DOI: 10.20514/2226-6704-2021-11-5-359-369

УДК 616.61-002.2-085.357+
616.379-008.64-085.357

С.С. Попов[1], Е.И. Ануфриева[2], Е.Д. Крыльский*[3], А.Н. Веревкин[3], К.К. Шульгин[3]

[1]— ФГБОУ ВО «Воронежский государственный медицинский университет им. Н.Н. Бурденко» Минздрава России, кафедра Организации фармацевтического дела, клинической фармации и фармакогнозии, Воронеж, Россия
[2]— ФГБОУ ВО «Воронежский государственный медицинский университет им. Н.Н. Бурденко» Минздрава России, кафедра Организации сестринского дела, Воронеж, Россия
[3]— ФГБОУ ВО «Воронежский государственный университет» Минобрнауки России, кафедра Медицинской биохимии и микробиологии, Воронеж, Россия

ВОЗДЕЙСТВИЕ КОМБИНИРОВАННОЙ ТЕРАПИИ С МЕЛАТОНИНОМ НА ФЕРМЕНТАТИВНОЕ ЗВЕНО ГЛУТАТИОНОВОЙ СИСТЕМЫ И УРОВЕНЬ ТРАНСФОРМИРУЮЩЕГО ФАКТОРА РОСТА-β1 У ПАЦИЕНТОВ С САХАРНЫМ ДИАБЕТОМ 2 ТИПА И ХРОНИЧЕСКОЙ БОЛЕЗНЬЮ ПОЧЕК

S.S. Popov[1], E.I. Anufrieva[2], E.D. Kryl'skii*[3], A.N. Verevkin[3], K.K. Shulgin[3]

[1]— Voronezh State Medical University named after N.N. Burdenko, Department of Organization of Pharmaceutical Business, Clinical Pharmacy and Pharmacognosy, Voronezh, Russia
[2]Voronezh State Medical University named after N.N. Burdenko, Department of Nursing Organization, Voronezh, Russia
[3]Voronezh State University, Department of Medical Biochemistry and Microbiology, Voronezh, Russia

The Effect of Combination Therapy with Melatonin on the Enzymes of Glutathione System and the Level of Transforming Growth Factor- β1 in Patients with Type 2 Diabetes Mellitus and Chronic Kidney Disease

Резюме

Цель работы. Целью работы являлась оценка воздействия комбинированной терапии с мелатонином на клинико-биохимические показатели развития хронической болезни почек (ХБП) и сахарного диабета (СД) 2 типа, функционирование ферментативного звена

*Контакты: Евгений Дмитриевич Крыльский, e-mail: evgenij.krylsky@yandex.ru
*Contacts: Evgeniy D. Kryl'skii, e-mail: evgenij.krylsky@yandex.ru
ORCID ID: https://orcid.org/0000-0002-8855-5515

глутатионовой антиоксидантной системы и активность ферментов — поставщиков NADPH, а также уровень трансформирующего фактора роста-β1 и липидный профиль пациентов. **Материалы и методы.** В исследовании принимало участие 60 пациентов (19 мужчин и 41 женщина, средний возраст — 65,6±9,3 года) с ХБП и СД 2 типа. Пациенты были разделены на 2 группы. Первая группа пациентов находилась на базисном лечении (n=30, 8 мужчин и 22 женщины, средний возраст — 64,1±7,9 года); вторая группа участников (n=30, 11 мужчин и 19 женщин, средний возраст — 69,0±10,5 года) дополнительно к базисной терапии получала 2 мг мелатонина. Контрольную группу составили 65 практически здоровых лиц (30 мужчин и 35 женщин, средний возраст — 42,3±17,7 года) с нормальными показателями общего и биохимического анализов крови. В ходе работы был осуществлен анализ клинико-биохимических показателей и липидного профиля в сыворотке крови, уровня трансформирующего фактора роста-β1 методом иммуноферментного анализа, активности ферментов глутатионовой антиоксидантной системы и NADPH-генерирующих ферментов спектрофотометрическим методом. **Результаты.** Применение мелатонина на фоне базисного лечения по сравнению со стандартной терапией способствовало снижению протеинурии (p=0,01), гипергликемии (p=0,019), концентрации мочевины (p=0,043), гликированного гемоглобина (p=0,045) и трансформирующего фактора роста-β1 (p=0,020) у пациентов с ХБП. Кроме того, использование данного препарата оказывало воздействие на липидный профиль и приводило к возрастанию активности ферментов глутатионовой антиоксидантной системы, ферментов — поставщиков NADPH, что отражает эффективность формирования компенсаторного ответа в условиях активации свободнорадикального окисления на фоне гипергликемии. **Заключение.** Наблюдаемые в ходе исследования различия, очевидно, были вызваны действием мелатонина, для которого характерен нефропротекторный и гипогликемический эффекты, способность нейтрализовывать свободные радикалы и активизировать функционирование компонентов антиоксидантной системы.

Ключевые слова: *хроническая болезнь почек, сахарный диабет 2 типа, окислительный стресс, антиоксидантная система, мелатонин, трансформирующий фактор роста — в1*

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

Источники финансирования

Авторы заявляют об отсутствии финансирования при проведении исследования

Статья получена 27.01.2021 г.
Принята к публикации 28.07.2021 г.

Для цитирования: Попов С.С., Ануфриева Е.И., Крыльский Е.Д. и др. ВОЗДЕЙСТВИЕ КОМБИНИРОВАННОЙ ТЕРАПИИ С МЕЛАТОНИНОМ НА ФЕРМЕНТАТИВНОЕ ЗВЕНО ГЛУТАТИОНОВОЙ СИСТЕМЫ И УРОВЕНЬ ТРАНСФОРМИРУЮЩЕГО ФАКТОРА РОСТА-β1 У ПАЦИЕНТОВ С САХАРНЫМ ДИАБЕТОМ 2 ТИПА И ХРОНИЧЕСКОЙ БОЛЕЗНЬЮ ПОЧЕК. Архивъ внутренней медицины. 2021; 11(5): 359-369. DOI: 10.20514/2226-6704-2021-11-5-359-369

Abstract

Aim. The aim of the work was to assess the effect of combination therapy with melatonin on the clinical and biochemical parameters of chronic kidney disease (CKD) and type 2 diabetes mellitus (DM), the level of transforming growth factor-β1, lipid profile, activity of the glutathione antioxidant system enzymes and the activity of NADPH-generating enzymes in patients. **Materials and methods.** The study involved 60 people (19 men and 41 women, average age 65.6 ± 9.3 years) with chronic kidney disease associated with type 2 diabetes. The patients were divided into 2 groups. The first group of patients received basic treatment (n = 30, 8 men and 22 women, mean age 64.1 ± 7.9 years); the second group of participants (n = 30, 11 men and 19 women, mean age 69.0 ± 10.5 years) received 2 mg of melatonin in addition to the basic therapy. The control group consisted of 65 apparently healthy individuals (30 men and 35 women, average age 42.3±17.7 years) with normal indicators of general and biochemical blood tests. In the course of the work, the analysis of clinical and biochemical indicators and lipid profile in blood serum, the level of transforming growth factor-β1 by enzyme immunoassay, the activity of enzymes of the glutathione antioxidant system and NADPH-generating enzymes by the spectrophotometric method were carried out. **Results.** The use of melatonin additionally with basic treatment compared with standard therapy led to a decrease in proteinuria (p=0.010), hyperglycemia (p=0.019), urea concentration (p=0.043), glycated hemoglobin (p=0.045) and transforming growth factor-β1 levels (p=0.020) in patients with CKD. In addition, the use of this drug led to a changing of the lipid profile, and the activity of glutathione antioxidant system enzymes and NADPH-generating enzymes. **Conclusion.** The differences observed during the study were apparently caused by the action of melatonin, which has nephroprotective and hypoglycemic properties, the ability to neutralize reactive oxygen species and activate the antioxidant system functioning.

Key words: *chronic kidney disease, type 2 diabetes mellitus, oxidative stress, antioxidant system, melatonin, transforming growth factor-β1*

Conflict of interests

The authors declare no conflict of interests

Sources of funding

The authors declare no funding for this study

Article received on 27.01.2021
Accepted for publication on 28.07.2021

For citation: Popov S.S., Anufrieva E.I., Kryl'skii E.D. et al. The Effect of Combination Therapy with Melatonin on the Enzymes of Glutathione System and the Level of Transforming Growth Factor- β1 in Patients with Type 2 Diabetes Mellitus and Chronic Kidney Disease. The Russian Archives of Internal Medicine. 2021; 11(5): 359-369. DOI: 10.20514/2226-6704-2021-11-5-359-369

AI — atherogenic index, CKD — chronic kidney disease, DM — diabetes mellitus, G6FDG — glucose-6-phosphate dehydrogenase, GFR — glomerular filtration rate, GP — glutathione peroxidase, GSH — reduced glutathione, GR — glutathione reductase, GT — glutathione transferase, HDL — high density lipoprotein cholesterol, LDL — low density lipoprotein cholesterol, NADP-IDG — NADP-dependent isocitrate dehydrogenase, TC — total cholesterol, TGF-β1 — transforming growth factor β1

Introduction

It is known that the main etiological factors in the development of chronic kidney disease (CKD) include diabetes mellitus (DM) and arterial hypertension [1]. Proteinuria is an important predictor of progressive kidney damage, which leads to tubulointerstitial kidney tissue damage and, as a result, a decrease in glomerular filtration rate, resulting in end-stage renal failure. The kidney is a metabolic organ where mitochondrial oxidation reactions take place. According to studies, increased production of reactive oxygen species and a weakening of the body's antioxidant defense have an adverse effect on the course of CKD [2].

Oxidative stress activates mitogen-activated protein kinases, various cytokines and transcription factors, which ultimately leads to fibrosis and end-stage renal failure. One of the factors activated due to proteinuria and oxidative stress is transforming growth factor β1 (TGF-β1), which is an important mediator of many processes in renal glomeruli and tubules. Also, high glucose concentration increases mRNA and TGF-β1 protein levels in cultured proximal tubular cells, epithelial and mesangial cells of renal glomeruli [3]. Numerous studies have shown that TGF-β1 plays a central role in the development of fibrosis. TGF-β1 increases mitochondrial production of reactive oxygen species in various cell types that mediate TGF-β-induced apoptosis, as well as many of the TGF-β-induced pro- and fibrotic effects [4].

The pathogenic action of free radicals is opposed by the antioxidant system, which is aimed at suppressing free radical oxidation processes. One of the main links of antioxidant defense is the glutathione system, which includes enzymes glutathione peroxidase (GP), glutathione reductase (GR), glutathione transferase (GT) and glutathione tripeptide, which plays the role of a reducing cofactor in glutathione peroxidase reaction. GP is a key enzyme that utilizes reactive oxygen species and lipid peroxidation products. GR facilitates the reduction of glutathione oxidized during glutathione peroxidase reaction without increasing its synthesis *de novo*. GT detoxifies lipid peroxidation products generated during the metabolism of xenobiotics in the endoplasmic reticulum. The glutathione antioxidant system requires a constant supply of the NADPH coenzyme to function. The primary sources of this compound are enzymes glucose-6-phosphate dehydrogenase (G6PDH) and NADP-dependent isocitrate dehydrogenase (NADP-IDH) [5].

Clinical guidelines for the management of CKD include the control of blood pressure, glycemia, lipid metabolism, and nephroprotective therapy [6]. In this regard, it seems appropriate to study the natural metabolites of the body that have a nephroprotective effect and are aimed at suppressing oxidative stress and TGF-β1. One of these metabolites is the hormone melatonin [7]. It is known that melatonin binds free radicals while simultaneously inducing the body's natural antioxidant defense system.

Therefore, the purpose of this work was to assess the clinical and biochemical parameters of CKD, lipid profile, TGF-β1 level, the activity of the glutathione antioxidant system and NADPH-generating enzymes in patients with CKD and type 2 DM during basic treatment and combination therapy with melatonin.

Experimental Part

This clinical study was a randomized, open-label, controlled trial. The study was approved by the Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education "Burdenko Voronezh State Medical University", extract of minutes No. 4 dated 09/29/2016. All patients signed informed consent to participate in the study. This clinical study was carried out in the endocrinology departments of the Voronezh Regional Clinical Center for Specialized Types of Medical Care and the Voronezh City Clinical Emergency Hospital No. 10. The study included 60 patients (29 males, mean age (65.6 ± 9.3) years) with type 2 DM and CKD. History of CKD was (13.2 ± 6.3) years. Stage 2 CKD was observed in 6 (10%) patients, stage 3a — in 42 (70%), stage 3b — in 12 (20%) patients. The diagnosis of CKD was established according to clinical guidelines [8]. Among comorbidities (Table 1), arterial hypertension (100%), diabetic retinopathy (100%), obesity (69%), chronic heart failure (66%) were the most common. The study had the following exclusion criteria: type 1 DM, viral hepatitis, acute infectious diseases, acute myocardial infarction, malignant neoplasms, acute cerebrovascular accident. After admission to the hospital, all patients underwent therapy adjustment. Group 1 (n = 30, 8 males and 22 females, mean age — (64.1 ± 7.9) years) received basic treatment. Group 2 (n = 30, 11 males and 19 women, mean age — (69.0 ± 10.5) years). In addition to similar basic therapy, they received an agent containing 2 mg of melatonin (Table 2). All patients of group 2 who were on inpatient

treatment had complaints of sleep disturbance. Therefore, with the exception of the administration of melatonin, there were no differences in the treatment of participants in groups 1 and 2. The duration of basic therapy and combined treatment with melatonin carried out in the hospital was two weeks. Clinical and biochemical parameters of the lipid profile, TGF-β1 level, the activity of the glutathione antioxidant system and NADPH-generating enzymes were analyzed upon admission to the hospital and before discharge. The control group included 65 apparently healthy individuals (30 males and 35 females, mean age — (42.3 ± 17.7) years) with normal values of general and biochemical blood tests, who underwent a planned preventive medical examination at the Voronezh City Polyclinic No. 10.

The glomerular filtration rate (GFR) was calculated using the CKD-EPI (2011) formula Daily proteinuria in urine was determined by a photocolorimetric method with pyrogallol red dye and the Brandberg — Roberts — Stolnikov method.

Fasting glucose and postprandial glucose were assessed by the hexokinase enzymatic method and using a Satellite Plus glucometer (ELTA, Russia). To assess lipid metabolism, the concentration of total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol in serum was determined using reagent kits (Bio-La-Test) using an enzymatic photocolorimetric method on a Klima 15MC biochemical analyzer (Spain). The atherogenic index (AI) was defined as the ratio of the difference between total cholesterol and HDL to HDL [AI = (TC – HDL) / HDL], which should normally be ≤ 3.

Glycated hemoglobin parameters were calculated using the reference method using a D 10 analyzer manufactured by Bio-Rad (USA).

Urea and creatinine concentration was analyzed using diagnostic kits manufactured by Olvex (Russia).

The content of melatonin sulfate in the urine of patients was measured by enzyme immunoassay using a kit manufactured by Buhlmann (Germany).

The activity of the studied enzymes was determined on a Hitachi U-1900 spectrophotometer (Japan). HP activity was measured in a spectrophotometric medium of the following composition: 50 mM potassium phosphate buffer (pH 7.4) containing 1.0 mM EDTA, 0.12 mM NADPH (AppliChem, Germany), 0.85 mM reduced glutathione (GSH) (AppliChem, Germany), 0.37 mM H_2O_2, 1 U/ml GR (Sigma Aldrich, USA). The control sample did not contain GSH [9]. GR activity was assessed in 50 mM potassium phosphate buffer (pH 7.4) containing 1.0 mM EDTA, 0.16 mM NADPH, and 0.8 mM GSH [9].

Table 1. *Comorbidities of study participants who received basic treatment (group 1, n=30) and patients who received melatonin in addition to basic therapy (group 2, n=30)*

Comorbidities, n (%)	Group 1, n=30	Group 2, n=30
Arterial hypertension	30(100)	30(100)
Diabetic retinopathy	30(100)	30(100)
Obesity	21(70)	20(67)
Chronic heart failure	22(73)	18(60)
Coronary artery disease	13 (43)	13 (43)
Acute disorders of cerebral circulation	5 (17)	4 (13)
Myocardial infarction	5 (17)	4 (13)
Chronic obstructive pulmonary disease	-	2 (7)
Peripheral artery disease	6 (20)	8 (27)

Table 2. *Prescribed drugs to study participants*

Prescribed drugs	Group 1	Group 2
Oral hypoglycemic drugs	Biguanides (metformin — 500-1500 mg once in the evening) Sulfonylurea preparations (Gliclazide — 30-90 mg once a day) Inhibitors of dipeptidyl peptidase-4 (Vildagliptin — 50-100 mg 1-2 times a day, Alogliptin — 12.5-25 mg once a day)	Biguanides (metformin — 500-1500 mg once in the evening) Sulfonylurea preparations (Gliclazide — 30-90 mg once a day) Inhibitors of dipeptidyl peptidase-4 (Vildagliptin — 50-100 mg 1-2 times a day, Alogliptin — 12.5-25 mg once a day)
Antihypertensive drugs	ACE blockers (Enalapril -5-20 mg 1-2 times a day, Lisinopril 5-20 mg once a day), B-blockers (Bisoprolol — 2.5-10 mg 1 once a day, Metoprolol succinate — 50-100 mg once a day)	ACE blockers (Enalapril -5-20 mg 1-2 times a day, Lisinopril 5-20 mg once a day), B-blockers (Bisoprolol — 2.5-10 mg 1 once a day, Metoprolol succinate — 50-100 mg once a day)
Lipid-lowering drugs	Statins (Atorvastatin — 20-40 mg once a day)	Statins (Atorvastatin — 20-40 mg once a day)
Diuretics	Thiazide diuretics (Indapamide 2.5 mg once a day)	Thiazide diuretics (Indapamide 2.5 mg once a day)
Melatonin	-	2 mg orally, 1 tablet once a day, after meals, in the evening, 1-2 hours before bedtime

GT activity was measured in 0.1 M potassium phosphate buffer (pH 7.4) containing 1.0 mM EDTA, 1.0 mM 1-chloro-2,4-dinitrobenzene, 5.0 mM GSH [10]. G6PDH activity was measured in 50 mM Tris-HCl buffer (pH 7.8) containing 3.0 mM glucose-6-phosphate (Sigma Aldrich, USA), 0.25 mM NADP (AppliChem, Germany), 1.0 mM $MnCl_2$ [11]. NADP-IDH activity was assessed in 50 mM Tris-HCl buffer (pH 7.8) containing 1.5 mM isocitrate (Sigma Aldrich, USA), 2.0 mM $MnCl_2$, 0.4 mM NADP [11]. The rate of the enzymatic reaction was judged by the change in optical density at 340 nm. The unit of enzymatic activity (E) was defined as the amount of enzyme catalyzing the formation of 1 μmol of the reaction product in 1 min at 25 °C. Enzyme activity was expressed in enzymatic units per ml of serum. GSH concentration was determined using a reaction with 5,5-dithiobis-(2-nitrobenzoic) acid (Sigma Aldrich, USA) [11]. Protein concentration in blood serum was assessed by the biuret method.

The level of TGF-β1 in blood serum was measured by enzyme immunoassay using a kit manufactured by Ray-Biotech (USA).

Statistical processing of the material was carried out using SPSS 23.0 software and standard methods of variation statistics (calculation of mean values, standard error of the mean, standard deviation, median values and interquartile range). Normality of distribution in groups was assessed using the Kolmogorov — Smirnov test. The significance of differences was analyzed using Student's t-test and the nonparametric Mann — Whitney test. The Pearson or Spearman rank correlation coefficient was used to identify correlations between the studied parameters depending on the distribution. This paper gives the values of average (0.30–0.69) and strong (> 0.70) correlation. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Baseline clinical and laboratory characteristics of patients are presented in Table 3.

Biochemical parameters, TGF-β1 level and lipid profile of the study participants are presented in Table 4.

The daily proteinuria level in groups 1 and 2 before therapy was 0.540 and 0.781 g/day, respectively. After treatment, this parameter decreased in both groups. However, combination therapy with melatonin more significantly reduced the proteinuria level ($p = 0.010$). Also, combined treatment with melatonin led to a decrease in fasting glucose concentration, postprandial hyperglycemia ($p < 0.001$) and the level of glycated hemoglobin ($p = 0.010$) relative to pre-treatment values. An assessment of melatonin sulfate level in urine confirmed that the level of this hormone increased during therapy in patients of group 2 ($p = 0.010$). As is known, melatonin has a protective effect on organs and tissues during hyperglycemia, which is due to its antioxidant, anti-inflammatory and antiapoptotic effects. This hormone has a positive effect on carbohydrate metabolism due to the ability to inhibit gluconeogenesis in the liver, increase insulin secretion and tissue sensitivity to it, as well as restore mitochondrial dysfunction in diabetes mellitus [12].

Table 3. *Baseline clinical characteristics of patients*

Indicator	Control group, n=65	Group 1, n=30	Group 2, n=30
Sex, m/f, n (%)	30/35 (46% мужчин)	8/22 (27% мужчин)	11/19 (37% мужчин)
Age, years	42,0 (27,5-51,0)	63,0 (59,0-69,0)*	69,0 (59,0-74,5)*
Average duration of diabetes mellitus, years	-	9,2 (7,8-10,9)	10,1 (8,3-11,7)
BMI	26,0 (23,5-27,4)	31,3 (26,4-34,4)	30,5 (26,0-35,6)
BP systolic mm Hg	115,0 (109,0-124,0)	160,0 (155,0-165,0)*	160,0 (151,3-170)*
BP diastolic mm Hg	71,0 (68,0-73,0)	90,0 (85,0-90,0)*	90,0 (85,0-95,0)*
Heart rate beats / min	71,0 (66,0-73,0)	78,0 (75,0-80,0)*	82,0 (76,5-87,5)*
Stages of CKD G2, n/% G3a, n/% G3b, n/%	-	 3 (10%) 20 (67%) 7 (23%)	 3 (10%) 22 (73%) 5 (17%)
GFR, ml / min	102,0 (95,7-108,3)	53,5 (45,8-58,0)*	54,0 (47,0-57,0)*
Concentration of creatinine, μM	87,3 (79,2-90,1)	114,0 (98,3-120,8)*	104,5 (94,5-124,8)*
Concentration of urea, mM	5,2 (4,6-5,5)	6,5 (5,4-8,4)*	7,7 (5,7-10,4)*
Daily proteinuria level, g / day	0,01 (0,007-0,015)	0,40 (0,24-0,65)*	0,54 (0,31-1,18)*
Glycated hemoglobin level, %	5,0 (4,6-5,5)	8,0 (6,8-9,7)*	8,4 (7,0-9,8)*
Fasting glucose concentration, mM	4,8 (3,5-5,2)	9,9 (7,9-12,2)*	10,4 (9,7-12,6)*
Postprandial glucose concentration, mM	5,5 (4,1-6,2)	12,3 (8,9-14,5)*	12,3 (8,9-14,5)*

Note: BMI — Body mass index, BP — Blood pressure, CKD — Chronic kidney disease
Data are presented as median value (Q1-Q3); * — differences from the control group, $p < 0.05$.

Also, an experiment on animals showed that melatonin normalizes the shape and organization of mitochondrial cristae in metabolic dysfunction of renal tubules and reduces the number of cells with an increased index of apoptosis in proximal tubules [13]. Therefore, the antioxidant and antiapoptotic effects typical for melatonin apparently contributed to a more pronounced change in glycemia and proteinuria parameters in patients compared with standard treatment. Among other things, combination treatment with melatonin reduced the level of TGF-β1 compared with standard therapy (p = 0.001). Lately, TGF-β1 is considered a multifunctional cytokine involved in cell growth, differentiation and migration, the formation and degradation of extracellular matrix components, chemotactic processes and apoptosis, as well as immune regulation. TGF-β1 is a key mediator of renal fibrosis [14]. Maintaining the physiological level of TGF-β is necessary for the normal functioning of most tissues and maintenance of organs. Increased expression of TGF-β1 is associated with pathological changes in tissues in various diseases such as pulmonary fibrosis, spinal muscular atrophy and kidney disease [15]. The concentration of TGF-β1 is high in the blood serum of patients with pathologies such as diabetic nephropathy, immunoglobulin A-nephropathy, focal segmental glomerulosclerosis, rapidly progressive glomerulonephritis and lupus nephritis. TGF-β1 also mediates kidney disease by inducing an epithelial-mesenchymal transition involving tubular epithelial cells that are believed to contribute to the pathogenesis of tubular atrophy [15].

Table 4. *Clinical and biochemical parameters and the level of TGF-β1 of the examined patients*

Indicator	Control group	Group 1, n=30			Group 2, n=30			p_2
		Before treatment	After treatment	p_1	Before treatment	After treatment	p_1	
Fasting glucose concentration, mM	4,8 (3,5-5,2)	9,9 (7,9-12,2)*	7,5 (6,6-8,8)	<0,0001	10,4 (9,7-12,6)*	6,8 (5,3-7,9)	<0,0001	0,019
Postprandial glucose concentration, mM	5,5 (4,1-6,2)	12,3 (8,9-14,5)*	8,9 (7,2-10,4)	0,002	12,3 (8,9-14,5)*	7,9 (6,5-9,3)	<0,0001	0,939
Glycated hemoglobin level, %	5,0 (4,6-5,5)	8,0 (6,8-9,7)*	6,70 (5,7-7,1)	0,020	8,4 (7,0-9,8)*	6,0 (5,2-6,2)	0,010	0,005
Daily proteinuria level, g / day	0,01 (0,007-0,015)	0,40 (0,24-0,65)*	0,14 (0,09-0,29)	<0,0001	0,54 (0,31-1,18)*	0,13 (0,08-0,48)	<0,0001	0,010
Concentration of urea, mM	5,2 (4,6-5,5)	6,5 (5,4-8,4)**	5,8 (4,8-7,9)	0,052	7,7 (5,7-10,4)*	6,5 (5,1-9,1)	0,043	0,301
Concentration of creatinine, μM	87,3 (79,2-90,1)	114,0 (98,3-120,8)*	98,0 (86,5-114,8)	0,021	104,5 (94,5-124,8)*	97,0 (81,5-105,5)	0,024	0,254
GFR, ml / min	102,0 (95,7-108,3)	53,5 (45,8-58,0)*	60,0 (55,0-63,8)	0,072	54 (47,0-57,0)*	63,0 (55-67,8)	0,159	0,034
Total cholesterol, mmol/l	4,1 (3,3-4,6)	6,4 (5,1-6,8)*	5,0 (4,4-5,6)	0,008	5,8 (5,1-6,7)*	4,6 (3,9-5,2)	<0,0001	0,321
Triglycerides, mmol/l	1,2 (0,8-1,4)	2,0 (1,4-2,5)*	1,7 (1,2-2,1)	0,29	2,7 (1,4-3,0)*	1,1 (0,9-1,8)	<0,0001	0,005
LDL, mmol/l	2,2 (1,8-2,4)	2,7 (2,2-3,3)*	1,8 (1,5-2,2)	<0,0001	2,6 (2,1-3,4)*	1,8 (1,5-2,2)	<0,0001	0,633
HDL, mmol/l	2,4 (2,2-2,7)	1,06 (0,75-1,20)*	1,10 (1,01-1,20)	0,114	1,02 (0,86-1,27)*	1,24 (1,10-1,35)	0,003	0,203
Atherogenic index	0,70 (0,4-1,0)	5,0 (4,1-5,7)*	3,5 (2,8-4,1)	0,001	5,0 (4,2-5,7)*	2,6 (2,1-3,2)	<0,0001	0,408
TGF-β1, ng/ml	21,0 (17,3-24,8)	128,0 (100,1-151,7)*	98,0 (80,1-115,2)	0,010	130,5 (108,2-153,6)*	80,0 (64,3-97,7)	<0,0001	0,001
Concentration of melatonin sulfate in urine, ng/ml	9,0 (8,1-9,8)	7,0 (6,0-7,8)*	7,5 (6,7-7,9)	0,326	7,1 (6,3-7,7)*	8,8 (8,0-9,5)	0,010	0,001
BP systolic mm Hg	115,0 (109,0-124,0)	160,0 (155-165,0)*	130,0 (121,3-130)	<0,0001	160,0 (151,3-170,0)*	125,0 (120-130,0)	<0,0001	0,592
BP diastolic mm Hg	71,0 (68,0-73,0)	90,0 (85,0-90,0)*	80,0 (80,0-80,0)	<0,0001	90,0 (85,0-95,0)*	80,0 (70,0-80,0)	<0,0001	0,309
Heart rate beats / min	71,0 (66,0-73,0)	78,0 (75,0-80,0)*	70,0 (66,0-70,0)	<0,0001	82,0 (76,5-87,5)*	66,0 (62,5-68,0)	<0,0001	0,611

Note: GFR — Glomerular filtration rate, LDL — Low density lipoproteins, HDL — High density lipoproteins, TGF-β1 — Трансформирующий фактор роста-β1, Transforming Growth Factor-β1, BP — Blood pressure, ЧСС — Частота сердечных сокращений
Data are presented as median value (Q1-Q3); * — differences from the control group, p <0.05

During treatment, the patients in our study showed positive changes in the lipid profile parameters in blood serum. There was a decrease in the concentration of cholesterol, LDL, and atherogenic index ($p < 0.05$) in both groups of patients. Also, in patients who received additional melatonin, the concentration of triglycerides significantly decreased ($p < 0.001$) and HDL level increased ($p = 0.003$). As is known from the literature, daily administration of melatonin helps reduce excess body weight. In addition, the ratio of the concentration of melatonin to insulin in blood negatively correlates with the LDL level and positively with the concentration of HDL [16]. Vasoregulatory effects of melatonin are complex and may involve both central and peripheral mechanisms. Melatonin receptors MT1 are responsible for vasoconstriction, and MT2 — for vasodilation, and depend on circadian time, duration and mode of exposure to endogenous or exogenous melatonin, as well as the functional sensitivity of receptors [17].

The study also showed that combined treatment with melatonin promoted an increase in the activity of antioxidant enzymes of the glutathione unit towards control values, which had a more pronounced trend compared with the corresponding changes with underlying basic therapy. In particular, the use of melatonin contributed to a more significant increase in the activity of GR ($p = 0.031$, compared with basic treatment) and GT ($p < 0.0001$, compared with basic treatment), as well as an increase in the activity of GH ($p < 0.0001$), while in patients in group 1, the activity of this enzyme decreased during treatment ($p = 0.034$) (Fig. 1). Antioxidant activity of melatonin contributes to a more significant increase in the activity of the glutathione system, which plays a key role in the compensatory response to oxidative stress induced under conditions of hyperglycemia [18]. It is known that the dysfunction of antioxidant system components is an important factor in the development of complications in a wide range of diseases, including type 2 DM. In particular, it was shown that the level of oxidative stress markers increases in the kidneys in diabetes, and in mice lacking the main cytosolic and mitochondrial antioxidant enzyme GP 1, the modeling of diabetes leads to a more significant increase in oxidative stress and the progression of kidney disease [19]. Changes in the specific activity of enzymes of the glutathione antioxidant system were of a similar nature (see Fig. 1).

With underlying combination therapy with melatonin, there was also an increase in the activity of NADP-IDG in blood serum compared with pre-treatment parameters ($p < 0.0001$), while with underlying basic therapy, there were no significant differences in this parameter (Fig. 2). Apparently, the increase in NADP-IDG activity is associated with an increase in the need for NADPH of the glutathione antioxidant system under conditions of its activation. Another supplier of NADPH is the pentose phosphate pathway. The key enzyme here is glucose-6-phosphate dehydrogenase (G6PDH). However, as the study shows, both basic treatment and combination therapy with melatonin led to a multidirectional change in the activity of this enzyme in patients. In particular, after using melatonin, 10 (33.3%) patients saw a decrease in the activity of G6PDH in blood serum, and 20 (66.7%) — an increase in comparison with pre-treatment parameters. There is evidence that during oxidative stress, an important mechanism of protection against the action of reactive oxygen species is the interaction in the nucleus of hemoxygenase-1 with the transcription factor Nrf2, which contributes to the enhancement of the expression of the second phase detoxification enzymes, which also include G6PDH [20]. Apparently, the intake of melatonin contributed to an increase in the content of transcription factor Nrf2 in the nuclear fraction and increased the expression of hemoxygenase-1 in patients with CKD, which, as was shown, can reduce oxidative stress [21]. In patients who were characterized by a decrease in G6PDH activity, the inhibition of the functioning of the pentose phosphate pathway was probably observed, and melatonin doses taken were not enough to change this parameter upward. Suppression of this enzyme can occur under conditions of DM with underlying inhibition of glucokinase synthesis and induction of glucose-6-phosphatase in the liver, reducing the availability of glucose-6-phosphate for G6PDH [22].

An analysis of the relationships between biomarkers of CKD, type 2 DM and indicators of the functioning of glutathione antioxidant protection components in the groups is presented in Table 5. Results showed that in patients of group 1, there was a negative correlation between the change in postprandial glucose concentration and shifts in NADP-IDG activity during treatment. This correlation confirms that the degree of oxidative stress caused by chronic hyperglycemia in CKD correlates with the degree of depletion of the GSH pool and the inhibition of the activity of one of the most important suppliers of NADPH for its restoration — NADP-IDG. Also, patients of group 1 were characterized by a positive correlation of changes in GP activity with changes in the concentration of urea and creatinine, as well as a negative correlation with changes in the glomerular filtration rate. The observed negative phenomenon could be due to a tendency towards a decrease in GP activity during standard treatment. For patients of group 2, there were no reliably significant relationships between shifts in the analyzed parameters.

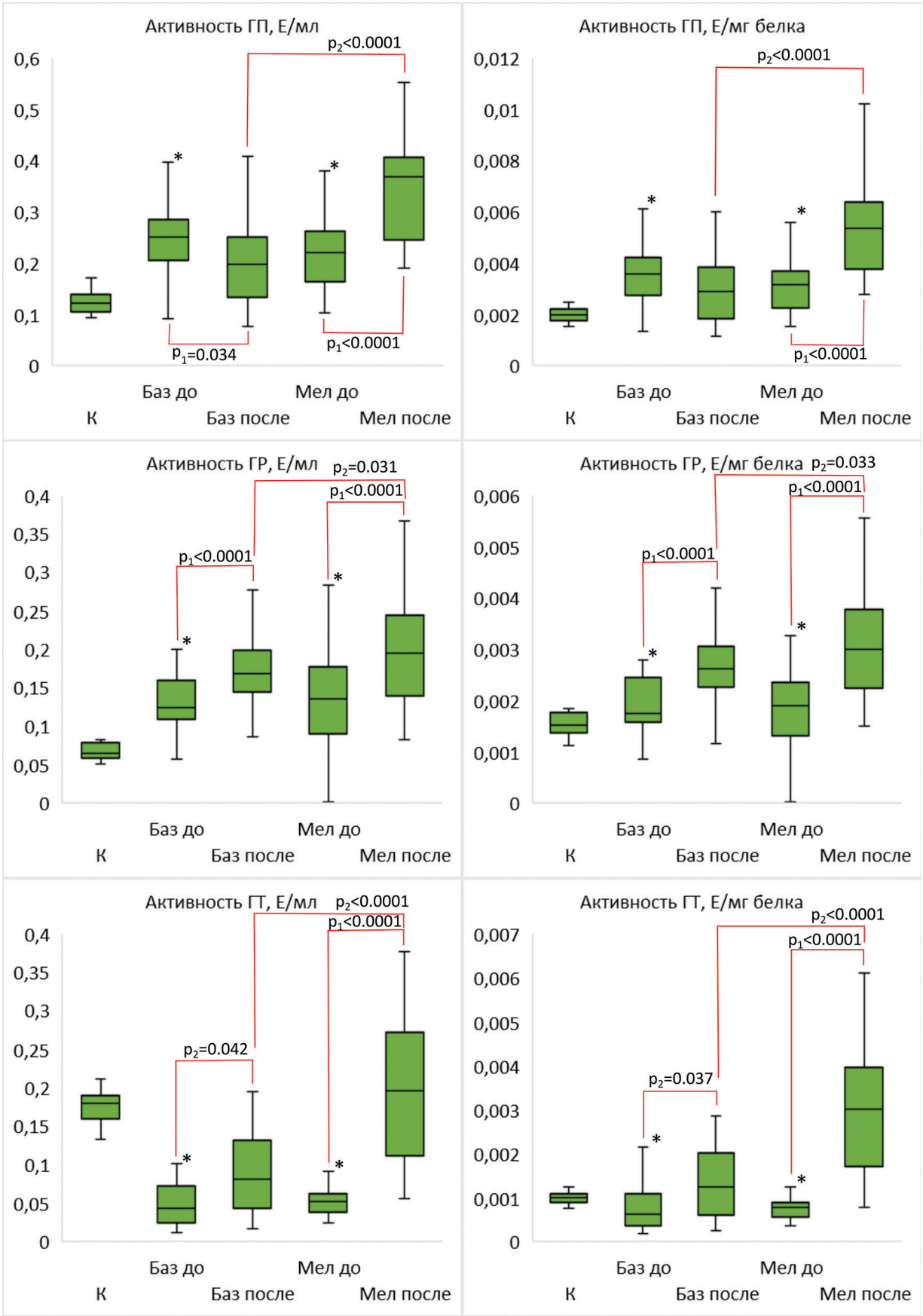

Figure 1. *Activity of glutathione peroxidase (GP), glutathione reductase (GR) and glutathione transferase (GT) in people of the control group (C), patients receiving basic therapy (Bas after) against the indicators before treatment (Bas before), as well as patients receiving combination therapy with melatonin (Mel after) against the indicators before treatment (Mel before)*

Note: * — differences from the control group are significant, p <0.05; p1 — the level of significance of differences between the indicators before and after treatment in groups; p2 — the level of significance of the differences between the changes in indicators that occurred during treatment in the second group compared to the first group

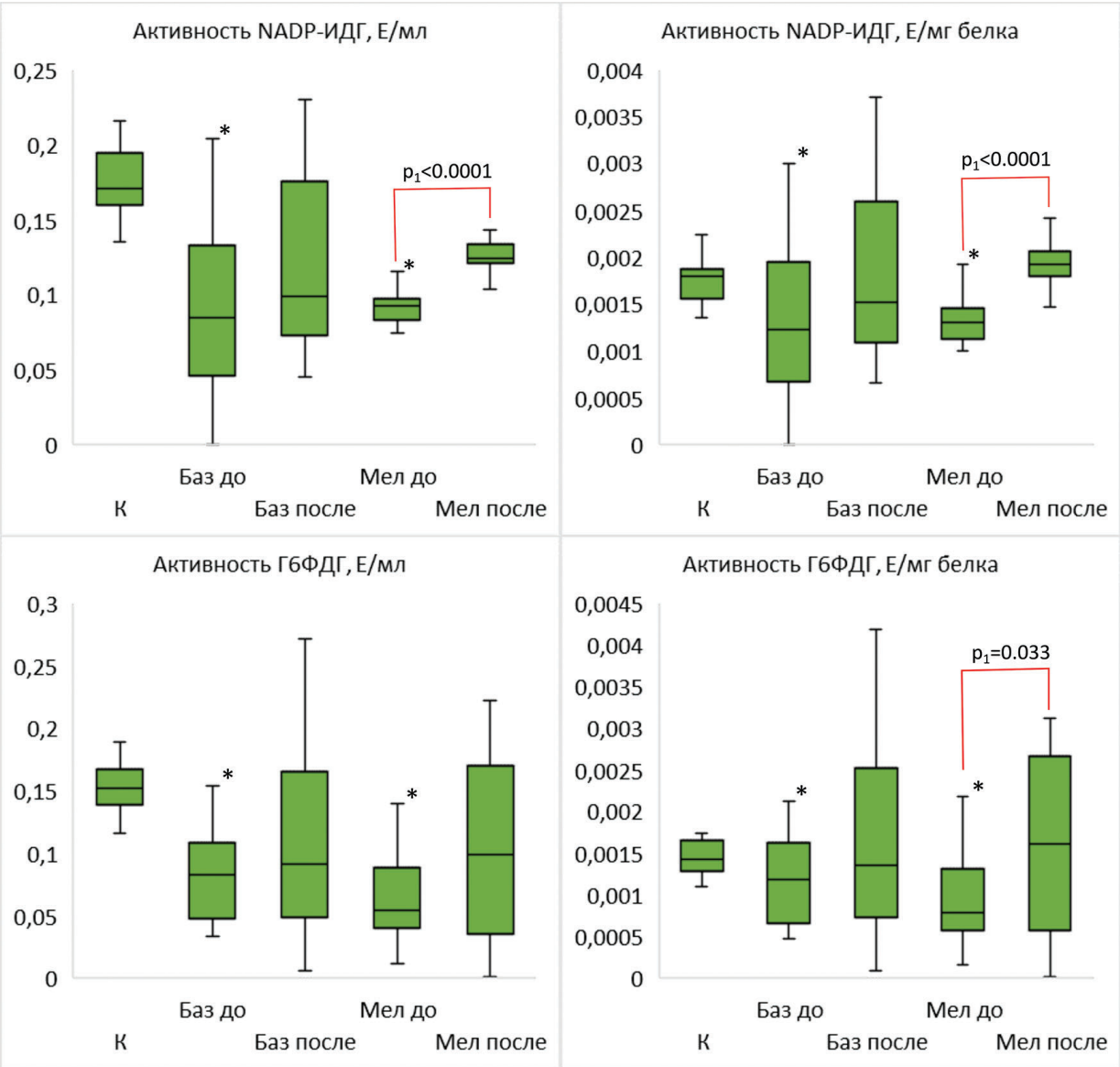

Figure 2. *Activity of NADP-dependent isocitrate dehydrogenase (NADP-IDH) and glucose-6-phosphate dehydrogenase (G6PDH) in people of the control group (C), patients receiving basic therapy (Bas after) against the indicators before treatment (Bas before), and also patients receiving combination therapy with melatonin (Mel after) against the indicators before treatment (Mel before)*

Note: * — differences from the control group are significant, p <0.05; p1 — the level of significance of differences between the indicators before and after treatment in groups; p2 — the level of significance of the differences between the changes in indicators that occurred during treatment in the second group compared to the first group

Table 5. Correlation between changes (Δ) of the studied parameters during treatment in groups of participants

Members of the first group	
Δ postprandial glucose concentration	Δ NADP-ИДГ Δ NADP-IDH r=-0,390 p=0,033
Δ urea concentration	Δ ГП Δ GP r=0,476 p=0,029
Δ creatinine concentration	Δ ГП Δ GP r=0,548 p=0,007
Δ glomerular filtration rate	Δ ГП Δ GP r=-0,571 p=0,004

Apparently, this is due to the more pronounced activating effect of combination therapy with melatonin on the activity of the components of the glutathione antioxidant system, which provides a more effective compensatory response in CKD.

The TGF-β1 level positively correlated with the parameters of proteinuria ($r = 0.800$, $p < 0.0001$), fasting glucose concentration ($r = 0.532$, $p < 0.0001$) and total cholesterol ($r = 0.681$, $p < 0.0001$).

Therefore, the positive effects of melatonin implemented in the treatment regimen for patients with type 2 DM and CKD contributed to an improvement in the oxidative status in the serum of patients. This was reflected in a more significant change in most clinical and biochemical parameters, the level of TGF-β1 and lipid profile compared with participants who received basic treatment.

Conclusion

The data obtained suggest that, compared with basic treatment, combination therapy with melatonin provides a more pronounced antioxidant, hypoglycemic and hypolipidemic effect that is apparently linked with the nephroprotective effect of this hormone. Melatonin promotes the triggering of a compensatory response to oxidative stress, which is one of the key factors in the pathogenesis of complications in type 2 DM. The use of melatonin led to a more significant increase in the activity of glutathione antioxidant system enzymes, as well as the activity of NADP-IDG, one of the main suppliers of NADPH. The observed differences in the effectiveness of the analyzed therapeutic approaches are due to the hypoglycemic activity and direct antioxidant effect of melatonin, as well as its ability to maintain and induce the functional activity of other antioxidants.

Вклад авторов:

Все авторы внесли существенный вклад в подготовку работы, прочли и одобрили финальную версию статьи перед публикацией

Попов С.С. (ORCID ID: https://orcid.org/0000-0002-4438-9201): концепция и дизайн исследования, отбор пациентов, интерпретация результатов, написание текста, окончательное утверждение рукописи

Ануфриева Е.И. (ORCID ID: https://orcid.org/0000-0001-8380-4765): отбор пациентов, сбор и обработка материалов, лабораторные исследования, анализ полученных данных, статистический анализ, подготовка публикации

Крыльский Е.Д. (ORCID ID: https://orcid.org/0000-0002-8855-5515): анализ полученных данных, подготовка публикации

Веревкин А.Н. (ORCID ID: https://orcid.org/0000-0002-7412-9988): лабораторные исследования, анализ полученных данных

Шульгин К.К. (ORCID ID: https://orcid.org/0000-0001-9234-8124): лабораторные исследования, анализ полученных данных

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

Popov S.S. (ORCID ID: https://orcid.org/0000-0002-4438-9201): study concept and design, patient selection, interpretation of results, text writing, final approval of the manuscript

Anufrieva E.I. (ORCID ID: https://orcid.org/0000-0001-8380-4765): selection of patients, collection and processing of materials, laboratory research, analysis of the data obtained, statistical analysis, preparation of a publication

Kryl'skii E.D. (ORCID ID: https://orcid.org/0000-0002-8855-5515): analysis of the data obtained, preparation of the publication

Verevkin A.N. (ORCID ID: https://orcid.org/0000-0002-7412-9988): laboratory research, analysis of the data obtained

Shulgin K.K. (ORCID ID: https://orcid.org/0000-0001-9234-8124): laboratory research, analysis of the data obtained

Список литературы/ References:

1. Hwang S.J., Tsai J.C., Chen H.C. Epidemiology, impact and preventive care of chronic kidney disease in Taiwan. Nephrology (Carlton). 2010; 15(S2): 3-9. doi: 10.1111/j.1440-1797.2010.01304.x.
2. Chawla L.S., Bellomo R., Bihorac A. et al. Acute kidney disease and renal recovery: consensus report of the Acute Disease Quality Initiative (ADQI) 16 Workgroup. Nat Rev Nephrol. 2017; 13(4): 241-257. doi: 10.1038/nrneph.2017.2.
3. Chang A.S., Hathaway C.K., Smithies O. et al. Transforming growth factor-β1 and diabetic nephropathy. Am J Physiol Renal Physiol. 2016; 310(8): F689-F696. doi: 10.1152/ajprenal.00502.2015.
4. Chung A.C. K., Dong Y., Yang W. et al. Smad7 suppresses renal fibrosis via altering expression of TGF-β/Smad3-regulated microRNAs. Mol Ther. 2013; 21(2): 388-398. doi: 10.1038/mt.2012.251.
5. Крыльский Е.Д., Попова Т.Н., Кирилова Е.М. Активность глутатионовой антиоксидантной системы и NADPH-генерирующих ферментов при экспериментальном ревматоидном артрите у крыс. Бюллетень экспериментальной биологии и медицины. 2015; 160(7): 30-33. doi: 10.1007/s10517-015-3089-0.
Kryl'skii E.D., Popova T.N., Kirilova E.M. Activity of Glutathione Antioxidant System and NADPH-Generating Enzymes in Rats with Experimental Rheumatoid Arthritis. Bull Exp Biol Med. 2015; 160(1): 24-27. doi: 10.1007/s10517-015-3089-0 [in Russian].

6. Ruiz-Ortega M., Rayego-Mateos S., Lamaset S. et al. Targeting the progression of chronic kidney disease. Nature Reviews Nephrology. 2020; 16: 269–288. doi: 10.1038/s41581-019-0248-y
7. Горбенко М.В., Попова Т.Н., Шульгин К.К., и др. Влияние мелаксена и вальдоксана на активность глутатионовой антиоксидантной системы и НАДФН-генерирующих ферментов в сердце крыс при экспериментальном гипертиреозе. Экспериментальная и клиническая фармакология. 2013; 76(10): 12-15. doi: 10.30906/0869-2092-2013-76-10-12-15.
Gorbenko M.V., Popova T.N., Shul'gin K.K., et al. Effects of melaxen and valdoxan on the activity of glutathione antioxidant system and NADPH-producing enzymes in rat heart under experimental hyperthyroidism conditions. Eksp Klin Farmakol. 2013; 76(10): 12-15. doi: 10.30906/0869-2092-2013-76-10-12-15 [in Russian].
8. Ассоциация нефрологов. Клинические рекомендации «Хроническая болезнь почек». 2019; 169 с.
Association of Nephrologists. Clinical guidelines «Chronic kidney disease». 2019; 169 p. [In Russian].
9. Kryl'skii E.D., Popova T.N., Safonova O.A. et al. Transcriptional Regulation of Antioxidant Enzymes Activity and Modulation of Oxidative Stress by Melatonin in Rats Under Cerebral Ischemia / Reperfusion Conditions. Neuroscience. 2019; 406: 653-666. doi: 10.1016/j.neuroscience.2019.01.046.
10. Iskusnykh I.Y., Kryl'skii E.D., Brazhnikova D.A. et al. Novel Antioxidant, Deethylated Ethoxyquin, Protects against Carbon Tetrachloride Induced Hepatotoxicity in Rats by Inhibiting NLRP3 Inflammasome Activation and Apoptosis. Antioxidants. 2021; 10(1): 122. doi: 10.3390/antiox10010122.
11. Popov S.S., Shulgin K.K., Popova T.N. et al. Effects of Melatonin-Aided Therapy on the Glutathione Antioxidant System Activity and Liver Protection. Journal of Biochemical and Molecular Toxicology. 2015; 29(10): 449-457. doi: 10.1002/jbt.21705.
12. Meng X., Li Y., Li S. et al. Dietary Sources and Bioactivities of Melatonin. Nutrients. 2017; 9(4): E367. doi: 10.3390/nu9040367.
13. Stacchiotti A., Favero G., Giugno L. et al. Mitochondrial and Metabolic Dysfunction in Renal Convoluted Tubules of Obese Mice: Protective Role of Melatonin. PLoS One. 2014;9(10): e111141. doi: 10.1371/journal.pone.0111141.
14. Xavier S., Vasko R., Matsumoto K. et al. Curtailing Endothelial TGF-β Signaling Is Sufficient to Reduce Endothelial-Mesenchymal Transition and Fibrosis in CKD. Journal of the American Society of Nephrology. 2015;26(4):817-829. doi: 10.1681/ASN.2013101137.
15. Gu Y.Y., Liu X.S., Huang X.R. et al. Diverse Role of TGF-β in Kidney Disease. Front Cell Dev Biol. 2020; 8: 123. doi: 10.3389/fcell.2020.00123.
16. Obayashi K., Saeki K., Iwamoto J. et al. Exposure to Light at Night, Nocturnal Urinary Melatonin Excretion, and Obesity/Dyslipidemia in the Elderly: A Cross-Sectional Analysis of the HEIJO-KYO Study. The Journal of Clinical Endocrinology & Metabolism. 2013; 98(1): 337–344. doi: 10.1210/jc.2012-2874.
17. Pandi-Perumal S.R., BaHammam A.S., Ojike N.I. et al. Melatonin and Human Cardiovascular Disease. J Cardiovasc Pharmacol Ther. 2017; 22(2): 122-132. doi: 10.1177/1074248416660622.
18. Lindblom R., Higgins G., Coughlan M. et al. Targeting Mitochondria and Reactive Oxygen Species-Driven Pathogenesis in Diabetic Nephropathy. Rev Diabet Stud. 2015; 12(1-2): 134-156. doi: 10.1900/RDS.2015.12.134.
19. Huang J.Q., Zhou J.C., Wu Y.Y. et al. Role of glutathione peroxidase 1 in glucose and lipid metabolism-related diseases. Free Radical Biology and Medicine. 2018; 127: 108-115. doi: 10.1016/j.freeradbiomed.2018.05.077.
20. Biswas C., Shah N., Muthu M. et al. Nuclear heme oxygenase-1 (HO-1) modulates subcellular distribution and activation of Nrf2, impacting metabolic and anti-oxidant defenses. J Biol Chem. 2014; 189(39): 26882-26894. doi: 10.1074/jbc.M114.567685.
21. Kilic U., Kilic E., Tuzcu Z. et al. Melatonin suppresses cisplatin-induced nephrotoxicity via activation of Nrf-2/HO-1 pathway. Nutr Metab (Lond). 2013; 10(1): 7. doi: 10.1186/1743-7075-10-7.
22. Haeusler R.A., Camastra S., Astiarraga B. et al. Decreased expression of hepatic glucokinase in type 2 diabetes. Molecular Metabolism. 2015; 4(3): 222-226. doi: 10.1016/j.molmet.2014.12.007.