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Mechanisms of tumor-associated cardiac muscle damage under different variants of melanoma growth

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Abstract

In case of the presence of a malignant process in an organism, the heart is subjected to an additional loading due to a unique combination of the external factors that is determined by the tumor biology and potentially cardiotoxic treatment actions and effects. It should be mentioned that cardiomyocytes have the same pathways of responding to stress and metabolic strategies as it is the case with the tumor cells, and this suggests that metabolic changes in the course of the tumor progression make their impact on the non-malignant tissue.

Aim. The aim of our research work has been to study the level of the indices of the activity of free-radical processes and the hypoxia factor in cardiac mitochondria in mice under the different rates of the growth of B16 melanoma, growing either independently or against the background of chronic neurogenic pain.

Materials and methods. In our experiment, we have used mice of both sexes of strain C57BL/6 (n = 56) and strain C57BL/6-Plautm1.1Bug — ThisPlauGFDhu/GFDhu (with uPA gene-knockout) (n = 56). We have composed the following experimental groups: an intact animal group (♂ n = 7; ♀ n = 7); a reference group (♂ n = 7, ♀ n = 7) with reproduction of the model of chronic neurogenic pain (CNP); a comparison group (♂ n = 63, ♀ n = 63) with a standard subcutaneous inoculation of melanoma (B16/F10), upon 2 weeks of the tumor growth; the main test group (♂ n = 63, ♀ n = 63) (CNP+B16/F10) with melanoma transplanted 3weeks after reproduction of the CNP model, with a tumor growing time of 2 weeks. After decapitation of the animals, the heart was harvested and mitochondria were isolated using the differential centrifugation with a high-speed refrigerated centrifuge. In the prepared mitochondria specimens with the use of ELISA assays we have determined concentrations of SOD 2 (pg/mg protein), GPx-1 (ng/mg protein), MDA mcM/g protein); AOPP (mcM/g protein); 8-hydroxy 2′ deoxyguanosine (8-OHdG) (ng/mg protein); the amount of protein (mg/mL) has been determined with a biochemical assay method, namely with the Biuret assay (Olvex Diagnosticum, Russia).

The obtained statistics data have been processed with software Statistica 10.0.

Results. In the female mice of strain C57BL/6, when comparing the studied data in cardiac mitochondria between the groups with the independent growth of melanoma and that in combination with CNP, we have revealed the higher levels of MDA increased by a factor of 1,8 ($p < 0,05$), AOPP increased by a factor of 1,7 ($p < 0,05$), 8-OHdG increased by a factor of 7,7, SOD 2 2,8 times higher and SOD 2/ GPx increased by a factor of 19,6 under the melanoma growth against the background of CNP. In this case, we have recorded lower values of GPx 1, namely, decreased by a factor of 6,9 and lower values of HIF, namely, reduced by a factor of 2,0 in cardiac mitochondria in female mice with combined pathology. In male mice we have recorded that the levels of SOD 2, MDA, 8-OHdG and SOD 2/GPx 1 are 1,5 times ($p < 0,05$), 1,3 times ($p < 0,05$), 5,5 times and 1,7 times ($p < 0,05$) higher than those found in cardiac mitochondria in the males under the independent melanoma growth. In this case, we should state that the HIF level is higher in cardiac mitochondria in the comparison group: it has been increased by a factor of 2,0. In the mutant mice of both sexes no differences in the studied data in cardiac mitochondria between the main test group and the comparison group have been revealed.

Conclusion. The revealed imbalance between the reactive oxygen species (MDA, AOPP) and the hypoxia factor (HIF) leading to damage of mtDNA (increased 8-OHdG) in cardiac mitochondria determines the degree of damage of the cardiac muscle in the normal genotype mice, namely, in mice of strain C57BL/6 of both sexes in case of melanoma B16 growing against the background of chronic neurogenic pain. In the uPA gene-knockout mice of both sexes, CNP stimulates the growth of melanoma, but in this case no infarctions appear and the processes of free-radical oxidation are inhibited, which are stimulators of heart damages in the normal genotype mice.

Keywords

Mitochondria, Heart, Lipid peroxidation, Antioxidant system, Chronic neurogenic pain, Melanoma B16/F10, Mice

Imprint

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Topicality

An increasing number of oncological patients face their risks of the cardiovascular dysfunction that results in the appearance of a new area, namely, cardiooncology, or oncocardiology [1-3]. Higher survival rates in oncological patients lead to a greater risk of some other life-threatening conditions, in particular cardiovascular diseases (CVD) that is considered to be a second leading cause of mortality and the CVD incidence in cancer survivors [4]. It is believed that cancer survivors demonstrate a higher risk of cardiovascular diseases that is partially associated with cardiac dysfunction after chemotherapy, including targeted therapy, radiation therapy and immunotherapy [5-9]. However research studies on an influence made by the propagation of the malignant process, or the presence of comorbid pathology, on the state of the cardiovascular system in oncological patients are few in number.

Metabolic reprogramming is the distinctive feature both of the malignant tumors and the cardial adaptation. Cardiomyocytes in the heart are capable of adapting to different types of stress, among them tumor-growth-induced stress, at the expense of optimization of the use of nutrients and, as a consequence, acquiring metabolic adaptation [10,11]. In case of a malignant process the heart is subjected to an additional load due to a unique combination of the external factors, which is determined by the biology of the tumor and potentially cardiotoxic treatment actions and effects [12]. Cardiomyocytes have the same pathways of responding to stress and metabolic strategies as it is the case with the tumor cells, and this suggests that metabolic changes in the course of the tumor progression make their impact on the non-malignant tissue.

For the last of few years the survival rate in cancer patients has been considerably increasing. However cancer survivors may demonstrate more chance in a long-term period to die from cardiovascular diseases, which are viewed as secondary diseases with respect not only to potential toxicity of antitumor medication agents, but also to the biology of the malignant tumor. In this context, every effort of the fundamental and translational researches is of primary importance for the proper understanding of molecular mechanisms, which induce cardiovascular diseases in oncological patients, as well as for defining new therapeutic targets, which may successfully prevent and treat both diseases [12].

Koelwyn, G. J. et al. (2020) have demonstrated that breast cancer patients, at an early state of their disease, having some cardiovascular complications after they had been diagnosed with cancer, can be at increased risk of recurrence and cancer-specific death. The preclinical and clinical evidence data obtained by the researchers have shown that myocardial infarction induces alterations in systemic homeostasis, triggering cross-disease communication that accelerates breast cancer [13].

Previously, in our experiments on animals we have demonstrated that the melanoma growth, stimulated by comorbidity like chronic neurogenic pain (CNP), produces a pronounced mitochondrial dysfunction of cardiomyocytes and results in myocardial infarction in the vast majority of the animals. That has been accompanied by disordering in the organization of polyenzimatic systems of apoptosis and a high level of oxidative stress, inducing chemiluminescence of mitochondrial associates in the framework of the pathogenetic mechanism of the death of cardiomyocytes. The standard growth of B16/F10 melanoma and CNP in an independent variant in the female mice has been followed by a reduction in the respiration- and energy-related function of cardiac mitochondria, but it has not led to myocardial infarction in any case [14,15]. Another comorbidity, namely, diabetes mellitus, against the background of which an experimental malignant tumor has been progressed, has been also resulted in dysfunction of cardiac mitochondria via activation of the peroxidation damage of lipids that has caused local damage to mtDNA [16,17].

Chronic widespread pain (CWP) represents a special case of chronic primary pain, when and where

certain biological causes may be either available or not, that makes impossible to identify whether it is pain of muscular skeletal or neuropatic type [18]. The sensitivity of baroreceptors and heart rate variability are the most important factors for an assessment of the health status and the performance of the cardiovascular system and the autonomous nervous system in patients [19-21].

Karekar P. et al. (2021) in their experimental studies reported on the immediately cancer-induced cardiac dysfunction [22]. The above researchers have demonstrated an early impact made by the tumor on the cardiac tissues that induces the cardiac dysfunction, and they have pointed to the importance of treatment of possible cardiac problems, which may appear under some kinds of malignant tumors, in order to avoid converting them to an aggravation factor of severe complications associated with the cardiac dysfunction. They have also identified the dysregulation of different oncogenic pathways, which produce specific systemic effects on the heart, and the proper understanding of these latter at the molecular level for the purpose of development of a specific and an effective treatment. The scientists have thoroughly looked into the issues on the Yki pathways and evidenced that reactive oxygen species (ROS) are mediators of the systemic effects made on the cardiac muscle. Hypoxia cause damage to the mitochondrial electron transport chain (ETC) that results in an increase in the production of ROS, which stimulate oxidation of proteins and lipids in mitochondria [23, 24].

Matsushima et al. (2013) have demonstrated that HIF-1α may protect cardiac fibroblasts from apoptosis, and it represents a potential therapeutic target for heart remodeling after the hypoxic damage [25]. In addition, a number of investigations have confirmed that HIF-1α prevents from ischemia and exhibits a cardioprotective effect [26]. Some research studies show that mitochondria may regulate stability of HIF-1α and that ROS, produced by the mitochondrial ETC, may stabilize HIF-1α under the hypoxia conditions [27].

The aim of this research work has been to study the levels of indices of the activity of the free-radical processes and the hypoxia factor in cardiac mitochondria in mice under different rates of the B16 melanoma growth.

Materials and methods

In our experiment we have used 112 mice of both sexes. The experimental animals have been kept under natural light conditions with a free access to water and food. Our manipulations have been conducted in accordance with the relevant regulations stated by EU Directive 86/609 EEC, the Declaration of Helsinki (DoH), 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. Manipulations with animals were carried out in the box in compliance with the generally accepted rules of asepsis and antisepsis.

Group one was composed by mice of strain C57BL/6 (n = 56) of both sexes with an initial body mass of 21-22 g in females and 28-30 g in males. The experimental animals have been delivered to us by the Federal State Medical & Biological Institution "Research Center of Biomedical Technologies", Branch Andreevka, (Moscow Region), at the Federal Medical & Biological Agency. In our research work we used the murine melanoma cell line B16/F10 supplied by the Russian National Medical Research Center of Oncology named after N.N. Blokhin, Ministry of Health, Russia. Our study protocol has been 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. 2) on May, 31, 2018.

Group two included mice of strain C57BL/6-Plautm1.1Bug — ThisPlauGFDhu/GFDhu of both sexes with uPA gene-knockout (n = 56) with an initial body mass of 24-26 g in females and 31-33 g in males, delivered by the Laboratory Animal Breeding Facility "Pushchino" at the Branch of the Research Institute for Bio-Organic Chemistry named after Full RAS Members M.M.Shemyakin and Yu.A.Ovchinnikov (Pushchino, Moscow Region). Mutant animals may be used in investigations of chronic tissue inflammation, mechanisms of fibrinolysis, oncogenesis and vascularization in a tumor and a tissue. Our study protocol has been 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. 22/102) on 24.12.2020.

The animals of each gender were randomly distributed in separate experimental groups as follows: the group of intact mice (♂ n = 7; ♀ n = 7); the reference group (♂ n = 7, ♀ n = 7) to reproduce the model of chronic neurogenic pain (CNP) [28]; the comparison

group (♂ n = 63, ♀ n = 63) with melanoma upon subcutaneous inoculation of the tumor cells (B16/F10), the growth time of the tumor covered 2 weeks; the main test group (♂ n = 63, ♀ n = 63) (CNP+B16/F10) where the B16/F10 line melanoma was transplanted 3 weeks after the completion of the CNP model and where the growth time of the tumor covered 2 weeks.

The chronic neurogenic pain model was reproduced in the mice of the reference group (CNP) and in the mice of the main test group (CNP +B16/F10) by ligation of the sciatic nerves under xyl-zoletil anesthesia: used were xylazine (Xyl) intramuscularly at a dose of 0.05 ml/kg of body weight (according to the instructions), then, 10 minutes later, Zoletil-50 at a dose of 10 mg per 100 g of body weight. B16/F10 melanoma was transplanted into the main test group mice, 3 weeks after healing of the surgery wound, in a standard manner under the skin of the right scapula in the volume of 0.5 ml of tumor cell suspension in a 1:10 dilution with saline solution. As to the comparison group animals, the B16/F10 melanoma cells were transplanted into them subcutaneously at the same dose and in the same volume as it was the case with the main test group, but without reproducing the CNP model. Decapitation of the animals from the main test group and the reference group was performed with the use of the guillotine upon expiration of 2 weeks of the B16/F10 melanoma growth (log-phase growth of the tumor).

Upon the decapitation, the heart was harvested from each animal without delay, with the use of coolants, and mitochondria were separated by the method by Egorova M. V., Afanasiev S. A. [29] with the differential centrifugation employing high-performance refrigerated centrifuge Avanti J-E, BECMAN COULTER, USA. The tissues were washed with an ice-cold 0.9% KCl solution. To disrupt the intercellular junctions, the cell walls and the plasma membranes, mechanical treatment of tissues with grinding using scissors and homogenization in a glass homogenizer with a Teflon pestle (Potter-Elvehjem homogenizer) was utilized. Per 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 minutes at 1000 g at a temperature of 0-2 °C, the second and third centrifugation procedures were completed at 20000 g for 20 minutes at a temperature of 0-2°C. Between the centrifugation procedures, the mitochondria sediment was resuspended in the isolation medium. Mitochondria were further purified from lysosomes, peroxisomes, melanosomes, etc. using the Percoll 23% density gradient centrifugation. The suspension of the 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 mitochondria washing procedure was carried out by centrifugation for 10 minutes at 15000 g at a temperature of 0 - 2°C. The prepared mitochondrial samples (protein concentration 4-6 g/l) were stored at -80° C in the isolation medium before performing an assay. In the mitochondrial samples, using ELISA assay equipment (Infinite F50 Tecan, Austria), we have determined concentrations of SOD 2 (pg/mg protein) (Ab Frontier, South Korea), GPx-1 (ng/mg protein) (Ab Frontier, South Korea), MDA (mcM/g protein) (BlueGene Biotech, China); AOPP (mcM/g protein) (BlueGene Biotech, China); 8-hydroxy 2' deoxyguanosine (8-OHdG) (ng/mg protein) (Enzo Life Sciences, Switzerland); the amount of protein (mg/mL) has been determined with a biochemical assay method, namely with the Biuret assay (Olvex Diagnosticum, Russia) employing Automated Analyzer ChemWell (Awareness Technology INC, USA).

The obtained statistics data have been processed with software Statistica 10.0.

The obtained evidence data were analyzed for the compliance of the features distribution with the normal distribution law using the Shapiro-Wilk test. Significance of differences between independent samplings has been assessed with the Mann-Whitney U test for small sample size. The data in Tables given herein are presented in the M±m form, where M is the arithmetic mean, and m is the standard error of the mean; in addition, for the purpose of the better description of the groups, we applied median (Me) and interquartile range [Q1-25%; Q3-75%]. In this case, $p < 0.05$ has been taken as the level of statistical significance.

The obtained statistics data have been processed in compliance with general applicable medical research recommendations.

Results

Table 1 given herein contains data on concentration indices of free-radical oxidation processes and antioxidant protection in mitochondria in the cardiac

cells in female mice of strain C57BL/6 under the independent growth of B16/F10 melanoma and under the growth of the latter against the background of chronic neurogenic pain.

In female mice of strain C57BL/6, CNP has produced a number of alterations in cardiac mitochondria as compared with the respective values reported in the intact cardiac mitochondria, namely, as follows: the SOD 2 value has decreased by a factor of 2,9 in parallel with an increase in the level of GPx-1 and AOPP by a factor of 2,2 and 1,5, respectively ($p < 0,05$) (see Table 1 herein). The values of the calculated ratio SOD 2/GPx-1 under the chronic pain syndrome has been found to be lowered by a factor of 6,2. The independent growth of melanoma has promoted a reduction in the level of the antioxidant enzymes SOD 2 and GPx-1 by a factor of 1,9 ($p < 0,05$) and by a factor of 1,6 ($p < 0,05$), respectively, and a rise in the HIF level by a factor of 1,3 ($p < 0,05$). The combination of the two pathological processes has led to more pronounced changes in the levels of the analyzed biochemistry data in cardiac mitochondria in the female mice of strain C57BL/6. So, as against the values recorded in cardiac mitochondria under CNP, the level of GPx-1 has decreased by a factor of 24,4 and the HIF value has been recorded to be 1,5 times lower ($p < 0,05$), while the SOD 2 level has

Table 1

Data on concentrations of components in system LPO – AOP in cardiac mitochondria in female mice of strain C57BL/6 under the B16/F10 melanoma growth against the background of chronic neurogenic pain

	Intact group	Group with CNP (Reference)	B16/F10 melanoma growth (Comparison group) Week 2	CNP + B16/F10 melanoma growth (Main test group) Week 2
SOD 2 pg/mg protein	581,741±33,778 581,74 [481,970; 667,230]	196,685±16,889 196,680 [154,77; 241,360] $p^1 = 0,0000$	303,414±17,851 303,410 [257,310; 323,470] $p^1 = 0,0000$	854,76±19,793 853,76 [808,22; 908,39] $p^2 = 0,0000$ $p^3 = 0,0000$
GPx-1 ng/mg protein	0,501±0,035 0,501 [0,406; 0,585]	1,124±0,0646 1,124 [0,972; 1,267] $p^1 = 0,0000$	0,319±0,024 0,318 [0,257; 0,386] $p^1 = 0,0011$	0,046±0,003 0,046 [0,037; 0,057] $p^2 = 0,0000$ $p^3 = 0,0000$
MDA mcM/g protein	3,728±0,189 3,728 [3,221; 4,145]	2,908±0,254 3,144 [2,114; 3,361]	3,254±0,227 3,112 [2,748; 4,003]	6,003±0,216 6,003 [5,5110; 6,498] $p^2 = 0,0000$ $p^3 = 0,0000$
AOPP mcM/g protein	2,989±0,234 3,011 [2,412; 3,527]	4,642±0,372 4,64 [3,744; 5,745] $p^1 = 0,0027$	3,604±0,241 3,604 [3,011; 4,045]	6,042±0,218 6,042 [5,511; 6,585] $p^2 = 0,0070$ $p^3 = 0,0000$
HIF ng/mg protein	5,327±0,207 5,327 [4,878; 5,752]	5,306±0,218 5,188 [4,822; 5,852]	7,039±0,306 7,039 [6,381; 7,836] $p^1 = 0,0005$	3,484±0,277 3,484 [2,812; 4,145] $p^2 = 0,0002$ $p^3 = 0,0000$
8-OHdG ng/mg protein	1,525±0,078 1,525 [1,301; 1,708]	1,63±0,082 1,630 [1,426; 1,809]	1,399±0,101 1,399 [1,111; 1,654]	10,785±0,387 10,785 [9,836; 11,817] $p^2 = 0,0000$ $p^3 = 0,0000$
SOD 2/GPx-1	1,28±0,176 1,16 [0,794; 1,702]	0,205±0,040 0,180 [0,105; 0,295] $p^1 = 0,0000$	0,969±0,059 0,966 [0,858; 1,107]	19,00±0,135 19,16 [17,20; 21,73] $p^2 = 0,0000$ $p^3 = 0,0000$

Note: p^1 is statistically significant compared with the intact group values; p^2 is statistically significant compared with the reference group values; p^3 is statistically significant compared with the comparison group values. M±m; Me [Q1-25%; Q3-75%]; p indicates absolute values.

increased by a factor of 4,3; in this case the products of free-radical oxidation (FRO), namely, the concentrations of MDA, AOPP and 8-OHdG have become 2,1 times, 1,3 times ($p < 0,05$) and 6,6 times higher. The value of the SOD 2/GPx-1 ratio has become 92,6 times greater. When comparing the recorded changes in the analyzed data in cardiac mitochondria between the group with the independent growth of melanoma and the group with the melanoma growth combined with CNP, we have identified higher levels of MDA, AOPP, 8-OHdG, SOD 2 and SOD 2/GPx-1 in case of the melanoma growth against the CNP background. It has been found that the MDA level is 1,8 times higher ($p < 0,05$), the AOPP level is 1,7 times greater ($p < 0,05$), the 8-OHdG value is 7,7 times greater, the SOD 2 value is 2,8 higher and SOD 2/GPx-1 is 19,6 higher, respectively. In this case, we have recorded much lower values of GPx-1: they have decreased by a factor of 6,9, and lower HIF indices: they were found halved in cardiac mitochondria in the female mice with combined pathology (see Table 1 herein).

At the same, in the male mice of strain C57BL/6 in their cardiac mitochondria under all experimental pathological processes we have determined somewhat different changes of the studied data (see Table 2 herein). So, under the CNP action, in the male mice in mitochondria of the heart we have recorded an increase in the concentration of SOD 2, MDA and AOPP by a factor of 1,3 ($p < 0,05$), by a factor of 1,6 ($p < 0,05$) and by a factor of 5,5, respectively.

In case of the independent melanoma growth in males, in their cardiac mitochondria, the values of the

Table 2
Data on concentrations of components in system LPO – AOP in cardiac mitochondria in male mice of strain C57BL/6 under the B16/F10 melanoma growth against the background of chronic neurogenic pain

	Intact group	Group with CNP (Reference)	B16/F10 melanoma growth (Comparison group) Week 2	CNP + B16/F10 melanoma growth (Main test group) Week 2
SOD 2 pg/mg protein	473,131±33,028 473,130 [402,84; 550,41]	630,016±38,415 630,010 [537,14; 728,33] $p^1 = 0,0092$	436,114±27,554 436,110 [366,64; 505,33]	654,981±33,826 685,010 [558,42; 725,24] $p^3 = 0,0003$
GPx-1 ng/mg protein	0,486±0,025 0,286 [0,22; 0,815]	0,644±0,076 0,644 [0,422; 0,815]	0,507±0,028 0,307 [0,235; 0,388]	0,442±0,028 0,442 [0,385; 0,511] $p^2 = 0,0282$
MDA mcM/g protein	4,069±0,378 4,069 [3,034; 5,002]	6,453±0,308 6,453 [5,803; 7,233] $p^1 = 0,0003$	5,077±0,346 5,077 [4,255; 6,007]	6,650±0,234 6,752 [6,017; 7,033] $p^3 = 0,0026$
AOPP mcM/g protein	0,975±0,084 0,975 [0,788; 1,158]	5,406±0,362 5,406 [4,415; 6,358] $p^1 = 0,0000$	22,808±1,775 22,809 [18,257; 26,361] $p^1 = 0,0000$	22,808±1,774 22,810 [18,257; 26,361] $p^2 = 0,0000$
HIF ng/mg protein	5,567±0,308 5,753 [4,653; 6,162]	5,407±0,266 5,653 [4,656; 6,007]	6,446±0,308 6,400 [5,803; 7,233]	3,233±0,235 3,230 [2,745; 3,902] $p^2 = 0,0000$ $p^3 = 0,0000$
8-OHdG ng/mg protein	1,415±0,071 1,326 [1,275; 1,651]	1,417±0,056 1,383 [1,286; 1,564]	1,761±0,056 1,383 [1,286; 1,564]	9,751±0,560 9,751 [8,656; 11,045] $p^2 = 0,0000$ $p^3 = 0,0000$
SOD 2/GPx-1	0,98±0,078 0,964 [0,874; 1,086]	1,01±0,177 0,978 [0,721; 1,335]	0,86±0,042 0,839 [0,807; 0,898]	1,50±0,041 1,481 [1,450; 1,551] $p^3 = 0,0000$

Note: p^1 is statistically significant compared with the intact group values; p^2 is statistically significant compared with the reference group values; p^3 is statistically significant compared with the comparison group values. M±m; Me [Q1-25%; Q3-75%]; p indicates absolute values.

majority of the studied indices have been found within the limits of the intact group values, and the exception is AOPP, the level of which has considerably increased by a factor of 23,3. The combination of CNP and melanoma has induced a rise in the level of AOPP by a factor of 4,2, in the level of 8-OHdG by a factor of 7,0 with a decrease in the level of GPx-1 by a factor of 1,4 ($p < 0,05$) and HIF by a factor of 1,7 ($p < 0,05$) as compared with the values recorded in cardiac mitochondria in the reference group males. In this case, it has been found that the level of SOD 2, MDA, 8-OHdG and the value of the SOD 2/GPx-1 ratio are 1,5 times ($p < 0,05$), 1,3 times ($p < 0,05$), 5,5 times and 1,7 times ($p < 0,05$) higher, respectively, than it is the case with the data recorded in cardiac mitochondria in the males with the independent growth of melanoma only. Nevertheless the HIF level has been reported to be higher in cardiac mitochondria in the comparison group: it has doubled.

Previously it has been demonstrated by us that in the mice of C57BL/6 strain of both sexes appear infarctions, which develop under the combinations of the two pathological processes: CNP and melanoma, while in case of the independent growth of melanoma no infarctions have been revealed [15]. The nature of the infarction occurrence is of a multi-component type, and pathogenesis of infarction addresses participation/re-formatting of different biochemical mechanisms and systems, a lot of times via activation of some biological agents, which indirectly or directly involve the subcellurar structures into a pathological process [14, 17]. In the presented study we can trace a correlation between an accumulation of the LPO products in cardiac mitochondria and the infarction occurrence in the mice of both sexes with the normal genotype.

At the second stage of the experiment, it has been necessary to study how all the above listed biochemical indices have changed under the experimental pathological processes to be studied in the mice of strain C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu of both sexes (see Table 3 and 4 herein). The modified genotype in this mice strain contributes to inhibition of the malignant growth of melanoma [30].

The mice strain C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu with uPA gene-knockout used in our experiment is precisely the model of mutant mice that demonstrates blocking of the activator of plasminogen of urokinase type, or simply "urokinase" (uPA), which is the key serine protease, participating in the conversion of inactive plasminogen to active plasmin, which in its turn functions in a number of events of cancerogenesis. Our research reports have shown that CNP cancels the genetically determined inhibition of the malignant tumor growth [31].

It has been established that in the female mice of strain C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu in their cardiac mitochondria in case of CNP, only one index has been changed: it is the GPx-1 value, the level of which has decreased by a factor of 1,7 ($p < 0,05$) as compared with the values recorded in the intact group mitochondria (see Table 3 herein). In case of the independent growth of melanoma, we have determined an increase in the level of MDA by a factor of 1,3 ($p < 0,05$), AOPP by a factor of 2,1 and HIF by a factor of 1,3 ($p < 0,05$) as against the values recorded in the intact group cardiac mitochondria.

The combination of the two pathological processes (CNP + melanoma) in the female mice of strain C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu has induced in their cardiac mitochondria a change in the one index only: it is the HIF value, the level of which has been recorded to be 1,3 times higher ($p < 0,05$) as compared with the values in cardiac mitochondria found under CNP.

At the same time, in the male mice of strain C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu, we have revealed in their cardiac mitochondria a decrease in the level of antioxidant enzymes as follows: a decrease in the level of SOD 2 by a factor of 1,4 ($p < 0,05$) and in the level of GPx-1 by a factor of 1,9 ($p < 0,05$), while there has been an increase in the level of AOPP by a factor of 1,5 ($p < 0,05$) as compared with the respective values recorded in the intact cardiac mitochondria (see Table 4 herein).

The independent growth of melanoma has promoted an accumulation of AOPP in cardiac mitochondria, the level of which has become 3,1 times higher as compared with the intact values. Besides, noted is also an AOPP accumulation in case of combined pathology (CNP + melanoma), when this index has been recorded to be 1,8 times greater ($p < 0,05$) than it is the case in cardiac mitochondria under CNP solely.

Despite the fact that CNP promotes the melanoma growth in the mice of strain C57BL/6-PlautmI.IBug-ThisPlau6FDhu/GFDhu of both sexes [31], during our experiment, when examining the internal organs in the mice of both genders under all experimental pathological processes, no myocardial infarctions have been detected.

Table 3

Data on concentrations of components in system LPO – AOP in cardiac mitochondria in female mice of strain *C57BL/6-PlautmI. IBug-ThisPlau6FDhu/GFDhu* under the B16/F10 melanoma growth against the background of chronic neurogenic pain

	Intact group	Group with CNP (Reference)	B16/F10 melanoma growth (Comparison group) Week 2	CNP + B16/F10 melanoma growth (Main test group) Week 2
SOD 2 pg/mg protein	321,094±33,635 321,090 [238,250; 414,520]	293,976±33,097 293,970 [214,480; 397,620]	387,519±18,982 387,520 [335,640; 435,85]	322,865±21,242 322,860 [278,340; 378,570]
GPx-1 ng/mg protein	3,187±0,268 3,190 [2,510; 3,880]	1,918±0,246 1,920 [1,383; 2,544] p^1 = 0,0044	2,994±0,252 2,990 [2,389; 3,565]	2,413±0,242 2,410 [1,814; 3,085]
MDA mcM/g protein	6,258±0,391 6,260 [5,220; 7,360]	6,492±0,310 6,490 [5,750; 7,160]	7,862±0,399 7,900 [6,845; 8,855] p^1 = 0,0141	7,849±0,424 7,850 [6,675; 8,835]
AOPP mcM/g protein	2,711±0,238 2,710 [2,104; 3,226]	3,483±0,245 3,500 [2,751; 4,082]	5,740±0,322 5,740 [5,015; 6,449] p^1 = 0,0000	4,638±0,367 4,640 [3,864; 5,267]
HIF ng/mg protein	4,596±0,370 4,596 [3,864; 5,137]	5,990±0,307 5,990 [5,215; 6,849]	6,244±0,385 6,244 [5,222; 7,263] p^1 = 0,0095	7,571±0,407 7,571 [6,515; 8,635] p^2 = 0,0091
8-OHdG ng/mg protein	1,705±0,063 1,678 [1,568; 1,881]	1,683±0,061 1,701 [1,524; 1,803]	1,814±0,061 1,778 [1,671; 1,967]	1,762±0,087 1,762 [1,528; 1,954]
SOD 2/GPx-1	100,78±6,389 106,577 [90,131; 110,573]	163,81±23,565 145,904 [136,406; 175,127]	135,19±13,515 129,605 [105,629; 163,915]	141,09±15,587 133,966 [115,787; 168,473]

Note: p^1 is statistically significant compared with the intact group values; p^2 is statistically significant compared with the reference group values; p^3 is statistically significant compared with the comparison group values. M±m; Me [Q1-25%; Q3-75%]; p indicates absolute values.

Table4

Data on concentrations of components in system LPO – AOP in cardiac mitochondria in male mice of strain *C57BL/6-PlautmI. IBug-ThisPlau6FDhu/GFDhu* under the B16/F10 melanoma growth against the background of chronic neurogenic pain

	Intact group	Group with CNP (Reference)	B16/F10 melanoma growth (Comparison group) Week 2	CNP + B16/F10 melanoma growth (Main test group) Week 2
SOD 2 pg/mg protein	490,114±27,339 490,110 [422,380; 565,160]	355,191±30,878 382,140 [265,160; 413,380] p^1 = 0,0066	470,400±24,586 463,610 [422,380; 535,660]	437,527±26,775 430,300 [375,160; 512,740]
GPx-1 ng/mg protein	1,522±0,223 1,520 [0,914; 2,103]	0,786±0,066 0,780 [0,609; 0,968] p^1 = 0,0083	1,151±0,118 1,150 [0,865; 1,415]	1,010±0,094 0,914 [0,809; 1,217]
MDA mcM/g protein	5,876±0,354 5,880 [4,970; 6,864]	5,593±0,274 5,590 [4,970; 6,164]	6,371±0,369 6,400 [5,571; 7,212]	6,182±0,337 6,180 [5,371; 7,012]
AOPP mcM/g protein	1,866±0,232 1,860 [1,230; 2,420]	2,858±0,197 2,860 [2,367; 3,250] p^1 = 0,0069	5,754±0,341 5,750 [4,857; 6,664] p^1 = 0,0000	5,152±0,307 5,150 [4,380; 6,004] p^2 = 0,0000
HIF ng/mg protein	5,658±0,353 5,658 [4,857; 6,365]	4,472±0,303 4,472 [3,738; 5,182]	4,268±0,296 4,268 [3,522; 5,004]	4,692±0,325 4,690 [3,837; 5,531]

	Intact group	Group with CNP (Reference)	B16/F10 melanoma growth (Comparison group) Week 2	CNP + B16/F10 melanoma growth (Main test group) Week 2
8-OHdG ng/mg protein	1,606±0,045 1,606 [1,494; 1,663]	1,583±0,052 1,583 [1,446; 1,731]	1,714±0,062 1,714 [1,557; 1,864]	1,662±0,069 1,662 [1,478; 1,821]
SOD 2/GPx-1	381,43±73,552 322,441 [195,946; 626,630]	488,274±75,468 455,372 [264,631; 663,908]	432,913±49,158 409,052 [378,558; 462,122]	452,769±45,449 426,039 [375,098; 590,586]

Note: p^1 is statistically significant compared with the intact group values; p^2 is statistically significant compared with the reference group values; p^3 is statistically significant compared with the comparison group values. M±m; Me [Q1-25%; Q3-75%]; p indicates absolute values.

Discussion

In the quest to study the indices of the free-radical processes and the hypoxia factor in mice cardiac mitochondria under different growth rates of B16 melanoma, both growing independently and against the background of chronic neurogenic pain, we have used two strains of animals, which genetically differ from each other in their predisposition to manifestations of a malignant growth, and CNP represents in this case the decisive factor modifying the malignant process.

Reactive oxygen species (ROS) have received a wide study in different diseases of a human, including cancer [32]. Oxidation-related events are connected with pathological conditions and the processes of normal ageing. Interestingly, that the physiological levels of antioxidants also modulate the cell functions via homeostatic redox-sensitive cell signal cascades [33-35].

Mitochondria are the main points of aerobic respiration, and at the same time they are the important targets in ischemic injury. Mitochondrial dysfunction plays the decisive role in ischemic damage of the heart [36,37]. In ischemia, a reduction in the oxygen level deteriorates production of mitochondrial adenosine triphosphate (ATP) and induces an increase in the intracellular Ca 2+. A rise in the ROS production and an increase in the Ca 2+ levels lead to opening of nonselective highly conductive permeability transition pores (PTP) in the mitochondrial inner membrane and induce alterations in the permeability of the mitochondrial membrane. Opening of PTP additionally increases the mitochondrial levels of Ca 2+ and ROS and stimulates oxidation of proteins and lipids in mitochondria [38]. Calcium overloading and oxidative stress may cause mitochondrial dysfunction that in its turn induces apoptosis or necrosis of cardiomyocytes.

In this research report we have shown that in case of melanoma development against the background of CNP in the female mice of strain C57BL/6, in their cardiac mitochondria, GPx-1 as the antioxidant component is inhibited against the background of an increase in the concentration of SOD 2 and an increase in the SOD 2/GPx-1 ratio as compared with the independent growth of the tumor. In the males of strain C57BL/6, the antioxidant protection has come into action in a somewhat another way: it works via accumulation of SOD 2 only that results in an increase in the SOD 2/GPx-1 ratio. At the same time, in the mutant mice of both genders, we have found no differences in the concentrations of the biochemical indices of the performance of system Lipid peroxidation – Antioxidant protection (LPO – AOP) in cardiac mitochondria, when comparing the data recorded under the independent growth of melanoma and those reported in case of the melanoma growth combined with CNP.

The obtained evidence data are consistent with the research by Hinch E. C. et al. (2013) who have demonstrated that a combination of higher expression of XDH and lower activity of the SOD enzyme is key contributors to oxidative stress and cardiac tissue injury in cardiac atrophy induced by cancer [39]. As to the gender specifics, there are obvious differences between the male and female organs and hormonal fluctuations, which exert their effects on progression both of cardiovascular diseases and cancer. Although the males more often are diagnosed with cardiovascular diseases at an earlier age, they are more frequently diagnosed with cancer, too [40].

A disorder in the physiological cascade of antioxidant enzymes leads to an accumulation of ROS, which are capable of damaging DNA in the cells.

One of the end products of lipid peroxidation (LPO) is MDA, which represents a highly toxic compound initiating polymerization of proteins, destructuring of DNA, sulfhydril antioxidants and modification of the lipid layer of the cell membranes. As a consequence, we observe suppression of the genera-

tion of high-energy compounds by mitochondria, in particular ATP, required to maintain the life performance of the cells, growth rates and development of the organism as a whole. MDA is considered as the most mutagenic product in lipid peroxidation [41].

Among the most essential biological markers of oxidative stress, we should separate out purines: 8-OHdG or its oxidized modification 8-oxo-7,8-dihydro-2'-deoxiguanosine (8-oxodG). Despite the fact that all living cells develop a wide range of mechanisms responsible for DNA reparation, it is not necessarily the case that their enzymatic system removes all the DNA modifications. So, it follows that improperly repaired DNA may be a serious danger for cells, mainly due to modified genetic information as well as due to mutagenesis and apoptosis associated with the latter [42].

In order to confirm prime importance of 8-OHdG as a marker of the DNA damage and the MDA mutagenicity and the relationship between the above two markers of oxidative stress, it should be noted that at the output of our experimental study in the mice with the unchanged genotype of both sexes we have found in cardiac mitochondria a momentary accumulation of MDA and 8-OHdG under the melanoma growth against the background of CNP. In the context of this research and the obtained evidence data, we think that the stored damaging potential of MDA addresses above all the property thereof to initiate polymerization of proteins and damage of DNA that is confirmed by an increase in the 8-OHdG level in cardiac mitochondria in case of the combined action of CNP and B16 melanoma.

Modifications of 8-OHdG and 8-oxodG appear as a result from an interaction between • OH or 1 O 2 and the G-chain in DNA. Free radicals are attacking the G-chains in DNA or free 2'-deoxyguanosine, and as a result radical adducts are being produced. Due to electron disconnections, 8-OHdG is formed, which as a result from the reaction, known as keto-enol tautomerism, is transformed into the main oxidized product: 8-oxodG [42,17].

In the course of our experiment, we have detected that the level of HIF in cardiac mitochondria in the C57BL/6 strain mice of both genders was decreasing under the melanoma growth against the background of CNP as compared with the case of the independent melanoma tumor growth. At the same time, in the mutant mice of both sexes, the HIF amount has been recorded to remain stable, and it does not depend on the variant of the pathological process.

On the one hand, a reduction in the intracellular concentration of oxygen may activate HIF-1α in ischemia. And on the other hand, HIF-1α is capable of regulating the levels of expression of mitochondrially specific genes in order to adapt to hypoxic stress and improve the function of mitochondria [43, 44]. Nanayakkara G. et al. (2015) [44] have assumed that HIF-1α may transcriptionally regulate the levels of expression of frataxin as a response to hypoxia and act as a cardioprotective factor against ischemia-reperfusion injury. Higher levels of frataxin can reduce mitochondrial iron overloading and the subsequent ROS production, and, by doing so, the integrity of the mitochondrial membrane will be preserved and the viability of cardiomyocytes will be kept. So, HIF-1α preserves the integrity of the mitochondrial membrane, contributes to the cell survival and protects from ischemia. Moreover, HIF-1α can also improve the mitochondrial respiration function by activating different cardioprotective signaling pathways like the PI3K/AKT and Janus kinase 2/STAT3 pathways in order to protect the heart after IRI [45]. Besides some researchers have shown that mitochondria can regulate the stability of HIF-1α and that ROS, produced by the mitochondrial electron transport chains, may stabilize HIF-1α under the hypoxia conditions [46]. They may disturb the function of mitochondria and contribute to the cardiomyocyte death, or they may stabilize HIF-1α in order to improve the function of mitochondria [47]. However this discovery is a matter of some dispute, and it may be connected with the level of oxygen, since the oxygen level makes its impact both on the mitochondria function and the stability and activity of HIF-1α. An identification of the oxygen levels required for an optimization of mutually beneficial effects of mitochondria and HIF-1α may offer a new approach to treatment of ischemic damage to cardiomyocytes [48].

Therefore, we believe that the changes in the LPO – AOP system and oxygen saturation in cardiac mitochondria in the C57BL/6 strain mice of both genders under the melanoma growth against the background of CNP are contributors to infarctions, and the absence of changes in the LPO – AOP system and oxygen saturation in cardiac mitochondria in the uPA gene-knockout mice protects the cardiac muscle from damages.

Conclusion

The revealed imbalance between the reactive species of oxygen (MDA, AOPP) and the hypoxia factor

(HIF) that leads to damage to mtDNA (an increase in 8-OHdG) in cardiac mitochondria determines the degree of injury of the cardiac muscle in the C57BL/6 strain mice with the normal genotype of both genders under the B16 melanoma growth against the background of chronic neurogenic pain. In the uPA gene-knockout mice of both sexes, CNP stimulates the melanoma growth, but however in this case no infarctions are recorded and no activation of free-radical oxidation processes, which stimulate cardiac damages in the normal-genotype mice, is observed. It turns out that CNP by cancelling the genetically determined inhibition of the malignant tumor growth has not made an impact on the functional state of the cardiac muscle, or, more specifically, the low level of the activation of the processes with participation of ROS and high levels of HIF-1 have determined invulnerability of the animals to myocardial infarctions.

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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