors of natural origin – a number of anti-inflammatory cytokines, natural antioxidants, release of glucocorticoid hormones (GCH), reperfusion after ischemia, etc., but all these measures have a compensatory effect, require careful monitoring of anti-inflammatory processes. [11, 3, 12, 13, 14]. At the same time, the list of drugs that have a proven antifibrotic effect is quite limited [11]. The use of GCH drugs in dysmetabolic and inflammatory liver disease – NASH is not justified and even contraindicated,

because GCH transfer all types of metabolism to carbohydrate rails, stimulate hyperglycemia and hyperlipidemia, increase hepatocyte steatosis [11]. In hepatitis of viral origin (B, C, B + D) the effectiveness of interferon- $\alpha$  2a and 2b with the implementation of a powerful anti-inflammatory and antifibrotic action was proved [15, 16, 2, 17]. However, in NASH, this therapy is not used due to inefficiency, clinical manifestation of a number of significant side effects of  $\alpha$ -interferons. There are reports of the use of a drug with hepatoprotective, anti-inflammatory action – heparizin, which contains glyceric acid, and has a mild antifibrotic effect in NASH [11, 12].

In the available literature there are a number of reports on the use of the drug Bicyclol for anti-inflammatory, antifibrotic purposes in liver diseases of various origins, primarily viral hepatitis and liver cirrhosis [2, 8–11, 13–29]. Bicyclol is a high-tech, original, synthetic drug [11, 16]. Based on the results of multicenter, randomized, blind, placebo-controlled studies conducted in accordance with the principles of evidence-based medicine, Bicyclol is able to eliminate the cytolytic syndrome – to reduce the increased activity of aminotransferases in viral hepatitis B, C, fatty liver disease and alcoholic liver disease – when the liver is affected by chloroform, D-galactosamine and acetaminophen, to restore the violation of the structure of liver tissue in different severity [2, 8–11, 13–17]. Bicyclol inhibits the production of active neutrophils, Kupffer cells and macrophages of tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), as well as removes from the cells and neutralizes free radicals of oxygen and nitrogen [11, 16]. Bicyclol suppresses oxidative stress, restores the structure of the nucleus and DNA, the functional state of hepatocyte mitochondria, prevents apoptosis and necrosis of hepatocytes, helps to restore the functional state of hepatocytes, inhibits the process of fibrosis of liver tissue [2, 8–11, 13–17]. Studies have also been performed to prove the effectiveness of Bicyclol in alcoholic and nonalcoholic fatty liver disease on the background of obesity [1, 3, 4, 8], toxic and drug-induced hepatitis [11, 14], for liver rehabilitation after chemotherapy and chemoprophylaxis of GCH posttransplant reactions in kidney transplantation [10], cancer [17]. At the same time, there are no detailed data on the use of Bicyclol in NASH with stage 1–3 fibrosis on the background of DM2 with DKD in the literature, or there are reports of studies conducted in the experiment. These circumstances led to research in this direction.

**The purpose of the study** was to determine the probable effect of a complex of metformin, rosuvastatin and bicyclol on markers of hepatocyte cytolysis in patients with nonalcoholic steatohepatitis and diabetes mellitus type 2 with DKD, the degree of hepatocyte steatosis and stage of liver fibrosis, as well as the content of extracellular protein and carbohydrate components in the blood tissues that are markers of the intensity of liver fibrosis and the progression of NASH.

**Material and methods.** Study include 60 patients with NASH, type 2 diabetes and stage I–IV DKD, among which 48 patients (80.0%) were diagnosed with mild NASH, and 12 (20.0%) – NASH of moderate activity. A comorbid disease, i.e. type 2 diabetes mellitus of moderate severity, was registered in 100% of patients with NASH: among them, 15 people (25.0%) had compensated diabetes, 45 (75.0%) people had subcompensated stage. All patients with NASH and diabetes mellitus had comorbid DKD, in particular, 21 cases of DKD stage I–II (35.0%), 20 people with DKD stage III (33.3%), 19 people with DKD stage IV (31.7%). In 15 (25.0%) examined have secondary arterial hypertension (AH) of renal genesis of the I–II degree was established, in 11 persons (18.3%) essential AH of the I–II stage, I–II degree was established. Patients were excluded from the study if they had chronic pathology in the active phase or in the stage of decompensation (heart, blood vessels, kidneys, digestive system, blood and hematopoiesis, neurological, psychiatric, cancer, endocrine, rheumatic diseases, fatty liver disease of alcoholic etiology), acute diseases, pregnancy, lactation.

Depending on the prescribed treatment on a random basis, the examined patients were divided into 2 groups: group 1 (n = 28 patients) received a low-calorie diet, essential phospholipids (EPL) 300 mg 2 capsules 3 times a day) 90 days for the treatment of active NASH, metformin hydrochloride for type 2 diabetes and hyperlipidemia prescribed 1000 mg per day, rosuvastatin (5 mg once daily) for 90 days. Group 2 (n = 32 patients) consisted of patients who, in addition to similar dietary recommendations, hypoglycemic and hypolipidemic therapy instead of EFL received additional drug Bicyclol (Beijing Union Pharmaceutical Factory, China) 25 mg 3 times a day for 90 days. The mean age of patients was (53.8  $\pm$  3.52) years. The comparison group for the presentation of reference values of homeostasis was 30 healthy people of the appropriate age.



NASH was diagnosed in accordance with the recommendations of the unified clinical protocol approved by the order of the Ministry of Health of Ukraine № 826 06.11.2014, in the presence of criteria for exclusion of chronic diffuse liver disease of viral, hereditary, autoimmune or drug genesis as a cause of cytolytic, mesenchymal, inflammatory FibroMax Test, which included "SteatoTest", "ASH" and "NASH-Test" (BioPredictive, France) – to determine the degree of liver steatosis and its nature (alcoholic or non-alcoholic), "FibroTest" – to determine the stage of liver fibrosis, and also on the basis of the results of ultrasonography (USG) on the US scanner Ultima PA ("Radmir", Kharkiv, Ukraine).

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 DKD was carried out according to the recommendations of clinical guidelines GA "Institute of Nephrology NAMS of Ukraine" (2012). Calculation of the glomerular filtration rate (GFR) was performed using a GFR calculator of the Institute of Nephrology of the National Academy of Medical Sciences of Ukraine, KDIGO (2012,2020) recommends using CKD EPI equation. Determination of the stages of DKD was carried out according to the classification of C.E. Mogensen (1983).

The condition of the connective tissue components (CT) of extracellular matrix (ECM) was determined by the content of free oxyproline (FOP) – by the method of S.S. Tetyanets, protein-bound oxyproline (PBOP) – by the method of M.A. Osadchuk, hexosamines (HA) – by the method of O.G. Arkhipova, non-protein fucose (NPF) – by the method of P.M. Sharayeva et al. Collagenolytic activity (CLA) of blood plasma was studied by the intensity of azocol lysis.

The dynamics of treatment was assessed by markers of damage and liver function tests, the results of "SteatoTest" and "FibroTest", the state of protein and carbohydrate-protein components of the extracellular matrix of liver tissue by 30 and 90 days.

The research was carried out in compliance with the basic provisions of the GSR (1996), the Council of Europe Convention on Human Rights and Biomedicine (04.04.1997), the Helsinki Declaration of the World Medical Association on the ethical principles of scientific medical research with human participation (1964–2013), order of the

Ministry of Health of Ukraine № 690 dated 23.09.2009, № 616 dated 03.08.2012.

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 of 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 equality of the general variances of the compared samples, which was verified 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 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).

**Results of the research.** Analysis of the results of the study of the content of metabolic products of CT ECM (Table 1) in the blood of patients with NASH on the background of diabetes and DKD indicates that the inflammatory and dysmetabolic process contributes to a significant imbalance of the components of CT. In particular, the activation of collagen anabolism processes was increased by a 2-fold increase in PBOP blood ( $p < 0.05$ ), as well as a decrease in the intensity of collagen catabolism processes by a 1.4-fold decrease in blood FOP content ( $p < 0.05$ ), which probably occurred due to inhibition of collagenolytic activity of blood plasma (1.4 times,  $p < 0.05$ ).

At the same time, we found that Bicyclol, even with treatment for 30 days, affects the metabolism of CT components and probably inhibits the development of fibrosing reactions in patients with NASH. Thus, after 30 days of treatment, a significant decrease in the content of PBOP in 1.4 times ( $p < 0.05$ ) was registered only in the 2nd observation group, as well as an increase in the intensity of collagenolysis FOP in 1.5 times ( $p < 0.05$ ), as well as the rate of CLA – 1.7 times ( $p < 0.05$ ) compared to the rate before treatment), in the absence of probable changes in the control group (1 group). These processes were accompanied by a significant inhibition of degradation of

fucoglycoproteins (1.4 times,  $p < 0.05$ ) in the dynamics of treatment of patients in group 2 and a significant decrease in the content of HA in the

blood (1.4 times,  $p < 0.05$ ), while in the comparison group, the parameters tended to decrease ( $p > 0.05$ ).

**Table 1 – Content of metabolic products of connective tissue components in the blood patients with non-alcoholic steatohepatitis, type 2 diabetes mellitus and diabetic kidney disease in the dynamics of treatment (M ± m)**

	Indicators	Groups of examined patients		
		Group 1 (n = 28)	Group 2 (n = 32)	Control group (n = 30)
Before treatment	PBOP, mkmol/L	79.64 ± 5.34 *	79.62 ± 5.37 *	40.28 ± 3.73
	FOP, mkmol/L	9.03 ± 0.17 *	9.01 ± 0.18 *	12.93 ± 0.03
	Fucosa, units	95.33 ± 8.45 *	95.35 ± 8.39 *	37.34 ± 5.45
	HA, mmol/L	8.41 ± 0.57 *	8.43 ± 0.55 *	5.51 ± 0.06
	CLA, mkmol/L per hour	0.63 ± 0.03 *	0.64 ± 0.04 *	0.87 ± 0.02
After 30 days of treatment	PBOP, mkmol/L	70.35 ± 4.54 *	57.63 ± 4.32 **/#	40.28 ± 3.73
	FOP, mkmol/L	10.25 ± 0.57 *	13.34 ± 0.17 */**/#	12.93 ± 0.03
	Fucosa, units	89.75 ± 6.13 *	68.12 ± 2.18 */**/#	37.34 ± 5.45
	HA, mmol/L	7.38 ± 0.22 *	5.91 ± 0.24 **/#	5.51 ± 0.06
	CLA, mkmol/L per hour	0.70 ± 0.03 *	1.08 ± 0.02 */**/#	0.87 ± 0.02
After 90 days of treatment	PBOP, mkmol/L	62.65 ± 6.35 *	43.21 ± 2.31 **/#	40.28 ± 3.73
	FOP, mkmol/L	11.08 ± 0.59 */**	13.52 ± 0.28 **/#	12.93 ± 0.03
	Fucosa, units	65.22 ± 5.38 */**	46.98 ± 2.39 **/#	37.34 ± 5.45
	HA, mmol/L	7.46 ± 0.40 *	5.74 ± 0.21 **/#	5.51 ± 0.06
	CLA, mkmol/L per hour	0.74 ± 0.07 *	0.98 ± 0.05 **/#	0.87 ± 0.02

Note: \* – the difference for examined patients versus control group ( $p < 0.05$ ); \*\* – a significant difference in comparison with the baseline indicator ( $p < 0.05$ ); # – the difference in comparison with the indicator after treatment in patients of group 1 ( $p < 0.05$ )

After 90 days of treatment with Bicyclol, the indicators of the state of the components of CT ECM liver in most parameters approached the normative values (Table 1).

In particular, in patients of group 2, a decrease in the content of PBOP in the blood by 1.8 times ( $p < 0.05$ ) compared with pre-treatment, as well as a increase in the content of FOP after treatment by 1.5 times ( $p < 0.05$ ), which indicates a decrease in the intensity of collagen abolism and enhancement

of collagen catabolism in ECM liver tissue. Significant changes in these indicators in the dynamics of treatment in patients of group 1 were not registered ( $p > 0.05$ ). Initially reduced blood CLA in patients with NASH after treatment increased 1.5 times ( $p < 0.05$ ) in patients of group 2, which indicates the probable activation of external (humoral, enzymatic, cytokine, etc.) mechanisms of regulation of CT metabolism under the influence of Bicyclol. Changes in the control

group after treatment relative to the content of POBP and CLA weren't significant ( $p > 0.05$ ), at the same time, the concentration in the blood of FOP in patients of group 1 probably increased 1.2 times ( $p < 0.05$ ), but the norm is not reached. We also found a corrective effect of Bicyclol on the metabolism of glycosaminoglycans: a decrease in the content of HA in group 2 by 1.5 times ( $p < 0.05$ ) with the actual normalization of the indicator. In group 1, changes in HA content weren't significant ( $p > 0.05$ ). In addition, we observed a

significant decrease after treatment of fucose levels in the blood: 1.5 and 2.0 times, respectively, in patients of groups 1 and 2 ( $p_{1,2} < 0.05$ ).

Analysis of cytolytic syndrome activity indicates that before treatment alanine aminotransferase (ALT) increased activity (3.6 times,  $p < 0.05$ ) – after treatment decreased in patients both groups: respectively 2.2 and 3, 7 times ( $p < 0.05$ ) with the presence of a intergroup difference ( $p < 0.05$ ) (Table 2).

**Table 2 – Indicators of cytolysis activity of hepatocytes, steato-test and fibro-test in patients with non-alcoholic steatohepatitis, type 2 diabetes mellitus with stage I–IV diabetic kidney disease in the dynamics of treatment (M ± m)**

	Parameters	Groups of examined patients		
		Group 1 (n = 28)	Group 2 (n = 32)	Control group (n = 30)
Before treatment	ALT, mmol/L per hour	1.52 ± 0.17 *	1.51 ± 0.15 *	0.42 ± 0.03
	Steato test	0.75 ± 0.02 *	0.76 ± 0.02 *	0.19 ± 0.02
	Fibro test	0.56 ± 0.01 *	0.55 ± 0.01 *	0.17 ± 0.01
In 90 days	ALT, mmol/L per hour	0.69 ± 0.05 */**	0.41 ± 0.04 **/#	0.42 ± 0.03
	Steato test	0.61 ± 0.02 */**	0.42 ± 0.01 */**/#	0.19 ± 0.02
	Fibro test	0.50 ± 0.01 */**	0.33 ± 0.01 */**/#	0.17 ± 0.01

Note: \* – the difference for examined patients versus control group ( $p < 0.05$ );

\*\* – the difference in comparison with the baseline indicator ( $p < 0.05$ );

# – the difference in comparison with the indicator after treatment in patients of group 1 ( $p < 0.05$ )

At the same time, the increased hepatocyte steatosis before treatment, which exceeded the reference values by 4.0 times ( $p < 0.05$ ) – under the influence of treatment also significantly decreased in patients of 1 and 2 observation groups – 1.2 and 1.8 times, respectively ( $p < 0.05$ ) with the presence of a significant intergroup difference ( $p < 0.05$ ) (Table 2). Thus, significantly FibroTest increased before treatment (3.2 times,  $p < 0.05$ ) in patients with NASH with comorbid diabetes and DKD in the dynamics of treatment in patients of group 1 decreased by 10.7% ( $p < 0.05$ ), and in patients of group 2 – by 40.0% ( $p < 0.05$ ) with the presence of a significant intergroup difference ( $p < 0.05$ ). The data obtained indicate a favorable anti-inflammatory effect of Bicyclol, which is aimed at inhibiting and preventing liver fibrosis.

**Discussion.** The problem of comorbid NASH, DM2 and DKD is the rapid progression of all comorbid diseases, decompensation of carbohydrate

metabolism, development of hepatocellular and renal failure.

To determine the effect of bicyclol on the state of the connective tissue components of the extracellular matrix of the liver in the complex therapy of nonalcoholic steatohepatitis with liver fibrosis in patients with type 2 diabetes mellitus with diabetic nephropathy, the investigation was conducted in the treatment of 60 patients with nonalcoholic steatohepatitis with comorbid DM2 of moderate severity and stage I–IV DKD. Addition of Bicyclol to the complex therapy of NASH and DM2 with DKD for 3 months helped to eliminate the syndrome of cytolysis of hepatocytes, a significant reduction in steato-test and fibrotest, a reduction in the intensity of hepatic tissue fibrosis due to optimization of blood (protein-bound oxyproline), increasing the blood content of markers of collagen catabolism in the blood (free oxyproline) due to increased total collagenolytic activity of blood plasma, reducing the content of hexosamines and carbohydrate-protein

markers of fucoglycoprotein degradation in the blood. We investigated that the activated processes of collagen synthesis are accompanied by inhibition of its degradation with accumulation in ECM. We also found a significant increase in the content of HA in the blood by 1.5 times ( $p < 0.05$ ) and accelerated degradation of carbohydrate-protein components of the matrix (with an increase in the content of fucose, not bound to protein, 2.6 times, ( $p < 0.05$ )).

The results of the study of the content of connective tissue metabolic products in patients with NASH, DM2 with stage I–IV DKD in the dynamics

of treatment showed that traditional treatment with essential phospholipids and Bicyclol actively affect and during 90 days of treatment the main components of the pathological process in the liver in NASH – cytolysis and fatty degeneration of hepatocytes, but Bicyclol in the complex hypoglycemic and hypolipidemic therapy has a more intense effect. It should also be noted that the effect of traditional therapy and liver fibrosis activity, according to previous studies, was significantly lower than the proposed therapy with Bicyclol.

### Conclusions/Висновки

Combination therapy with metformin, rosuvastatin, Bicyclol in individuals with comorbid nonalcoholic steatohepatitis, type 2 diabetes mellitus and diabetic kidney disease for 3 months helped to eliminate the syndrome of cytolysis of hepatocytes, a significant reduction in fiatoma spectrum of

connective tissue components of the extracellular matrix in the blood with a decrease in the content of markers of collagen anabolism, increase in the content of markers of collagen catabolism due to increased total collagenolytic activity of blood plasma, decrease in the content of hexosamines and carbohydrate protein markers.

### Prospects for future research/Перспективи подальших досліджень

The prospect of our further research in this direction is an establishment of other pleiotropic effects of Bicyclol under the conditions of complex treatment of various comorbid pathologies against the background of non-alcoholic steatohepatitis.

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The authors declare no conflict of interest.

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