Innov Biosyst Bioeng, 2023, vol. 7, no. 2, 3–12
doi: 10.20535/ibb.2023.7.2.285863

Original article

EFFECTS OF METFORMIN AND PREPARATIONS WITH PLEIOTROPIC EFFECTS ON TESTICULAR BIOCHEMICAL INDICES OF RATS WITH JUVENILE-ONSET METABOLIC SYNDROME

L.B. Bondarenko[1*], G.M. Shayakhmetova[1], O.Ye. Tkachenko[1], M.M. Kalachinskaya[2], V.M. Kovalenko[1]

[1]SI "Institute of Pharmacology & Toxicology of the National Academy of Medical Sciences of Ukraine", Kyiv, Ukraine
[2]Open International Human Development University "Ukraine", Kyiv, Ukraine

*Corresponding author: larabon04@yahoo.com

Received 20 December 2022; Accepted 10 April 2023

Background. Metabolic syndrome (MS) is a complex of disorders characterized by abdominal obesity, insulin resistance and glucose tolerance, arterial hypertension, and all types of metabolic disorders. Taking into account the wide range of symptoms accompanying MS, the use of preparations with pleiotropic effects on metabolic processes in the body could be promising for its treatment.
Objective. The aim of this study is comparative estimation of metformin or its combination with vitamins' complex or liposomal preparation treatment effects on DNA, RNA, histones, ATP, ADP, AMP contents, and DNA fragmentation processes in testes of rats with MS induced in juvenile age.
Methods. MS model was induced by full replacement of drinking water with 10% fructose solution in Wistar male rats of 21−23 days age (50−70 g). DNA, RNA, histones, ATP, ADP, AMP contents, and DNA fragmentation processes investigations were carried out after 60 days of MS modeling and metformin or its combination with vitamins' complex or liposomal preparation treatment.
Results. In experiments with pubertal rats with MS and metformin or its combination vitamins' complex or liposomal preparation treatment, we established partially corrective effects of these medications for DNA, RNA, histones, ATP, ADP, AMP contents, and DNA fragmentation processes changes caused by MS development.
Conclusions. A comparative analysis of the studied preparations' effects under MS simulation in the juvenile age showed that none of these drugs was able to completely normalize the disorders in studied indicators caused by MS. However, both combinations of metformin with vitamins' complex or liposomal preparation were still more effective in these negative changes' correction then metformin itself. Metformin with vitamins' complex caused a more pronounced influence on the processes of DNA fragmentation, the levels of adenyl nucleotides, and the energy charge of rat testicular cells, while the corrective effect of metformin with liposomal preparation was more noticeable with respect to the content of chromatin components.

Keywords: metabolic syndrome; metformin; vitamins' complex; liposomal preparation; testes; juvenile age.

Introduction

Metabolic syndrome (MS) is a complex of disorders characterized by abdominal obesity, insulin resistance and glucose tolerance, arterial hypertension, and all types of metabolic disorders [1]. Its severe consequences for men reproductive function necessitate the search for new approaches to its pharmacological treatment [2].

Ineffectiveness of non-pharmacological methods prompts patients to use metformin, which is included in the list of drugs recommended by the WHO for the treatment pre-diabetic conditions such as MS [3].

Metformin increases sensitivity to insulin and improve glucose metabolism. However, information regarding the effect of metformin on the reproductive function of MS patients is quite contradictory [4, 5].

On the other hand, taking into account the wide range of symptoms accompanying MS, the use of preparations with pleiotropic effects on metabolic processes in the body could be promising. To such medicines belongs phosphatidyl choline-quercetin liposomal preparation, which has a wide range of pharmacological effects [6−10]. This preparation has an antioxidant, antihypoxic and anti-inflammatory effect. Its therapeutic effect is realized via the blockade of the 5-lipoxygenase pathway of arachidonic acid metabolism by quercetin and simultaneous antihypoxic and antioxidant action of lecithin liposomes. This medicine restores the functional activity of the vascular endothelium, synthesis and/or release of endothelial relaxation factor (nitric oxide), inhibits the processes of lipid peroxidation in blood and tissues, supports the activity of the body's antioxidant systems, prevents a decrease in the energy metabolism of cells, and ex-

hibits a membrane- and endothelium-protective effect. It is prescribed in the complex treatment of acute myocardial infarction without a Q wave, unstable and stable angina pectoris (IHD), myocarditis, to prevent toxic damage to the myocardium during cycles of polychemotherapy for breast cancer.

It could also be promising to use (together with metformin) the multivitamin preparation containing DL-methionine, vitamins E, B_1, B_3 and zinc sulfate heptahydrate. Previously, we demonstrated the gonadoprotective effect of this vitamins' complex under the conditions of antituberculosis agents' administration to male rats [11]. In our previous investigations, it was found that metformin, phosphatidyl choline-quercetin liposomal preparation, and to a lesser extent, this multivitamin preparation with MS, have gonadoprotective action, mediated by the involvement of various mechanisms. The range of pharmacological correction indicators for each of the preparations was not the same and none of them could completely remove the negative impact of MS on the gonads [12].

The aim of this study is comparative estimation of metformin or its combination with vitamins' complex or liposomal preparation treatment effects on DNA, RNA, histones, ATP, ADP, AMP contents, and DNA fragmentation processes in testes of rats with MS induced in juvenile age.

Materials and methods

A total of 50 Wistar male rats of 21–23 days age (50–70 g) were used in the study. They were kept under a controlled temperature (from 22 to 24 °C), relative humidity of 40% to 70%, lighting (12 hours light-dark cycle), and on a standard pellet feed (Research Limited Liability Company "F.U.D", Tetiiv, Ukraine). The procedure followed the 1986 UK Animals (Scientific Procedures) Act and the EU Directive 2010/63/EU and was approved by the Institute's Animal Care and Use Committee (approval number 01/07/15).

In experiments were used phosphatidyl choline-quercetin liposomal preparation (PQ) (which includes egg phosphatidylcholine-1,2-diacyl-sn-y-glycero-3-phosphocholine, lecithin – 550 mg and quercetin – 3,3',4',5,7-pentahydroxy flavonein terms of dry substance 15 mg per 1 portion) and multivitamin preparation (MV) (containing DL-methionine – 291 mg; α-tocopherol acetate (vitamin E) – 7.5 mg; thiamine hydrochloride (vitamin B1) – 0.9 mg; nicotinamide (vitamin B3) – 0.68 mg; zinc sulfate heptahydrate, which is equivalent to zinc 0.03 mg – 0.14 mg per 1 capsule).

The model of MS was reproduced according to the protocol of Bettaieb *et al.* [13]. Animals were divided into 5 groups (10 animals in each group): 1 – Control 1 (intact rats), 2 – MS model – complete replacement of drinking water with 10% fructose solution for 60 days [13], 3 – MS + metformin (rats with MS and metformin treatment [266 mg/kg of body weight (b.w.), *per os*, in a 1% starch gel] after 30 days from the start of MS simulation, 30 days]), 4 – MS + metformin + PQ (rats with MS and metformin [as in group 3] + PQ treatment [3.1 mg/kg of body weight (b.w.), (in terms of quercetin), in the form of a suspension in physiological solution 2 hours before the administration of metformin, *intraperitoneally*, after 30 days from the start of MS simulation, 30 days]), 5 – MS + metformin + MV (rats with MS and metformin [as in group 3] + MV treatment [77.3 mg/kg of body weight (b.w.), (in terms of methionine), in a 1% starch gel 2 hours before the introduction of metformin, *per os*, after 28 days from the start of MS simulation, during three five-day courses with a break of 9 days]).

The solutions were prepared by mixing crystalline fructose (Shandong Xiwang Sugar Industry Co., Ltd., Binzhou, China) with drinking water on a daily basis and given to the MS groups *ad libitum* for 60 days. Metformin manufactured by LEK SA, Poland, DN0372 series, phosphatidyl choline-quercetin liposomal preparation manufactured by NanoMedTech LLC, Ukraine, experimental series 050715, and multivitamin preparation produced by PrJSC "Technolog", Ukraine, R.P. No. UA/1553/01/01 were used for administration to animals. The coefficient for conversion of human doses to animal equivalent doses based on body surface area was taken into account for all drugs [14].

After 60 days of 10%, fructose solution consumption and metformin, PQ, or MV treatment rats were sacrificed under mild ether anesthesia by decapitation. Testes were removed and used for further investigations. A comprehensive assessment including determination of testes' DNA, RNA, histones, ATP, ADP, AMP contents, and DNA fragmentation, was carried out after 60 days of MS modeling. Testes DNA and RNA were isolated as previously described [15].

Chromatin DNA fragmentation evaluation (as an apoptosis marker) was carried out according to

Bondarenko *et al.* [16]. DNA were dissolved in TBE buffer (10 mm Tris-HCl and 1 mm EDTA, pH 8); and then were fractionated through 2% agarose gels (50–60 V; 3.5 h). After electrophoresis, gels were stained with ethidium bromide and visualized under a UV transilluminator (BIORAD, USA). Electrophoresis data analysis was carried out with Quantity One Software (USA).

Histone content was determined according to the modified method of N. Smirnova [17].

Spectrophotometric determination of the concentration of DNA and RNA was carried out according to the generally accepted method [18]. Light absorption at a wavelength of 260 nm was measured. The DNA concentration was calculated using a conversion factor of 6.5 according to

$$C_{\text{DNA}}[\mu\text{g/ml}] = A_{260} \times 6.5.$$

RNA content was similarly determined and its concentration was calculated using a conversion factor of 5.2 according to

$$C_{\text{RNA}}[\mu\text{g/ml}] = A_{260} \times 5.2.$$

Determination of the content of AMP, ADP and ATP was carried out by the method of thin-layer chromatography [19].

The energy charge (*EC*) was calculated according to

$$EC = \frac{2\text{ATP} + \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}}.$$

The protein content in different fractions of testes was determined according to the method of O.H. Lowry *et al.* [20].

Statistical analysis

The obtained data were expressed as the mean ± standard error of the mean ($M \pm SEM$) and analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test using OriginPro 7.5 Software. Differences were considered to be statistically significant at $p < 0.05$.

Results

The verification of the MS model in rats of different ages was carried out by us previously using glucose tolerance test and blood pressure evaluation. Glucose intolerance and hypertension development were demonstrated [16].

In the current study we have established that testicular cells functions changes, caused both by MS itself and by the action of metformin, PQ, and MV on its background, were accompanied by intensification and changes in the nature of nuclear DNA fragmentation processes (Figure).

Under the conditions of MS, DNA fragmentation in the testes of rats was significantly increased compared to the control (Figure, Table 1). The development of MS in pubertal rats, in contrast to controls, was accompanied by the activation of DNA fragmentation processes in the testes with the formation of 13 fractions of low molecular weight fragments at once. The percentage of fragmentation increased almost 4 times: from 7.04% in the control to 27.63% – in animals with MS. At the same time, 8 fractions consisted of fragments with masses from 20 to 100 base pairs (b.p.), while relatively more massive fragments were represented by 5 fractions with a length of 350–500 b.p.

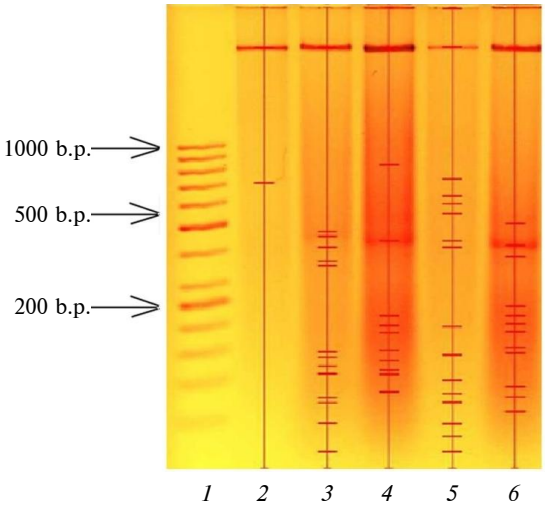

Figure: The level of DNA fragmentation in the testes of rats with juvenile-onset metabolic syndrome and metformin or its combination with MV or PQ treatment ($M \pm m$, $n = 10$): *1* – marker, 2 – control; *3* – MS; *4* – MS + metformin; 5 – MS + metformin + PQ; *6* – MS + metformin + MV (MS – metabolic syndrome, MV – multivitamin preparation, PQ – phosphatidyl choline-quercetin liposomal preparation)

Table 1: Relative % of DNA fragmentation in testes of rats with juvenile-onset metabolic syndrome and metformin or its combination with MV or PQ treatment

Group	Relative % of DNA fragmentation
Control	7.04
MS	27.63
MS + metformin	20.91
MS + metformin + PQ	21.93
MS + metformin + MV	3.14

Notes. MS – metabolic syndrome, MV – multivitamin preparation, PQ – phosphatidyl choline-quercetin liposomal preparation.

Under the conditions of metformin treatment, a certain normalization of DNA fragmentation was noted in the testes of rats: the number of fragment fractions decreased to 10, of which only 2 fractions of fragments with masses from 800 to 450 b.p. were formed, and the rest were represented by low-molecular fragments (from 100 to 200 b.p.).

Therefore, the metformin treatment to some extent weakened the negative consequences of MS development for DNA fragmentation processes: the number of fractions decreased compared to untreated animals (Figure, Table 1).

When applying metformin with PQ in the testes of rats, there was no normalization of DNA fragmentation: 14 fractions of fragments were formed with masses from 700 to 20 b.p. with a predominance of low molecular weight ones (from 20 to 200 b.p. – 8 fractions). The percentage of fragmentation was 21.93%. Therefore, treatment by PQ with metformin did not reduce the negative consequences of MS for DNA fragmentation processes: the number of fragment fractions and the level of fragmentation did not decrease compared to MS animals.

Under the conditions of MS treatment by metformin with MV in the testes of rats, there was also no normalization of DNA fragments number: 12 fractions of fragments with masses from 500 to 100 b.p. were formed, with a predominance of the number of low molecular weight ones (from 20 to 200 b.p. – 9 fractions). However, the content of these fractions decreased significantly: the percentage of fragmentation in this group was normalized up to 3.14% (Table 1). Therefore, the simultaneous use of MV with metformin weakened the negative consequences of MS for the processes of DNA fragmentation: the percentage of fragmentation decreased compared to MS animals.

Changes in testicular cells chromatin components stability with MS and tested medicines administration were accompanied by quantitative changes in these compounds. The results of the study of the content of nucleic acids, indices of their ratio and the content of histones in testicular cells of rats with juvenile-onset metabolic syndrome and metformin or its combination with MV or PQ treatment are shown in Table 2.

From the given data, it can be seen that there is an increase in the content of DNA, DNA-bound proteins, RNA, and histones in testicular cells with MS. At the same time, the RNA /DNA and RNA/total nucleic acids (NA) ratio – decreased. Tested medicines administration allowed partially normalizing abovementioned parameters. The exception was the histones content, which remained elevated.

The study results on ATP, ADP, AMP and energy charge level changes in testicular cells of rats with juvenile-onset metabolic syndrome and administration of metformin or its combination with MV or PQ are shown in Table 3.

It can be seen from the given data that in testes of rats with MS, ATP and ADP contents decrease (–27% and –49% correspondently) with a simultaneous minute increase in the AMP content (+3%).

Table 2: Nucleic acids and histones changes in testicular cells of rats with juvenile-onset MS and administration of metformin or its combination with MV or PQ

Indices	Groups				
	Control	MS	MS + metformin	MS + metformin + PQ	MS + metformin + MV
RNA content, μg/ mg of tissue	28.90 ± 3.71	38.70 ± 1.70*	30.50 ± 2.02#	29.33 ± 3.42#	29.54 ± 1.51#
DNA content, μg/ mg of tissue	17.82 ± 2.50	30.19 ± 3.91*	17.03 ± 4.23#	14.80 ± 3.60#	15.10 ± 2.24#
DNA-bound proteins, μg/mg of tissue	109.83 ± 14.02	178.40 ± 25.50*	97.39 ± 26.21#	86.70 ± 23.40#	92.05 ± 22.70#
RNA/DNA	1.67 ± 0.17	1.39 ± 0.16	2.43 ± 0.60	2.48 ± 0.58	2.53 ± 0.61
RNA/NA	0.62 ± 0.03	0.57 ± 0.03	0.67 ± 0.05	0.68 ± 0.04	0.66 ± 0.06
Histones, μg /mg of tissue	26.11 ± 3.03	36.02 ± 1.01*	30.02 ± 5.01	40.14 ± 1.04*,#	38.15 ± 1.00*,#

Notes. * – $p \leq 0.05$ compared to the control group, # – $p \leq 0.05$ compared to the MS group; MS – metabolic syndrome, MV – multivitamin preparation, PQ – phosphatidyl choline-quercetin liposomal preparation.

Table 3: ATP, ADP, AMP and energy charge levels changes in testicular cells of rats with juvenile-onset MS and administration of metformin or its combination with MV or PQ

Indices	Groups				
	Control	MS	MS + metformin	MS + metformin + PQ	MS + metformin + MV
ATP content, μg/mg of protein	439.83 ± 18.71	336.93 ± 28.69*	353.45 ± 17.99*	337.74 ± 40.24*	1131.61 ± 56.72*,#
ADP content, μg/mg of protein	845.76 ± 38.56	429.68 ± 29.50*	709.78 ± 87.81#	482.17 ± 44.84*	850.45 ± 59.72#
AMP content, μg/mg of protein	497.10 ± 18.99	510.55 ± 14.10	565.79 ± 54.52	507.72 ± 98.04	974.35 ± 174.67*,#
Energy charge, CU	0.48	0.43	0.35	0.44	0.47

Notes. * – $p \leq 0.05$ compared to the control group, # – $p \leq 0.05$ compared to the MS group; MS – metabolic syndrome, MV – multivitamin preparation, PQ – phosphatidyl choline-quercetin liposomal preparation.

The treatment with metformin and PQ did not lead to the normalization of these indices, while the combination of metformin with MV made it possible to significantly increase the content of ATP and ADP and completely normalize the cells' energy charge.

Discussion

In this work, our task was to assess the possibility of additional correction of the reproductive system state with juvenile-onset metabolic syndrome, by preparations that demonstrated a certain gonadoprotective activity in our previous studies on adult animals [11, 12]. Since preliminary experiments showed that metformin monotherapy in this case was not sufficiently effective [12], it was of interest to compare the results obtained in a single experiment of including MV and PQ into the treatment regimen.

Damage to any intracellular macromolecules with MS development can be of fundamental importance for cell's vital activity, and can lead to cell's damage and death.

There is a clear correlation between systemic changes in DNA structure and a number of changes characterizing the MS development [21]. Stimulation of DNA fragmentation processes under the conditions of metabolic syndrome development is obviously connected with stimulation of oxidative processes with this pathology [22]. Oxidative processes intensification can lead to oxidative modifications of purine, pyrimidine, and deoxyribose residues, disruption of DNA interaction with chromatin proteins and enzymes, and therefore, as a result, to significant changes in the stability of the DNA molecule [22]. The violation of testicular cells apoptosis normal process due to MS can lead to the limitation of their regeneration and the deepening of pathological changes in these organs [21, 23]. Our results on the increase in the level of DNA fragmentation with metformin administration against the background of MS are fully consistent with the data of other authors establishing the presence of this medicine significant effect on the processes of adenyl nucleotide metabolism and its ability to stimulate cell autophagy [24].

Quercetin has a lot of pharmaceutical applications, many of which arise from its potent antioxidant properties and liposomal form can significantly improve its solubility and bioavailability [9]. However, we did not reveal positive effect of PQ on testicular DNA fragmentation rate in our experiments. Such results are fully consistent with the data [25, 26], which showed the ability of phosphatidylcholine and quercetin, (PQ components), to affect directly the processes of cells proliferation, stimulating their apoptosis. Pro-apoptotic effects of quercetin could be realized via different mechanisms involving antioxidant effects and the suppression of p53 gene and BCL-2 protein [27]. The suppression of BCL-2 gene transcription diminishes the inhibitory effects on BAD protein in the mitochondria, which is considered as the initiator of apoptosis for the intrinsic pathway [28]. The inhibition of p53 leads to susceptibility of cells toward cytotoxicity induced by quercetin [29]. It should be noted that p53 also acts as a modulator of intracellular levels of ROS exerting antioxidant effects in cells with no or low stress through the regulation of genes involved in such activity, which comprises microsomal GSH transferase homolog

PIG12, aldehyde dehydrogenase 4 family member A1 (ALDH4A1), Gpx1, manganese superoxide dismutase (SOD2) and catalase) [30–34]. Apart of p53, such proteins as p63 and p73 may be involved in apoptosis stimulation [34]. Additionally, the apoptotic processes caused by quercetin could be mediated by the dissociation of Bax from Bcl-xL and the activation of caspases [35].

The MV vitamins' complex normalizing effect (used together with metformin) on DNA fragmentation processes under MS could be possibly due to the ability of Zn^{2+} ions to replace Ca^{2+} ions, thus affecting the Ca-dependent ways of autophagy processes stimulation by metformin. At the same time, non-calcium mediated metformin influence on lipid metabolism and glycolysis processes is not disturbed [24]. In addition, maintaining discrete subcellular pools of Zn^{2+} is critical for cells' functional and structural integrity. Among the essential biological processes influenced by Zn^{2+} is apoptosis, which is important in cellular homeostasis [36].

No less can be the contribution of methionine contained in the MV to the overall normalizing effect of the preparation. It was shown that methionine can influence directly on chromatin components state and structure, as soon as indirectly – via changing nucleosome mobility, metabolic enzymes, chromatin remodelers and chromatin modifiers activities [37]. Oxidative DNA damage and apoptosis could also be inhibited by another MV component – tocopherol [38].

Differences in the nature and intensity of DNA fragmentation processes noted in our experiments may be caused by changes in the activity levels of nucleases and other enzymes involved in maintaining the stability of the structure of DNA molecules in response to the administration of the studied substances. Depending on the set and effectiveness of nucleases, DNA fragmentation occurs to varying degrees in cells of different lineages [39, 40].

An increase in the content of chromatin components against the background of MS may be associated with a reduced release of spermatozoa from the testes due to a violation of their formation and metabolism of nuclear DNA as such [41]. Immature spermatocytes accumulate in the testicles. Their transformation into spermatozoa is inhibited by hormonal imbalance in MS. Changes in the DNA content under conditions of obesity caused by a high-energy diet are confirmed by the data of other authors [42]. In addition, the development of oxidative stress with MS may cause changes in the kinetics of DNA methylation processes due to the effect on DNA methyltransferase activity, thereby reducing DNA methylation [22]. The above-mentioned possible disturbances in the interaction of DNA with chromatin proteins and enzymes as a result of stimulation of oxidative processes irreversibly change the kinetics of transcription processes [22].

A decrease in RNA to DNA and RNA to NC ratios is evidence of the predominance of chromatin components changes at the transcriptional rather than translational and posttranslational levels, which was also noted in the works of other authors [22].

The effect of PQ on the studied parameters might be due to the quercetin ability to modify the RNA polymerase activity [43], as soon as to form quercetin-DNA complexes [44] and to interact with DNA-binding proteins [45]. The MV components multiple effects on quantity and quality of RNA, DNA and chromatin proteins, both direct and indirect, have already been mentioned by us above [36–38].

Histones, protamines, and non-coding RNA molecules are also targets of oxidative stress [22]. Therefore, the changes in their contents noted with MS are quite expected and are fully consistent with the data of other authors. In particular, it was recently established that tRNA has become a new factor contributing to the pathogenesis of diabetes. Changes in aminoacylation processes, as well as tRNA modification and fragmentation accompany β-cell failure, obesity, and insulin resistance [46].

Quercetin can influence on histones contents via histone deacetylases [47] or another histone epigenetic modifications [48]. The effects of MV components on histones might be mainly to methionine influence on chromatin architecture [37].

Our results on ATP, ADP, AMP, and energy charge levels changes in testicular cells of rats with juvenile-onset metabolic syndrome and administration of metformin or its combination with MV or PQ are fully consistent with the data of other authors, established the existence of a clear dependence of the content of ATP and ADP on the concentration of glucose in the blood [49]. Such changes in the content of ATP, ADP, and AMP with MS may be caused by disturbances in the activity of adenosine deaminase, similar to those observed with diabetes [50]. Due to the inhibition of adenosine deaminase activity, 2′-deoxyadenosine accumulates and is converted to 2′-deoxyadenosine-5′-triphosphate (dATP), which inhibits ribonucleotide reductase (RNR), a crucial enzyme in DNA synthesis. And the lack of AMP is compensated by its formation from ATP and ADP through their dephosphorylation.

Also, indirect mechanisms of influence on ATP, ADP, and AMP contents probably causing the above-mentioned changes in chromatin components and DNA fragmentation processes with MS cannot be excluded.

Our data regarding the metformin effect on the content of ATP, ADP, and AMP are in good correspondence with the results of other authors, established that the final intracellular target for metformin was 5'-adenosine monophosphate-activated protein kinase, a key energy sensor of the cell [51]. According to these data, metformin penetrates mitochondria through the intracellular space and accumulates in them. Here metformin causes a decrease in ATP production and an increase in [AMP]/[ATP] and [ADP]/[ATP] ratios. In addition, it reduces the activity of AMP-deaminase, which converts AMP into inosine monophosphate, inducing the accumulation of AMP inside the cell, as a result of which the activation of 5′-adenosine monophosphate-activated protein kinase occurs.

Therefore, metformin could influence on the content of ATP, ADP, and AMP in our experiments via stimulation of AMP and inhibition of ADP and ATP formation, suppressing the processes of adenyl nucleotides phosphorylation [24] during the mediated activation of adenosine 5′-monophosphate protein kinase. The multistage apoptosis-inducing effect of metformin is apparently triggered by the same mechanism.

The absence of a pronounced normalizing effect of PQ on energy metabolism in our experiments is possibly due to the fact that quercetin acts as the energy transfer inhibitor and can modify metabolisms of ATP, ADP, AMP and the ATP/ADP ratio [52].

The B group vitamins and zinc ions (contained in the MV) could cause the defining contribution to the overall preparation's normalizing effect on ATP, ADP, AMP, and energy charge levels [53, 54] as it was previously shown that alpha-tocopherol normalised the levels of MDA but did not substantially affect the energy metabolic indices [55.]

The MV pronounced effect on adenyl nucleotides, and energy charge levels looks very promising, since numerous studies have demonstrated the ability of extracellular nucleotides (mainly ATP and to a lesser extent ADP) to regulate a number of biological processes, including blood coagulation, wound healing and inflammation, which are significantly impaired in multiple sclerosis and diabetes [56].

Conclusions

In experiments on rats with MS and metformin or its combination with vitamins' complex or liposomal preparation treatment, we established the effects of these medications on DNA, RNA, histones, ATP, ADP, AMP contents, and DNA fragmentation processes. A comparative analysis of the studied preparations' effects under MS simulation in the juvenile age showed that none of these drugs was able to completely normalize the disorders in studied indices caused by MS. However, both combinations of metformin with vitamins' complex or liposomal preparation were still more effective in these negative changes' correction then metformin itself. Metformin with vitamins' complex caused a more pronounced influence on the processes of DNA fragmentation, the levels of adenyl nucleotides, and the energy charge of rat testicular cells, while the corrective effect of metformin with liposomal preparation was more noticeable with respect to the content of chromatin components.

Conflict of interests

Larysa Bondarenko is the member of the Editorial Council of *Innovative Biosystems and Bioengineering* and was not involved in the editorial evaluation or decision to accept this article for publication. The other authors have no conflicts of interest to declare.

Acknowledgements

The authors gratefully acknowledge the financial support of this study by the National Academy of Medical Sciences of Ukraine as a project "Features of the impact of metabolic syndrome in juvenile age and its pharmaco correction on the state of male reproductive function in an experiment" (state registration number 0116U006003).

References

[1] Rochlani Y, Pothineni NV, Kovelamudi S, Mehta JL. Metabolic syndrome: pathophysiology, management, and modulation by natural compounds. Ther Adv Cardiovasc Dis. 2017 Jun 22;11(8):215-25. DOI: 10.1177/1753944717711379

[2] Kasturi SS, Tannir J, Brannigan RE. The Metabolic Syndrome and Male Infertility. J Androl. 2008 Jan 9;29(3):251-9. DOI: 10.2164/jandrol.107.003731

[3] Reduction in the Incidence of Type 2 Diabetes with Lifestyle Intervention or Metformin. New Eng J Med. 2002 Feb 7;346(6):393-403. DOI: 10.1056/nejmoa012512

[4] Shegem NS, Alsheek Nasir AM, Batieha AM, El-Shanti H, Ajlouni KM. Effects of short term metformin administration on androgens in diabetic men. Saudi Med J. 2004 Jan;25(1):75-8.

[5] Ozata M, Oktenli C, Bingol N, Ozdemir IC. The effects of metformin and diet on plasma testosterone and leptin levels in obese men. Obesity Res. 2001 Nov;9(11):662-7. DOI: 10.1038/oby.2001.90

[6] Posokhova K, Stechyshyn I, Krynytska I, Marushchak M, Birchenko I, et al. Comparative study of the effect of various forms of quercetin on experimental diabetes. Roman J Diabetes Nutr Metab Dis. 2018 Dec 1;25(4):383-8. DOI: 10.2478/rjdnmd-2018-0046

[7] Liu D, Hu H, Lin Z, Chen D, Zhu Y, Hou S, et al. Quercetin deformable liposome: Preparation and efficacy against ultraviolet B induced skin damages in vitro and in vivo. J Photochem Photobiol B Biol. 2013 Oct;127:8-17. DOI: 10.1016/j.jphotobiol.2013.07.014

[8] Rivera F, Costa G, Abin A, Urbanavicius J, Arruti C, Casanova G, et al. Reduction of ischemic brain damage and increase of glutathione by a liposomal preparation of quercetin in permanent focal ischemia in rats. Neurotox Res. 2008 Jun;13(2):105-14. DOI: 10.1007/bf03033562

[9] Rezaei-Sadabady R, Eidi A, Zarghami N, Barzegar A. Intracellular ROS protection efficiency and free radical-scavenging activity of quercetin and quercetin-encapsulated liposomes. Artif Cells Nanomed Biotechnol. 2014 Jun 24;44(1):128-34. DOI: 10.3109/21691401.2014.926456

[10] Asmolov OK, Rybak TA. Lipophlavon influence on functional activity of the bronchial epithelium cells in patients suffering from chonic obstruction lung disease of the III degree of severety with a long term of smoking. Adv Biol Med. 2009;2:83-6.

[11] Shayakhmetova GM, Bondarenko LB, Voronina AK, Kovalenko VM. Comparative investigation of methionine and novel formulation Metovitan protective effects in Wistar rats with testicular and epididymal toxicity induced by anti-tuberculosis drugs co-administration. Food Chem Toxicol. 2017 Jan; 99:222-30. DOI: 10.1016/j.fct.2016.12.001

[12] Kovalenko VN, Tkachenko AE, Shayakhmetova AM, Matvienko AV. Impact of metformin on testis morpho-functional characteristics in male rats induced by metabolic syndrome developed at the juvenile age. Pharmacol Drug Toxicol. 2018;2(58):32-40.

[13] Bettaieb A, Vazquez Prieto MA, Rodriguez Lanzi C, Miatello RM, Haj FG, Fraga CG, et al. (-)-Epicatechin mitigates high-fructose-associated insulin resistance by modulating redox signaling and endoplasmic reticulum stress. Free Radic Biol Med. 2014 Jul;72:247-56. DOI: 10.1016/j.freeradbiomed.2014.04.011

[14] Guidance for industry. Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy Volunteers. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER); 2005 Jul. 30 p.

[15] Mannervik B, Jemth P. The glutathione pathway. Measurement of glutathione transferases. In: Costa LG, Hodgson E, Lawrence DA, Ozolins TR, Reed DJ, Greenlee WF, editors. Current protocols in toxicology. New York: John Wiley & Sons Inc; 2005. 2758 p.

[16] Bondarenko LB, Karatzuba TA, Shayakhmetova GM, Voronina AK, Matvienko AV, Strelkov IV, et al. Specificity of metabolic syndrome model reproduction at pubertal and adult male rats. Roman J Diabetes Nutr Metab Dis. 2015 Sep 1;22(3):251-60. DOI: 10.1515/rjdnmd-2015-0031

[17] Smirnova NV. Fractionation of rat liver histone proteins. In: Modern methods in biochemistry. Moscow: Medicine; 1977:349-52.

[18] Velikov VA. Molecular biology. Practical guide. Saratov: Publishing house "Saratov source"; 2013. p. 15-34.

[19] Figueira MA, Ribeiro JA. Separation of adenosine and its derivatives by thin-layer chromatography on silica gel. J Chromatogr A. 1985 Jan;325:317-22. DOI: 10.1016/s0021-9673(00)96036-9

[20] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem. 1951 Nov;193(1):265-75. DOI: 10.1016/s0021-9258(19)52451-6

[21] Erol A. Systemic DNA damage response and metabolic syndrome as a premalignant state. Curr Mol Med. 2010 Apr 1;10(3):321-34. DOI: 10.2174/156652410791065282

[22] Chianese R, Pierantoni R. Mitochondrial reactive oxygen species (ROS) production alters sperm quality. Antioxidants. 2021 Jan 11;10(1):92. DOI: 10.3390/antiox10010092

[23] Ray SD, Kamendulis LM, Gurule MW, Yorkin RD, Corcoran GB. Ca^{2+} antagonists inhibit DNA fragmentation and toxic cell death induced by acetaminophen. FASEB J. 1993 Mar;7(3):453-63. DOI: 10.1096/fasebj.7.5.8462787

[24] Cioce M, Pulito C, Strano S, Blandino G, Fazio VM. Metformin: metabolic rewiring faces tumor heterogeneity. Cells. 2020 Nov 9;9(11):2439. DOI: 10.3390/cells9112439

[25] Wang Y, Zhang W, Lv Q, Zhang J, Zhu D. The critical role of quercetin in autophagy and apoptosis in HeLa cells. Tumor Biol. 2015 Aug 11;37(1):925-9. DOI: 10.1007/s13277-015-3890-4

[26] Purwoko RY, Rosliana I, Sobariah S, Hermana N, Permatasari S, Wulandari D, et al. Apoptosis of adipose-derived stem cells induced by liposomal soybean phosphatidylcholine extract. Avicenna J Med Biotechnol, 2018 Jul-Sep;10(3):126-133.

[27] Hsu C-L, Yen G-C. Phenolic compounds: Evidence for inhibitory effects against obesity and their underlying molecular signaling mechanisms. Mol Nutr Food Res. 2008 Jan;52(1):53-61. DOI: 10.1002/mnfr.200700393

[28] Vargas AJ, Burd R. Hormesis and synergy: pathways and mechanisms of quercetin in cancer prevention and management. Nutr Rev. 2010 Jun 25;68(7):418-28. DOI: 10.1111/j.1753-4887.2010.00301.x

[29] Kuo PC, Liu HF, Chao JI. Survivin and p53 modulate quercetin-induced cell growth inhibition and apoptosis in human lung carcinoma cells. J Biol Chem. 2004 Dec;279(53):55875-85. DOI: 10.1074/jbc.m407985200

[30] Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. Nature. 1997 Sep;389(6648):300-5. DOI: 10.1038/38525

[31] Yoon KA, Nakamura Y, Arakawa H. Identification of ALDH4 as a p53-inducible gene and its protective role in cellular stresses. J Human Gen. 2004 Feb 25;49(3):134-40. DOI: 10.1007/s10038-003-0122-3

[32] Hussain SP, Amstad P, He P, Robles A, Lupold S, Kaneko I, et al. p53-Induced up-regulation of MnSOD and GPx but not catalase increases oxidative stress and apoptosis. Cancer Res. 2004 Apr 1;64(7):2350-6. DOI: 10.1158/0008-5472.can-2287-2

[33] O'Connor JC, Wallace DM, O'Brien CJ, Cotter TG. A novel antioxidant function for the tumor-suppressor gene p53 in the retinal ganglion cell. Invest Ophthalmo Vis Sci. 2008 May 16;49(10):4237-44. DOI: 10.1167/iovs.08-1963

[34] Ozaki T, Nakagawara A. p73, a sophisticated p53 family member in the cancer world. Cancer Science. 2005 Nov;96(11):729-37. DOI: 10.1111/j.1349-7006.2005.00116.x

[35] Lee DH, Szczepanski M, Lee YJ. Role of Bax in quercetin-induced apoptosis in human prostate cancer cells. Biochem Pharmacol. 2008 Jun;75(12):2345-55. DOI: 10.1016/j.bcp.2008.03.013

[36] Truong-Tran AQ, Ho LH, Chai F, Zalewski PD. Cellular zinc fluxes and the regulation of apoptosis/gene-directed cell death. J Nutrition. 2000 May;130(5):1459S-1466S. DOI: 10.1093/jn/130.5.1459s

[37] Xiao Z, Locasale JW. Epigenomic links from metabolism – methionine and chromatin architecture. Curr Opin Chem Biol. 2021 Aug;63:11-8. DOI: 10.1016/j.cbpa.2021.01.011

[38] Gosangi M, Ravula V, Rapaka H, Patri SV. α-Tocopherol-anchored gemini lipids with delocalizable cationic head groups: the effect of spacer length on DNA compaction and transfection properties. Org Biomol Chem. 2021;19(20):4565-76. DOI: 10.1039/d1ob00475a

[39] Singh DK, Ahn B, Bohr VA. Roles of RECQ helicases in recombination based DNA repair, genomic stability and aging. Biogerontology. 2008 Dec 15;10(3):235-52. DOI: 10.1007/s10522-008-9205-z

[40] Håkansson A, Zhivotovsky B, Orrenius S, Sabharwal H, Svanborg C. Apoptosis induced by a human milk protein. Proc Natl Acad Sci U S A. 1995 Aug 15;92(17):8064-8. DOI: 10.1073/pnas.92.17.8064

[41] Jia YF, Feng Q, Ge ZY, Guo Y, Zhou F, Zhang KS, et al. Obesity impairs male fertility through long-term effects on spermatogenesis. BMC Urol. 2018 May 16;18(1). DOI: 10.1186/s12894-018-0360-5

[42] Rato L, Alves MG, Dias TR, Lopes G, Cavaco JE, Socorro S, et al. High-energy diets may induce a pre-diabetic state altering testicular glycolytic metabolic profile and male reproductive parameters. Andrology. 2013 Mar 14;1(3):495-504. DOI: 10.1111/j.2047-2927.2013.00071.x

[43] Gansukh E, Nile A, Kim DH, Oh JW, Nile SH. New insights into antiviral and cytotoxic potential of quercetin and its derivatives – A biochemical perspective. Food Chem. 2021 Jan;334:127508. DOI: 10.1016/j.foodchem.2020.127508

[44] Song X, Wang Y, Gao L. Mechanism of antioxidant properties of quercetin and quercetin-DNA complex. J Mol Model. 2020 May 12;26(6). DOI: 10.1007/s00894-020-04356-x

[45] Lin ES, Luo RH, Huang CY. A complexed crystal structure of a single-stranded DNA-binding protein with quercetin and the structural basis of flavonol inhibition specificity. Int J Mol Sci. 2022 Jan 6;23(2):588. DOI: 10.3390/ijms23020588

[46] Arroyo MN, Green JA, Cnop M, Igoillo-Esteve M. tRNA biology in the pathogenesis of diabetes: Role of genetic and environmental factors. Int J Mol Sci. 2021 Jan 6;22(2):496. DOI: 10.3390/ijms22020496

[47] Bai X, Yao L, Ma X, Xu X. Small molecules as SIRT modulators. Mini Rev Med Chem. 2018 Jul 9;18(13):1151-7. DOI: 10.2174/1389557516666160620095103

[48] Ebert T, Tran N, Schurgers L, Stenvinkel P, Shiels PG. Ageing − Oxidative stress, PTMs and disease. Mol Aspects Med. 2022 Aug;86:101099. DOI: 10.1016/j.mam.2022.101099

[49] Yang M, Dart C, Kamishima T, Quayle JM. Hypoxia and metabolic inhibitors alter the intracellular ATP:ADP ratio and membrane potential in human coronary artery smooth muscle cells. PeerJ. 2020 Nov 10;8:e10344. DOI: 10.7717/peerj.10344

[50] Kutryb-Zajac B, Mierzejewska P, Slominska EM, Smolenski RT. Therapeutic perspectives of adenosine deaminase inhibition in cardiovascular diseases. Molecules. 2020 Oct 12;25(20):4652. DOI: 10.3390/molecules25204652

[51] Shpakov AO. Improvement effect of metformin on female and male reproduction in endocrine pathologies and its mechanisms. Pharmaceuticals. 2021 Jan 8;14(1):42. DOI: 10.3390/ph14010042

[52] Slovacek RE, Hind G. Correlation between photosynthesis and the transthylakoid proton gradient. Biochim Biophys Acta. 1981 Apr;635(2):393-404. DOI: 10.1016/0005-2728(81)90037-2

[53] Peterson CT, Rodionov DA, Osterman AL, Peterson SN. B vitamins and their role in immune regulation and cancer. Nutrients. 2020 Nov 4;12(11):3380. DOI: 10.3390/nu12113380

[54] Roth HP, Kirchgeßner M. Effect of zinc deficiency on 3',5'-cyclic-AMP content and parameters of energy metabolism in the rat. Z Ernahrungswiss. 1983 Jun;22(2):116-23. DOI: 10.1007/bf02026208

[55] Moosavi SMS, Ashtiyani SC, Hosseinkhani S, Shirazi M. Comparison of the effects of l-carnitine and α-tocopherol on acute ureteral obstruction-induced renal oxidative imbalance and altered energy metabolism in rats. Urol Res. 2009 Nov 26;38(3):187-94. DOI: 10.1007/s00240-009-0238-9

[56] Cruz AM, Beall C. Extracellular ATP increases glucose metabolism in skeletal muscle cells in a P2 receptor dependent manner but does not contribute to palmitate-induced insulin resistance. Front Physiol. 2020 Sep 25;11. DOI: 10.3389/fphys.2020.567378

Л.Б. Бондаренко[1], Г.М. Шаяхметова[1], О.Є. Ткаченко[1], М.М. Калачинська[2], В.М. Коваленко[1]

[1]ДУ “Інститут фармакології та токсикології НАМН України”, Київ, Україна
[2]Відкритий міжнародний університет розвитку людини “Україна”, Київ, Україна

ВПЛИВ МЕТФОРМІНУ ТА ПРЕПАРАТІВ ІЗ ПЛЕЙОТРОПНОЮ ДІЄЮ НА БІОХІМІЧНІ ПОКАЗНИКИ СІМ’ЯНИКІВ ЩУРІВ З МЕТАБОЛІЧНИМ СИНДРОМОМ, ЩО РОЗВИНУВСЯ У ЮВЕНІЛЬНОМУ ВІЦІ

Проблематика. Метаболічний синдром (МС) є комплексом порушень, що характеризуються абдомінальним ожирінням, інсулінорезистентністю та толерантністю до глюкози, артеріальною гіпертензією та всіма видами метаболічних порушень. З урахуванням широкого спектра симптомів, що супроводжують МС, застосування препаратів із плейотропною дією на метаболічні процеси в організмі може бути перспективним для його лікування.
Мета. Метою дослідження є порівняльна оцінка впливу метформіну або його комбінацій із вітамінним комплексом чи ліпосомальним препаратом на вміст ДНК, РНК, гістонів, АТФ, АДФ, АМФ, процеси фрагментації ДНК у сім‘яниках щурів із МС, індукованим у ювенільному віці.
Методика реалізації. Модель МС індукували повною заміною питної води 10 %-вим розчином фруктози у щурів самців лінії Вістар у віці 21–23 днів (маса тіла 50–70 г). Визначення вмісту ДНК, РНК, гістонів, АТФ, АДФ, АМФ і дослідження процесів фрагментації ДНК проводили через 60 днів моделювання МС і введення метформіну або його поєднання з вітамінним комплексом чи ліпосомальним препаратом.
Результати. В експериментах на статевозрілих щурах із МС і введенням метформіну або його поєднання з вітамінним комплексом чи ліпосомальним препаратом ми встановили наявність часткових коригувальних ефектів цих препаратів на зміни вмісту ДНК, РНК, гістонів, АТФ, АДФ, АМФ, процесів фрагментації ДНК, спричинені розвитком МС.
Висновки. Порівняльний аналіз ефектів досліджуваних препаратів за умов моделювання МС в ювенільному віці показав, що жоден із цих препаратів не зміг повністю нормалізувати порушення досліджуваних показників, викликані МС. Однак обидві комбінації метформіну з вітамінним комплексом чи ліпосомальним препаратом були ефективнішими в корекції цих негативних змін, ніж сам метформін. Метформін із вітамінним комплексом чинив більш виражений вплив на процеси фрагментації ДНК, рівні аденілнуклеотидів та енергетичний заряд клітин сім’яників щурів, тоді як коригувальна дія метформіну з ліпосомальним препаратом була більш помітною щодо вмісту компонентів хроматину.

Ключові слова: метаболічний синдром; метформін; вітамінний комплекс; ліпосомальний препарат; сім'яники; ювенільний вік.