

# The effect of diabetes mellitus under tumor growth on respiratory function and free radical processes in heart cell mitochondria in rats

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## Abstract

The aim is to study the effect of comorbid pathology, namely, diabetes mellitus, on free radical oxidation in the mitochondria in the heart cells in female rats with experimental Guerin carcinoma.

## Materials and methods

The study has been performed in female outbred rats (n=32) weighed 180-220 g. The animals have been randomly assigned to the following experimental groups: intact group (n=8), control group with diabetes (n=8), comparison group (n=8) to include rats with standard subcutaneous transplantation of Guerin's carcinoma, and the main group (n=8) to cover rats which were first reproduced with diabetes and which after 1 week of persistent hyperglycemia were transplanted with Guerin's carcinoma. In the samples of the heart mitochondria, the following concentrations were measured by the ELISA method: cytochrome C (ng/mg protein) (Bioscience, Austria), 8-hydroxy-2'-deoxyguanosine (8-OHdG) (ng/mg protein); malondialdehyde (MDA) (mmol/mg protein); the Statistica 10.0 software was used.

## Results

In diabetes mellitus (control group), an increase in 8-OHdG by 6.3 times, MDA by 1.9 times (p<0.05), and a decrease in cytochrome C by 1.5 times (p<0.05) were found in the mitochondria in the heart cells compared to the values in the intact group. The effect of Guerin's carcinoma on the mitochondria of heart cells was expressed only as a change in the MDA level, which exceeded the intact level by 1.8 times (p<0.05). The combination of two pathological processes in the animal's body, namely,

diabetes mellitus and the malignant tumor, caused an increase in the level of 8-OHdG by 14 times, MDA by 1.7 times (p<0.05) and a decrease in cytochrome C by 1.46 times (p<0.05).

## Conclusions

It has been found that the destabilization of the respiratory chain in mitochondria in female rats' heart cells occurs as a result of the activation of free radical oxidation processes under the influence of diabetes mellitus, both in its independent variant and linked with the tumor process.

## Keywords

Mitochondria, Heart, Free radical oxidation, Antioxidant defense, Diabetes, Guerin's carcinoma, Female rats

## Imprint

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## Introduction

Diabetes mellitus (DM) is one of the world's biggest health problems in the 21st century. DM is also considered a major risk factor for cardiovascular disease, which is the most common cause of death among adults with DM [1]. In addition to the well-known microvascular complications triggered by DM, such as nephropathy and retinopathy, there is an increasing epidemic of macrovascular complications, including diseases of the coronary arteries, peripheral arteries and carotid vessels [2]. The diagnostics of such macrovascular complications becomes more precise and accessible owing to the development of a new fundamental science: CARDIOMETRY, created on the basis of discovery of the hemodynamics mechanisms involved therein [3]. A significant breakthrough has been made in the study of the pathogenetic mechanisms of combined pathologies, which allow us to evaluate multisystem complications and their correction in tumor growth models [4,5,6,7].

Pathophysiological changes in DM, including a disorder in the energy metabolism regulation (glucose, amino acids, and fatty acids), insulin resistance, oxidative stress, and inflammation lead to various complications, which contribute to damage to target organs, such as the heart, the kidneys, the liver, the eyes and others [8]. In particular, DM is most involved in the pathogenesis of various cardiovascular diseases, including chronic conditions such as atherosclerosis [9] and congestive heart failure [10] as well as acute processes: myocardial infarction and unstable angina [11]. Moreover, cardiovascular diseases, which include peripheral vascular diseases, coronary heart disease, and cerebrovascular diseases, make a significant contribution to the mortality of people suffering from diabetes. At the same time, a unique event is diabetic cardiomyopathy, which itself causes functional and structural aberrations of the heart, when there are no other concomitant diseases, such as IHD, dyslipidemia and hypertension [12]. Cardiovascular diseases in diabetes and diabetes itself contribute to the deterioration of the state of the body as a whole, so that they can be considered as factors responsible for high mortality risks. The pathogenesis of the diabetic complications involves many mechanisms; however, oxidative stress mediated by reactive oxygen species (ROS) is an important key component [13]. Under the normal conditions, ROS are necessary for maintaining homeostatic cellular signal communication, as well as for triggering antioxidant reactions in response to stress [13,14]. When the ROS levels become excessive, as in DM, there are dangerous consequences for the organism [13]. In DM, elevated and stable levels of ROS make a direct effect on the integrity of mitochondria and the ability of cellular signal transmission [13]. In the heart of a diabetic patient, the production of toxic products of lipid peroxidation can disorder the function of the myocardium and contribute to the progression both of acute and chronic heart diseases. DM is known to be associated with a malignant process, and patients with malignant tumors often suffer from diabetes [15]. Possible biological links between DM and malignancy include hyperinsulinemia, hyperglycemia, and chronic inflammation induced by obesity. Although the strongest association between these pathologies is found in the pancreas and the liver, there are many other organs involved in carcinogenesis in patients with DM, including the mammary gland, the endometrium, the bladder, and the kidneys.

In connection with the above, it is relevant to study the mitochondrial dysfunction of cardiomyocytes both in diabetes mellitus and in the malignant process combined with diabetes mellitus.

### Aims

The aim is to study the effect of comorbid pathology, namely, diabetes mellitus, on free radical oxidation in the mitochondria in the heart cells in female rats with experimental Guerin carcinoma.

### Materials and methods

The research has been carried out using female outbred rats (n=32) weighed 180-220 g. The animals have been delivered to us by the Federal State Medical & Biological Institution "Research Center of Biomedical Technologies" (Branch Andreevka, Moscow Region). The animals were kept under natural lighting conditions with no restrictions on their access to water and food. The research in animals was conducted in accordance with the Directive 86/609/EEC on the Protection of Animals Used for Experimental and Other Scientific Purpose, as well as in accordance with the International Guiding Principles for Biomedical Research Involving Animals and Order No. 267 "Approval of the Rules of Laboratory Practice" dated June, 19, 2003 issued by the Ministry of Health of the Russian Federation. The research record was approved by the Commission on Bioethics at the Federal State Budgetary Institution "National Medical Research Center of Oncology", the Ministry of Health of the Russian Federation (Record No. 21/99 dated September, 1, 2020). Manipulations with animals were performed in the box in compliance with the generally accepted rules of asepsis and antisepsis.

We used the strain of Guerin's carcinoma supplied by the Russian National Medical Research Center of Oncology named after N.N.Blokhin, Ministry of Health, Russia. The material for transplantation was obtained from donor rats on day 12-16 of the tumor growth. The Guerin's carcinoma transplantation was performed by a standard subcutaneous injection of the tumor suspension under the right scapula in a volume of 0.5 ml of the cell suspension in a 1:10 dilution with saline solution. To reproduce experimental diabetes, the animals were once intraperitoneally injected with Alloxan at a dosage of 150 mg/kg of weight. Then, during a week, their blood glucose was measured. A high blood glucose level, in the range of 15-30 nmol

/ L, indicated the development of diabetes mellitus. Tools, dishes, and hands were disinfected in a generally accepted way in compliance with the generally accepted rules of asepsis and antisepsis.

The animals have been randomly assigned to the following experimental groups: the intact group (n = 8), the control group with diabetes (n=8), the comparison group (n=8) to include rats with standard subcutaneous transplantation of the Guerin's carcinoma, the main group (n=8) to cover rats which were first reproduced with diabetes and which after 1 week of persistent hyperglycemia were transplanted with Guerin's carcinoma in a volume of 0.5 ml of tumor cell suspension in a 1:5 dilution with saline solution. At the time of transplantation of the Guerin's carcinoma in the animals of the main group (n=8), the average blood glucose values were  $25.4 \pm 1.2$  mmol/l, whereas in the group of the intact animals (n=8) the values were recorded to be  $5.2 \pm 0.3$  mmol/l.

Decapitation of the animals was performed with the guillotine 14 days after transplantation of the Guerin's carcinoma or the reproduction of experimental diabetes. After decapitation the animal hearts have been quickly extracted with the use of refrigerants, and mitochondria were isolated by the method of Egorova M. V., Afanasiev S. A. [16] (using refrigerants and differential centrifugation on a high-speed refrigerated centrifuge Avanti J-E, BECMAN COULTER, USA). The tissues were washed with an icy 0.9% KCl solution. To destroy the intercellular bonds, the cell wall and plasma membranes, we used mechanical treatment of tissues with grinding with scissors and homogenization in a glass homogenizer with a Teflon pestle (the Potter-Elvehjem homogenizer). We added per gram of tissue, 10 ml of the isolation medium (0.22 M mannitol, 0.3 M sucrose, 1 mm EDTA, 2 mM TRIS-HCL, 10 mm HEPES, pH 7.4). The tissues were homogenized and centrifuged for the first time for 10 minutes at a speed of 1000 g, at a temperature of 0 - 2°C, the second and third centrifugation was carried out at 20000 g, for 20 minutes, at a temperature of 0 - 2°C. Between the centrifugation procedures, the mitochondrial sediment was resuspended in the isolation medium. Further the mitochondria were purified from lysosomes, peroxisomes, melanosomes, etc., with the use of a 23% Percoll gradient. The suspension of subcellular structures was layered on the Percoll gradient, centrifuged for 15 min at 21000 g, after which the separation into 3 phases was observed; the

lower layer of mitochondria was left and resuspended with the isolation medium. The next washing of the mitochondria was performed by centrifugation for 10 minutes at 15000 g, at a temperature of 0 - 2°C. The mitochondrial samples (protein concentration 4-6 g/l) were stored at -80°C in the isolation medium before their analysis. By the ELISA method determined were the following concentrations: cytochrome C (ng/mg protein) (Bioscience, Austria), 8-hydroxy-2' - deoxyguanosine (8-OHdG) (ng/mg protein) (Enzo Life Sciences, Switzerland), malonic dialdehyde (MDA) (nM/g protein) (BlueGene Biotech, China); protein (mg/ml) was estimated by the Biuret method (Olvex Diagnosticum, Russia) with the ChemWell automatic analyzer (Awareness Technology INC, USA).

The Statistica 10.0 software was applied for statistical analysis of the obtained data. The data were analyzed for the compliance of the features distribution with the normal distribution law using the Shapiro-Wilk test (for small samples). The comparison of the quantitative data in the groups (independent sampling) was carried out using the Kruskal-Wallis test (multiple comparisons). The table data are presented in the  $M \pm m$  form, where M is the arithmetic mean, and m is the standard error of the mean;  $p < 0.05$  was taken as the level of statistical significance. The obtained results were statistically processed in compliance with the applicable general recommendations for medical research.

## Results

In the course of the experiment, first of all, it was necessary to determine the effect made by diabetes mellitus (control group) on the mitochondria in the heart cells. Thus, as compared to the intact animal values, an increase in 8-OHdG by 6.3 times, MDA by 1.9 times ( $p < 0.05$ ) and a decrease in cytochrome C by 1.5 times ( $p < 0.05$ ) were detected (see Table 1 herein).

At the next stage of the experiment, the effect of Guerin's carcinoma on the mitochondria of the heart cells was studied, and changes were recorded only in the MDA level, which was 1.8 times ( $p < 0.05$ ) higher than the recorded intact level.

The combination of two pathological processes in the animal body, namely, diabetes mellitus and the malignant tumor, caused the following changes in the studied parameters: the level of 8-OHdG increased by 14 times, MDA by 1.7 times ( $p < 0.05$ ), and cytochrome C decreased by 1.46 times ( $p < 0.05$ ), respec-

Table 1

Changes in the parameters of free radical oxidation and respiration of mitochondria in heart cells in female rats with diabetes mellitus and Guerin's carcinoma

	8-OHdG (ng/mg protein)	MDA (nM/g protein)	Cytochrome C (ng/mg protein)
Intact group ( n=8 )	0.927±0.048	14.912±1.110	1.382±0.137
Control group - diabetes (n=8)	5.890±0.5291 p1=0.0000	27.866±0.8131 p1=0.0000	0.900±0.0481 p1=0.0053
Comparison group - Guerin's carcinoma (n=8)	0.748±0.058	16.992±0.599	1.215±0.101
Main group – diabetes+Guerin's carcinoma (n=8)	13.050±0.9421,2,3 p1=0.0000 p2=0.0000 p3=0.0000	26.092±0.6421,3 p1=0.0000 p3=0.0000	0.949±0.0411 p1=0.0093

Notes: p<sup>1</sup> statistically significant differences compared to the values in the intact group; p<sup>2</sup> statistically significant differences compared to the values in the control group; p<sup>3</sup> statistically significant differences relative to the values in the comparison group.

tively. Statistically significant differences between the groups of animals were determined only by 8-OHdG: the indicator, when compared between the control group and the main group, was 2.2 times higher in the main group, and, when compared with the values in the comparison group, was recorded to be 17.4 times higher in the main group. The MDA level in the main group exceeded the values in the comparison group by 1.53 times (p<0.05).

Thus, the most pronounced changes in the level of the studied parameters (8-OHdG, MDA, cytochrome C) in the heart cells mitochondria of female rats were revealed in the group of animals, where the malignant process developed against the background of diabetes mellitus as comorbid pathology.

## Discussion

At present, due to its mutagenicity, 8-OHdG is the most studied product from nucleic acid oxidation. An accumulation of 8-OHdG is considered to be a sensitive marker of oxidative stress to DNA [17]. In addition, 8-OHdG is also treated as a risk factor for diabetes, correlating with the severity of vascular complications, suggesting the presence of oxidative stress involved in the pathogenesis of vascular complications caused by diabetes [18]. In animal experiments, hyperglycemia has been shown to increase the production of 8-OHdG and suppress antioxidant enzymes like MnSOD [18].

In the offered study, the data obtained on the content of 8-OHdG in the mitochondria of the heart cells in diabetes correlate with the reference literature data [18], and indeed, the accumulation of 8-OHdG has been revealed in diabetes.

The accumulation of 8-OHdG in nuclear DNA and mtDNA is a consequence of the accumulation process and the overproduction of ROS caused by a chronic

disease such as diabetes [19]. Regarding the presented study, an increased content of the secondary product of lipid peroxidation (LPO), MDA, has been found in all experimental groups that is consistent with the concept of the above research work. MDA is considered as the most mutagenic product of lipid peroxidation [20]. MDA can directly affect DNA [21], and there is probably a relationship between 8-OHdG and MDA, and it is also suggested that the formation of 8-OHdG may be associated with lipid peroxidation [22].

We believe that the detected changes in MDA and 8-OHdG in this experiment indicate the ability of MDA to damage DNA through an increase in the 8-OHdG concentration as a biomarker of oxidative stress.

During the experiment, the investigations were conducted focused not only on the influence of diabetes and the malignant process on the mitochondria in the heart cells, but also on the tumor process linked with diabetes mellitus. As a result, changes in the same direction in the content of MDA and 8-OHdG in the mitochondria in the heart cells were recorded in diabetes and combination of comorbid pathology with the tumor growth. At the same time, the effect of the malignant process in its independent version led only to an increase in the level of MDA. Perhaps it is diabetes mellitus, i.e. hyperglycemia, that plays a leading role in triggering the cascade of reactions that lead to such an excessive production of reactive oxygen species and, as a result, damage to mtDNA.

It is known that hyperglycemia can induce some morphological changes in mitochondria [23], the hyperproduction of reactive oxygen species (ROS) [24] and the release of cytochrome C [25]. Cytochrome C is an important mitochondrial enzyme involved in the respiratory chain. Oxidative stress mediated by ROS increases the release of cytochrome C for cell apopto-

sis [26] Under the action by ROS, the permeability of the mitochondrial pores increases, and cytochrome C is released into the cytoplasm from the inner space of the mitochondrial membrane, and Bax is translocated from the cytosol to the mitochondria that leads to cell apoptosis. Thus, ROS can indirectly induce apoptosis. In addition to the above, it is known that 8-OHdG affects mtDNA causing damage, then, damaged mtDNA is able to disrupt the transcription of most proteins of the respiratory chain, resulting in a vicious cycle of the ROS action [28].

In our study, we revealed a decrease in cytochrome C with an increase in MDA and 8-OHdG in the mitochondria of the heart in groups of animals with diabetes and a combination of diabetes with the tumor growth. We believe that diabetes plays a decisive role in destabilizing the mitochondrial respiratory chain and triggering apoptosis due to an indirect effect made by ROS on the proteins in the mitochondrial respiratory chain.

## Conclusions

As a result of our experiment to study the effect made by diabetes mellitus in its independent variant and linked with the tumor process on the function of mitochondria in the heart cells in female rats, it has been found that the destabilization in the respiratory chain is mediated by free radical oxidation processes. Based on the results obtained, we can conclude that cardiovascular complications, both in diabetes mellitus and in the malignant process against the background of diabetes, are associated with the dysfunction of the heart cells mitochondria through the activation of the lipid peroxidation processes that causes local damage to mtDNA.

## Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

## Conflict of interest

None declared.

## Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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