South Russian Journal of Cancer 2021, v.2, №3, p. 13-22
https://doi.org/10.37748/2686-9039-2021-2-3-2
ORIGINAL ARTICLE

FUNCTIONAL STATE OF CARDIOMYOCYTE MITOCHONDRIA IN MALIGNANT PROCESS IN PRESENCE OF COMORBID PATHOLOGY IN EXPERIMENT

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ABSTRACT

Purpose of the study. An analysis of indices of free radical oxidation and respiration of mitochondria of heart cells in a malignant process in presence of diabetes mellitus and chronic neurogenic pain in experimental animals.
Materials and methods. The study included outbred female rats (*n*=32) and C57BL/6 female mice (*n*=84). Experimental groups of rats were: intact group 1 (*n*=8), control group 1 (*n*=8) with diabetes mellitus (DM), comparison group 1 (*n*=8) with standard subcutaneous transplantation of Guerin's carcinoma, main group 1 (*n*=8) with Guerin's carcinoma transplanted after 1 week of persistent hyperglycemia. Experimental groups of mice were: intact group 2 (*n*=21), control group 2 (*n*=21) with a model of chronic neurogenic pain (CNP), comparison group 2 (*n*=21) with standard subcutaneous transplantation of melanoma (B16/F10), main group 2 (*n*=21) (CNP+B16/F10) with melanoma transplanted 3 weeks after the CNP model creation. Heart mitochondria were isolated by differential centrifugation. Levels of cytochrome C (ng/mg of protein), 8-hydroxy-2'-deoxyguanosine (8-OHdG) (ng/mg of protein), and malondialdehyde (MDA) (μmol/g of protein) were measured in mitochondrial samples by ELISA. Statistical analysis was performed using the Statistica 10.0 program.
Results. DM in rats upregulated 8-OHdG by 6.3 times and MDA by 1.9 times (p=0.0000) and downregulated cytochrome C by 1.5 times (p=0.0053) in heart cell mitochondria, compared to intact values. DM+Guerin's carcinoma in rats increased 8-OHdG by 14.0 times and MDA by 1.7 times (p=0.0000) and decreased cytochrome C by 1.5 times (p=0.0000), compared to intact values. CNP in mice did not affect the studied parameters in mitochondria of the heart. CNP+B16/F10 in mice increased 8-OHdG by 7.1 times and MDA by 1.6 times (p=0.0000) and decreased cytochrome C by 1.6 times (p=0.0008).
Conclusions. Comorbidity (diabetes mellitus, chronic neurogenic pain) together with malignant pathology aggravates mitochondrial dysfunction of heart cells with destabilization of the respiratory chain mediated by free radical oxidation processes.

Keywords:
mitochondria, heart, experimental animals, Guerin's carcinoma, B16/F10 melanoma, cytochrome C, 8-hydroxy-2'-deoxyguanosine, malondialdehyde.

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Information about funding: no funding of this work has been held.
Conflict of interest: authors report no conflict of interest.

For citation:
Frantsiyants E.M., Neskubina I.V., Cheryarina N.D., Surikova E.I., Shikhlyarova A.I., Bandovkina V.A., Nemashkalova L.A., Kaplieva I.V., Trepitaki L.K., Kachesova P.S., Kotieva I.M., Morozova M.I., Pogorelova Yu.A. Functional state of cardiomyocyte mitochondria in malignant process in presence of comorbid pathology in experiment. South Russian Journal of Cancer. 2021; 2(3): 13-22. https://doi.org/10.37748/2686-9039-2021-2-3-2

Received 08.06.2021, Review (1) 08.07.2021, Review (2) 28.07.2021, Published 09.09.2021

Южно-Российский онкологический журнал 2021, т.2, №3, с. 13-22
https://doi.org/10.37748/2686-9039-2021-2-3-2
ОРИГИНАЛЬНАЯ СТАТЬЯ

ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ МИТОХОНДРИЙ КАРДИОМИОЦИТОВ ПРИ ЗЛОКАЧЕСТВЕННОМ ПРОЦЕССЕ НА ФОНЕ КОМОРБИДНОЙ ПАТОЛОГИИ В ЭКСПЕРИМЕНТЕ

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РЕЗЮМЕ

Цель исследования. Изучение показателей свободнорадикального окисления и дыхания митохондрий клеток сердца при злокачественном процессе на фоне сахарного диабета и хронической нейрогенной боли у экспериментальных животных.

Материалы и методы.Работа выполнена на нелинейных крысах-самках (*n*=32) и мышах-самках линии C57BL/6 (*n*=84). Экспериментальные группы крыс: интактная 1 (*n*=8), контрольная группа 1 (*n*=8) с сахарным диабетом (СД), группа сравнения 1 (*n*=8) – стандартная подкожная перевивка карциномы Герена, основная группа 1 (*n*=8) – через 1 неделю стойкой гипергликемии перевивали карциному Герена. Экспериментальные группы мышей: интактная 2 (*n*=21), контрольная 2 (*n*=21) – воспроизведение модели хронической нейрогенной боли (ХНБ), группа сравнения 2 (*n*=21) – стандартная подкожная перевивка меланомы (B16/F10), основная группа 2 (*n*=21) (ХНБ+B16/F10) – меланому перевивали через 3 недели после создания модели ХНБ. Митохондрии сердца получали методом дифференциального центрифугирования. В образцах митохондрий методом ИФА определяли концентрацию: цитохрома С (нг/мг белка), 8-гидрокси-2'-дезоксигуанозина (8-OHdG) (нг/мг белка), малонового диальдегида (МДА) (мкмоль/г белка). Статистический анализ – Statistica 10.0.

Результаты. Наличие СД у крыс способствовало повышению 8-OHdG в 6,3 раза, МДА в 1,9 раза (*p*=0,0000) и снижению цитохрома С в 1,5 раза (*p*=0,0053) в митохондриях клеток сердца по сравнению с интактными значениями. СД+карцинома Герена у крыс вызывало повышение уровня 8-OHdG в 14,0 раз, МДА в 1,7 раза (*p*=0,0000) и снижение цитохрома С в 1,5 раза (*p*=0,0093) по сравнению с интактными значениями. Присутствие ХНБ у мышей не повлияло на уровень изучаемых показателей в митохондриях сердца. ХНБ+меланома B16/F10 у мышей приводило к повышению уровня 8-OHdG в 7,1 раза, МДА в 1,6 раза (*p*=0,0000) и снижению уровня цитохрома С в 1,6 раза (*p*=0,0008).

Заключение. Коморбидная патология (сахарный диабет, хроническая нейрогенная боль), сопряженная со злокачественным процессом, усугубляет дисфункцию митохондрий клеток сердца с дестабилизацией дыхательной цепи, опосредованной процессами свободнорадикального окисления.

Ключевые слова:
митохондрии, сердце, экспериментальные животные, карцинома Герена, меланома B16/F10, цитохром С, 8-гидрокси-2'-дезоксигуанозин, малоновый диальдегид.

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Информация о финансировании: финансирование данной работы не проводилось.
Конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

Для цитирования:
Франциянц Е.М., Нескубина И.В., Черярина Н.Д., Сурикова Е.И., Шихлярова А.И., Бандовкина В.А., Немашкалова Л.А., Каплиева И.В., Трепитаки Л.К., Качесова П.С., Котиева И.М., Морозова М.И., Погорелова Ю.А. Функциональное состояние митохондрий кардиомиоцитов при злокачественном процессе на фоне коморбидной патологии в эксперименте. Южно-Российский онкологический журнал. 2021; 2(3): 13-22. https://doi.org/10.37748/2686-9039-2021-2-3-2

Получено 08.06.2021, Рецензия (1) 08.07.2021, Рецензия (2) 28.07.2021, Опубликовано 09.09.2021

RELEVANCE

Currently, a new multidisciplinary direction is actively developing – cardiac oncology, based on a comprehensive personalized approach to the prevention and treatment of cardiovascular diseases (CVD) in cancer patients. Advances in oncology have led to an increase in their survival rate, which in turn has increased the number of patients who live to develop long-term cardiac complications [1]. In general, such complications are not uncommon and can occur in almost every cancer patient [2]. The reasons are not only the cardiotoxic effects of antitumor therapy, but also the fact that the oncological disease itself provokes the appearance of cardiovascular problems, especially in patients with prerequisites for this, as well as CVD can be associated with the decompensation of chronic comorbid pathology existing in an oncological patient. The addition of a cardiological disease reduces the quality of life, and sometimes makes it impossible to continue special treatment.

In addition to cardiological pathology, the malignant process is often accompanied by other comorbid diseases. The connection of diabetes mellitus with a malignant process is known, often diabetes is present in patients with malignant tumors [3]. Pain is quite often an accompanying component of the tumor process and is present in 30-50 % of cancer patients after antitumor therapy, in 65-90 % of patients due to the progression of the disease, 33 % of patients suffer from pain after the end of antitumor treatment [4]. The origin of pain in cancer patients is usually multifactorial: concomitant diseases, direct and indirect effects of tumor growth, side effects of antitumor therapy [4].

The heart is an organ that consumes a lot of energy, and its function largely depends on ATP (adenosine triphosphate) produced by mitochondria. It is well known that mitochondria ensure the functioning of various organs under normal and pathological conditions [5-7]. Mitochondrial dysfunction is one of the main causes of various heart diseases, which requires a close study and understanding of the mechanisms of mitochondrial remodeling in the heart [5, 8, 9]. The most well-known role of mitochondria is energy production, which is of paramount importance for organs with a high level of metabolism, such as the heart. Moreover, mitochondria mediate the fate of cells, including proliferation, differentiation and viability, and can also change the normal development of the body [10]. It is proved that the mitochondria of cardiomyocytes are the largest producers of reactive oxygen species (ROS), and hydroxyl radicals damage mitochondrial proteins, mitochondrial DNA (mtDNA) and membrane lipids. The latter, called lipid peroxidation, can disrupt the functions of mitochondria, including the oxidation of fatty acids (FA) [11] and the production of ATP, which in turn can potentially cause systolic dysfunction [12, 13]. Significant oxidative damage detected in mitochondria can thus contribute to their functional deficiency in the heart [5].

Diabetes mellitus occurs when glucose tolerance is impaired due to insulin resistance. Various factors interact in the pathogenesis and progression of diabetes mellitus [14]. Diabetic cardiomyopathy is the main cause of heart failure and death in patients with diabetes [15].

Chronic neuropathic pain is defined as "pain resulting from a direct lesion or disease of peripheral or central neurons that affects the somatosensory system" [16] and is associated with a violation of the structural and functional connections of the brain [17, 18]. At the moment, the question of the effect of pain syndrome on the cardiovascular system has not been fully studied, therefore it is an area of science that requires more careful research.

In connection with the above, it is very relevant to study the influence of comorbid diseases accompanying the malignant process and to identify dysfunctional parameters at the subcellular level in organs not affected by the malignant process.

The purpose of the study was to study the indicators of free radical oxidation and respiration of mitochondria of heart cells in a malignant process against the background of diabetes mellitus and chronic neurogenic pain in experimental animals.

MATERIALS AND METHODS

The work was performed on non-linear female rats (*n*=32) 180-220 g and female mice of the C57BL/6 line (*n*=84) 8 weeks of age with an initial weight of 21-22 g. The animals were obtained from FSBI NMRC Scientific Center for Biomedical Technolo-

gies "Andreevka" FMBA (Moscow region). The study used a cell line of mouse melanoma B16/F10 and a strain of Guerin's carcinoma. The tumor strains were obtained from the N. N. Blokhin National Research Center of Oncology. The work with animals was carried out in accordance with the rules of the "European Convention for the Protection of Animals Used in Experiments" (Directive 86/609/EEC) and the Helsinki Declaration, as well as in accordance with the "International Recommendations for conducting Biomedical research using Animals" and Order No. 267 of the Ministry of Health of the Russian Federation dated June 19, 2003 "On approval of the rules of laboratory practice". The animals were kept under natural lighting conditions with free access to water and food. The Commission on Bioethics of the National Medical Research Centre for Oncology of the Ministry of Health of Russia of 31.05.2018 approved the protocol of the study (protocol of the ethical committee No. 2) on working with mice of the C57BL/6 line and on working with nonlinear rats of 01.09.2020, protocol of the ethical committee No. 21/99. Manipulations with animals were performed in the box in compliance with the generally accepted rules of asepsis and antiseptics.

Female rats (*n*=32) were randomly assigned to the following experimental groups: intact group 1 (*n*=8), control group 1 with diabetes (*n*=8), comparison group 1 (*n*=8) – rats with standard subcutaneous transplantation of Guerin's carcinoma, main group 1 (*n*=8) – rats who were first reproduced diabetes mellitus (DM) (once, intraperitoneal alloxan was administered at a dose of 150 mg/kg of weight) and after 1 week Guerin's carcinoma was transplanted to persistent hyperglycemia with 0.5 ml of a suspension of tumor cells in saline solution at a dilution of 1:5. At the time of transplantation of Guerin's carcinoma in animals of the main group 1 (*n*=8), the average blood glucose values were 25.4±1.2 mmol/l, whereas in the group of intact animals 1 (*n*=8) 5.2±0.3 mmol/l. Decapitation of animals was performed on a guillotine 14 days after transplantation of Guerin's carcinoma and 21 days after reproduction of experimental DM.

Female mice of the C57BL/6 line (*n*=84) were randomly assigned to the following experimental groups: intact group 2 (*n*=21), control group 2 (*n*=21) – reproduction of a model of chronic neurogenic pain (CNP) [19]. Comparison group 2 (*n*=21) – mice with standard subcutaneous melanoma grafting (B16/F10), main group 2 (*n*=21) (CNP+B16/F10) – mice that had melanoma grafted 3 weeks after the creation of the CNP model.

Female mice of the main group 2 (CNP+B16/F10) underwent ligation of the sciatic nerves from 2 sides under xyl-zoletil anesthesia: xylazine (Xyl preparation) intramuscularly, at a dose of 0.05 ml/kg (according to the instructions), after 10 minutes Zoletil-50 was administered at a dose of 10 mg/100g. 3 weeks after the healing of the surgical wound, 0.5 ml of a suspension of melanoma B16/F10 tumor cells was injected subcutaneously under the right shoulder blade in a 1:10 saline solution. Animals from the comparison group were transplanted with melanoma B16/F10 subcutaneously in the same dose and volume as in the main group, but without reproducing the model of chronic pain. Decapitated mice on the guillotine. Animals from the main group 2 and the comparison group 2 were decapitated 14 days after transplantation of experimental melanoma B16/F10.

After decapitation, the heart was quickly extracted from the animals using refrigerants and mitochondria were isolated according to the method of Egorova MV, Afanasiev SA (2011) [20] using 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 connections, the cell wall and plasma membranes, mechanical processing of tissues with grinding with scissors and homogenization in a glass homogenizer with a Teflon pestle (Potter-Elvegame homogenizer) was used. For each gram of tissue, 10 ml of the isolation medium was added (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 min at a speed of 1000 g, temperature 0-2 °C, the second and third centrifugation is carried out at 20,000 g, 20 min, temperature 0-2 °C. Between centrifugation, the procedure of resuspending the mitochondrial sediment in the isolation medium was performed. The mitochondrial fraction was additionally purified from lysosomes, peroxisomes, melanosomes, etc., by centrifuging in a 23 % Percoll

gradient. The suspension of subcellular structures was layered on a Percoll gradient, centrifuged for 15 minutes at 21000 g, after which separation into 3 phases was observed, the lower layer of mitochondria was left and resuspended with an isolation medium. The next washing of the mitochondria was carried out by centrifugation for 10 minutes at 15,000 g, temperature 0-2 °C. The obtained mitochondrial samples (protein concentration 4-6 g/l) were stored at-80 °C in the isolation medium before analysis. The concentration of cytochrome C (ng/mg protein) (Bioscience, Austria), 8-hydroxy-2'-deoxyguanosine (8-OHdG) (ng/mg protein) (Enzo Life Sciences, Switzerland), malondialdehyde (MDA) (μmol/g protein) was determined in mitochondrial samples of all groups using test systems on an ELISA analyzer (Infinite F50 Tecan, Austria) (BlueGene Biotech, China); by the biochemical method-the amount of protein (mg/ml) – the biuret method (Olvex Diagnosticum, Russia) on the ChemWell automatic analyzer (Awareness Technology INC, USA).

Statistical analysis of the results was carried out using the Statistica 10.0 software package. The obtained data were analyzed for compliance of the distribution of features with the normal distribution law using the Shapiro-Wilk criterion (for small samples). Comparison of quantitative data in groups (independent samples) was carried out using the Kraskel-Wallis criterion (multiple comparisons). The data of the tables are presented in the form M±m, where M is the arithmetic mean, 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 general recommendations for medical research.

RESEARCH RESULTS AND DISCUSSION

When analyzing experimental data, obtained using the model of diabetes mellitus and the growth of Guerin's carcinoma, it was necessary to establish the effect of diabetes mellitus (control group 1) on the mitochondria of female rat heart cells. Thus, compared with the values in intact group 1, 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 (Table 1).

No significant changes were found in comparison group 1. The combination of two pathological processes in the animal's body – diabetes mellitus and a malignant tumor caused the following changes in the studied parameters: the level of 8-OHdG increased by 14.0 times, MDA by 1.7 times ($p<0.05$), and cytochrome C decreased by 1.5 times ($p<0.05$) compared to the values in intact group 1. Statistically significant differences between animal groups were determined only in the level of 8-OHdG, this indicator was 2.2 times higher in the main group when compared between the control 1 and the main 1 groups, and when compared with the values in the comparison group it was 17.4 times higher in the main 1. The level of MDA in the main group 1 exceeded the values in the comparison group 1 by 1.5 times ($p<0.05$).

Table 1. Changes in indicators of free radical oxidation and respiration of mitochondria of female rat heart cells in diabetes mellitus and Guerin's carcinoma

	8-OHdG ng/mg protein	MDA μmol/g protein	Cytochrome C ng/mg protein
Intact group 1 (n=8)	0.927±0.048	14.912±1.110	1.382±0.137
Control group 1 – diabetes mellitus (n=8)	5.890±0.529[1] p^1=0.0000	27.866±0.813[1] p^1=0.0000	0.900±0.048[1] p^1=0.0053
Comparison group 1-Guerin's carcinoma (n=8)	0.748±0.058	16.992±0.599	1.215±0.101
Main group 1-diabetes mellitus + Guerin's carcinoma (n=8)	13.050±0.942[1,2,3] p^1=0.0000 p^2=0.0000 p^3=0.0000	26.092±0.642[1,3] p^1=0.0000 p^3=0.0000	0.949±0.041[1] p^1=0.0093

Note: p^1 – statistically significant differences compared to the values in the intact group; p^2 – statistically significant differences compared to the values in the control group; p^3 – statistically significant differences in relation to the values in the comparison group.

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

The next section of the work was devoted to the study of the influence of another comorbid pathology-chronic neurogenic pain accompanying the growth of melanoma B16/F10 in female mice in the mitochondria of heart cells. First of all, it was necessary to assess the state of free radical processes and respiration of the mitochondria of the heart cells of female mice, under the influence of CNP, as a result, it was determined that there were no significant differences between all the studied parameters (8-OHdG, MDA, cytochrome C) with the values in intact group 2 (Table 2).

With the independent growth of melanoma B16/F10 in female mice, as in the case of the independent growth of Guerin's carcinoma in rats, no significant changes were found compared to the level in intact group 2.

The combination of two pathological processes (CNP+melanoma B16/F10) in the animal's body led to a change in all the studied biochemical parameters in the mitochondria of the heart of female mice. Thus, with the growth of melanoma against the background of chronic neurogenic pain, compared with the values in intact group 2, an increase in the level of 8-OHdG was recorded by 7.1 times, MDA by 1.6 times ($p<0.05$) and a decrease in the level of cytochrome C by 1.6 times ($p<0.05$). Compared with the values in control group 2, an increase in the level of 8-OHdG was determined by 6.6 times, the level of MDA – by 2.0 times, and the level of cytochrome C, on the contrary, decreased by 2.1 times. When comparing the results obtained between the main group 2 (CNP+melanoma B16/F10) and the comparison group 2 (melanoma B16/F10), it was determined that the level of peroxidation products – 8-OHdG and MDA exceeded the corresponding indicators for comparison group 2 by 7.7 times and 1.8 times ($p<0.05$), and cytochrome C was reduced by 1.3 times ($p<0.05$).

The totality of the presented changes in the indicators of 8-OHdG, MDA and cytochrome C in the case of standard melanoma growth and concomitant CNP in female mice characterizes different mitochondrial dysfunction depending on the presence of comorbid pathology – CNP. At the same time, more pronounced dysfunctional changes in the mitochondria of the heart cells of female mice were noted when a malignant process was superimposed on the comorbid pathology – CNP.

Among the most important biological markers of oxidative stress, purines can be distinguished: 8-OHdG or its oxidized form-8-oxo-7,8-dihydro-2' – deoxyguanosine (8-oxodG). Although all living cells develop a wide range of DNA repair mechanisms, their enzymatic repair system does not always lead to the complete removal of all DNA modifications. Therefore, incorrectly reconstructed DNA is a serious problem for cells, mainly due to changes in genetic information, as well as related mutagenesis and apoptosis of cells [21]. Modifications of 8-OHdG and 8-oxodG occur as a result of the interaction of hydroxyl or superoxide radicals and guanine G in the

Table 2. Indicators of free radical processes and respiration of mitochondria of heart cells in female mice with chronic neurogenic pain and the growth of melanoma B16/F10

	8-OHdG ng/mg protein	MDA μmol/g protein	Cytochrome C ng/mg protein
Intact group 2 (n=21)	1.525±0.078	3.728±0.189	4.611±0.57
Control group 2 –CNP (n=21)	1.63±0.082	2.909±0.254	6.18±0.59
Comparison group 2 – melanoma B16/F10 (n=21)	1.399±0.101	3.254±0.227	3.81±0.47
Main group 2 – CNP+melanoma B16/F10 (n=21)	10.785±0.387^{1,2,3} p^1=0.0000 p^2=0.0000 p^3=0.0000	6.003±0.216^{1,2,3} p^1=0.0000 p^2=0.0000 p^3=0.0000	2.934±0.47^{1,2,3} p^1=0.0008 p^2=0.0010 p^3=0.0024

Note: $p1$ – statistically significant differences compared to the values in the intact group; p^2 – statistically significant differences compared to the values in the control group; p^3 – statistically significant differences in relation to the values in the comparison group.

DNA chain. Free radicals attack the G chains of DNA or free 2' – deoxyguanosine, resulting in the formation of radical adducts. The separation of electrons forms 8-OHdG, which, as a result of a reaction known as keto-enol tautomerism, turns into the main oxidized product of 8-oxodG [21].

Numerous experimental data emphasize the direct connection between oxidative stress and diabetes by measuring biomarkers of oxidative stress both in patients with diabetes and in experimental animals with reproduced diabetes mellitus [15, 22, 23]. A hyperglycemic condition can lead to increased levels of markers of DNA damage caused by oxidative stress, such as 8-hydroxy-2' – deoxyguanosine (8-OHdG) and 8-oxo-7,8-dihydro-2' – deoxyguanosine; lipid peroxidation products, measured as substances reacting with thiobarbituric acid (TBARS); protein oxidation products, such as the level of nitrothyrosine and carbonyl, and also reduce the activity of antioxidant enzymes [15].

In the presented study on experimental animals, an increase in the processes of lipid peroxidation was confirmed by an increase in the oxidation products of 8-OHdG, MDA and a decrease in the component of the respiratory chain – cytochrome C in diabetes mellitus in the mitochondria of heart cells.

One of the end products of lipid peroxidation (LP) is MDA. The formation of MDA occurs as a result of free radical oxidation of polyunsaturated fatty acids of phospholipids of cell membranes by active oxygen forms [24]. At the sites of the addition of peroxide radicals, fatty acids are torn into fragments, on the edges of which there are aldehyde groups with high reactivity. If the gap occurred on both sides, an MDA is formed. Reacting with the SH-and CH3-groups of proteins, MDA suppresses the activity of enzymes: cytochrome oxidase, hydroxylase, etc. MDA is a highly toxic compound that causes polymerization of proteins, destruction of DNA, sulfhydryl antioxidants, modification of the lipid layer of cell membranes. As a result, there is a suppression of the generation of high-energy compounds by mitochondria, in particular, adenosine triphosphate, which is necessary to ensure the vital activity of cells, growth rates, and development of the whole organism. MDA is considered to be the most mutagenic product of lipid peroxidation [24].

We believe that the detected changes in MDA and 8-OHdG in a rodent experiment using a model of diabetes mellitus and chronic neurogenic pain combined with two strains of a malignant tumor (Guerin's carcinoma, melanoma B16/F10) may indicate the ability of MDA to damage DNA, through an increase in the biomarker of oxidative stress – 8-OHdG.

Cytochrome C is an "extremely multifunctional" protein [25]. It mediates the transfer of electrons in the respiratory chain and acts as a detoxifying agent to get rid of ROS. In addition, cytochrome C participates in cell apoptosis as a precursor of internal apoptosis mediated by mitochondria [25, 26]. As a protein of the mitochondrial peripheral membrane, cytochrome C acts between the inner and outer mitochondrial membrane of healthy cells, where it mediates electron transfer between complexes III and IV of the respiratory chain [25].

Within the framework of this experiment, it was shown that the level of cytochrome C in the mitochondria of the heart with the standard growth of Guerin's carcinoma and melanoma B16/F10 had no statistically significant changes, whereas with Guerin's carcinoma and the growth of melanoma against the background of comorbid pathology – diabetes mellitus and CNP, its decrease was noted.

In this work, using two models of comorbid pathology (diabetes mellitus, chronic neurogenic pain) and two strains of malignant tumor (Guerin's carcinoma, melanoma B16/F10) on different experimental animals (rats, mice), both general dysfunctional changes in the mitochondria of heart cells and fundamental differences were demonstrated. As a result, differences between the two comorbid pathologies were revealed, so in diabetes mellitus, an increase in free radical oxidation with suppression of mitochondrial respiration was revealed, and in chronic neurogenic pain, the mitochondria of heart cells were stable. It is worth noting that the independent development of the tumor process-Guerin's carcinoma and melanoma B16/F10 in the body of female rats and female mice did not affect the functional state of the mitochondria of heart cells. General dysfunctional disorders in the mitochondria of heart cells were recorded with a combination of comorbid pathologies and a malignant process, which resulted in an increase in all products of lipid peroxidation and suppression of cytochrome C.

Recently, mitochondria have attracted considerable attention from both academic circles and pharmacological concerns, since mitochondrial dysfunction is a sign of many diseases, including heart failure. In general, many studies confirm the leading role of impaired activity of the mitochondrial electron transport chain, oxidative stress, etc.in cardiomyocyte dysfunction and, as a result, in the establishment or progression of heart failure [23, 27]. Why do mitochondria play such a central role in heart failure? First of all, the mitochondria are the powerhouse of the cell. Mitochondria make up ~ 35 % of the volume of cardiomyocytes and form a long, dynamic and well-organized network that facilitates both physical and chemical interactions between mitochondria and other intracellular structures. Recent studies on cellular and molecular mechanisms involved in the pathophysiology of heart failure and in the field of cardioncology point to mitochondria as strategic and dynamic nodes that actually affect every biochemical process in heart cells [23]. A dysfunctional mitochondrial network of cardiomyocytes can quickly spread damage inside cardiomyocytes in heart failure [23]. Therefore, the identification of points or markers of the dysfunctional state of mitochondria can be considered as a promising scientific direction, the results of which can be used to develop various new therapeutic strategies, including small molecules and peptides aimed at various mitochondrial anomalies that can improve heart function.

CONCLUSION

Summarizing the results obtained for all the used models of comorbid pathology (diabetes mellitus, chronic neurogenic pain) and malignant process (Guerin's carcinoma, melanoma B16/F10) in rodents, it can be argued that comorbid pathology associated with a malignant process exacerbates the dysfunction of the mitochondria of heart cells with the destabilization of the respiratory chain mediated by the processes of free radical oxidation.

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