LIVER MITOCHONDRIAL ENZYME SYSTEM IN THE DYNAMICS OF EXPERIMENTAL LEUKEMIA

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ABSTRACT

In rats with leukemia activity of liver mitochondrial enzymes are decreased at 6 months. Later, we noted compensatory activation of complexes II and III of the respiratory chain and transition to decompensation stage by final deadline. The activity of complex IV of the respiratory chain remains high. These changes result from increased lipid peroxidation and decreased activity of antioxidant protection enzymes

UDC CLASSIFICATION & KEYWORDS

■ 619:616.36-074 ■ Experimental Leukemia ■ Liver ■ Mitochondria ■ Enzyme System■

INTRODUCTION

Interorgan and intersystem relationship in various pathologies is one of the urgent problems of modern medicine. This is associated with increasing numbers of mixed pathologies, development of polyorgan failure in combination of various diseases. Ivashkin et al. [1] noted impairment of liver function in many pathological states that often leads to fulminant liver disease. Particularly, this problem is relevant for oncohematological patients, whose number is reported by Abdulkadyrov, Ibragimova and Vorobyov et al. [2,3,4] to increase annually. The pathogenesis of functional and structural liver disorders in leukemia is complex and results from many factors such as: (1) specific lesion of the liver (leukemic infiltration of the liver), (2) toxic effects of drugs, (3) hepatitis, herpes, cytomegalovirus and other viruses infections, (4) associated fungal and bacterial infections, and (5) complications of underlying disease [1,5,6].

In leukemias the total concentration of bound and physically dissolved oxygen is reduced. Hemoglobin oxygen saturation is decreased. This leads to development of metabolic acidosis, which is exacerbated when conducting massive chemotherapy. Taking into account that the main oxygen consumer (about 80-85% of oxygen) in cells is the mitochondrial oxidative system, which is responsible for energy production, we can assume that developing hypoxia may impair processes of oxidative phosphorylation and energy deficit.

The purpose of our study was to identify features of changes of liver mitochondrial enzyme system in the dynamics of experimental leukemia.

Materials and methods of investigation

We selected and developed benzyl model of leukemia performed by Blair et al. [7] in 157 male rats by subcutaneous injection of 40% oil solution of benzyl (0.01 ml per 100 g of body weight) throughout the experiment. Common mortality was 40.1%. Development of leukemia was assessed by changes in peripheral blood and bone marrow each month within 8 months from onset of the experiment by method of Anderson and Poulsen [8]. By the end of 6 month, 50% of animals had leukemia, which rate was increased to 77.4% and 86.4%, respectively at 7 and 8 months. At these terms, animals with signs of leukemia were sacrificed under Rausch-anesthesia, according to the rules outlined by the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986). Liver mitochondrial fraction was isolated by differential centrifugation. In this fraction we determined the activity of succinate dehydrogenase (SDH), rotenone-insensitive cytochrome C reductase (RI-cytochrome C reductase), succinate-cytochrome C reductase, cytochrome oxidase (CCO) [9] and mitochondrial protein [10]. The intensity of lipid peroxidation was assessed by the level of malondialdehyde (MDA) [11]. The antioxidant protection activity was estimated by the levels of superoxide dismutase (SOD) [12] and catalase [13].

Table 1. Activities of liver mitochondrial enzymes in the dynamics of development of leukemia, M±m, n = 6 - 7

Groups and periods of investigation	SDH, Umol/min mg protein	RI-cyt. C red., Umol/min mg protein	Suc.-cyt. C red., Umol/min mg protein	CCO, Umol/min mg protein
Intact	124.58±2.54	43.45±0.83	49.02±0.96	145.15±6.33
Leukemia, at				
6 month	38.38±1.63	11.80±0.81	16.37±0.58	376.68±6.65
p-value	<0.001	<0.001	<0.001	<0.001
7 month	72.12±3.35	30.28±1.38	38.12±1.51	357.83±13.83
p-value	<0.001	<0.05	<0.05	<0.001
8 month	54.18±1.92	20.48±1.10	24.53±1.29	219.17±12.63
p-value	<0.001	<0.001	<0.001	<0.001
Source: Authors				

The data obtained were processed by using statistical computer program Statistica 5. The significant difference was established at $P < 0.05$.

Results and discussion

Table 1 demonstrates that in rats with leukemia activities of respiratory chain enzymes of liver mitochondria changed in different directions. At 6 month after the experiment, activities of SDH, RI-NADH-cytochrome C reductase and succinate-cytochrome C reductase were decreased in 3.25 ($P < 0.001$), 3.68 ($P < 0.001$) and 3 ($P < 0.001$) times, respectively, whereas activity of CCO was increased dramatically 2.59-fold ($P < 0.001$), in comparison with the values of intact rats. At 7 month, the enzyme activities of complexes II and III of the respiratory chain were increased, however, these values still remained low, in comparison with those of intact rats: SDH 1.73-fold ($P < 0.01$), RI-NADH-cytochrome C reductase 1.43-fold ($P < 0.05$) and succinate-cytochrome C 1.29-fold ($P < 0.05$), respectively. We can assume that at 7 months in the inner mitochondrial respiratory chain there are marked compensatory adjustments in complexes II and III of the respiratory chain, aimed at maintaining energy balance in hepatocytes. However, these processes are poor efficient due to low activity of enzymes in these sites. Activity of CCO had only a tendency to decrease relative to that in the previous period, remained within the previous values, being still higher than the parameters of intact rats in 2.46 ($P < 0.001$) times. By final deadline of the experiment, activities of SDH, RI-NADH-cytochrome C reductase and succinate-cytochrome C reductase of the respiratory chain of liver mitochondria were re-inhibited in 2.3 ($P < 0.001$), 2.12 ($P < 0.001$) and 2 times, respectively, in comparison with the values of intact rats. Activity of CCO was also sharply inhibited, but still remained high, significantly exceeding the values of intact rats in 1.51 ($P < 0.05$) times.

Our studies show inhibition of activities of enzymes of complexes II and III and activation of complex IV in development of leukemia. These changes have compensatory-adaptive character, transforming into decompensation stage by final deadline. This is, probably, because of leaching of cytochrome C from external surface of inner mitochondrial membrane.

Prolonged hypoxia, which is also observed in oncohematological diseases, is accompanied by transition to a new level of regulation of oxygen homeostasis. According to several researchers [14,15,16], it is characterized by economisation of energy metabolism: decrease of body ventilation and temperature, weight loss, increase of hematocrit, decrease of respiratory rate, changes in the kinetic properties of enzymes of oxidative metabolism. This leads to increase in the efficiency of oxidative phosphorylation, appearance of new populations of small mitochondria with a set of enzymes that enable them to work in a new regime, vascular remodeling, aimed at reducing the distance between capillaries (angiogenesis), increase of glycolysis and glucose transport through histohematogenous barriers, etc. [14,15,16].

Therefore, the main flow from extracellular environment (called as "oxygen concentration gradient") is directed to mitochondria that explains the possibility of existence of areas with high and low pO_2 values in the cell [15]. In cells with high oxidative metabolism (neurons, cardiomyocytes, podocytes), 80-90% of incoming oxygen is consumed by mitochondria. Tissue hypoxia develops in leukemic infiltration of organs, decrease in the number of erythrocytes and hemoglobin, change in the affinity of hemoglobin for oxygen, which are observed in leukemic patients. Long-term comprehensive studies of the functioning of mitochondrial respiratory chain in hypoxia allowed Lukyanova et al. [15,16] to formulate the following postulates:

1. Disturbances of energy-synthesizing function of the respiratory chain, which are characteristic for hypoxia, are the result of a number of consistently developing changes in activity of its different enzymes that depend on the severity and/or duration of hypoxic exposure and determine phase of process as a whole.
2. Changes in electron-transporting function of the respiratory chain begin on its substrate (NAD-dependent) site and are primarily associated with impairment of function of mitochondrial complex I. At the beginning occurs amplification as response to decrease of oxygen concentration, and later inhibition of its activity (inhibition of NAD-dependent pathway of substrate oxidation of tricarboxylic acid cycle – the major supplier of recovered equivalents in the respiratory chain).
3. As a rule, this process is accompanied by inclusion of compensatory ways of oxidation of substrates, including activation of mitochondrial complex II (succinate oxidase path of oxidation), which plays the special role (compensatory stage of tissue or bioenergetic hypoxia).
4. With increasing of severity and/or duration of hypoxic exposure violations of electron-transporting function of the respiratory chain consecutively develop in mitochondrial complex III (in the region of cytochromes B and C (decompensation stage), and then in complex IV (cytochrome oxidase), which is inactivated only at very low pO_2 values (terminal stage of bioenergetic hypoxia).

Looking from this point of view at changes in the activity of liver mitochondrial enzymes of leukemic rats, we should note that at 6 month in the liver mitochondria activities of enzymes of the Krebs cycle are decreased at substrate level. It is characterized by compensatory activation of succinate oxidase path of oxidation, which is manifested by activation of SDH and enzymes of complex III at 7 month, although they do not reach normative values (compensation stage). Amplification of pathological process and hypoxia (at 8 month) leads to disruption and development of decompensation stage, which is manifested by inhibition of complexes II and III by final term of the experiment. In this case, the third (terminal) stage of bioenergetic hypoxia in the liver is not observed to develop, since activity of CCO remains high. Apparently, this is due to the peculiarities of blood supply in the liver, 70% of which comes through the portal vein and 30% through the hepatic artery, and this, in terms, determines the prevalence of lower concentrations of oxygen and, to some extent, anaerobic conditions. Therefore, activities and LDH4 and LDH5 in the liver are high. This allows the hepatocytes to functioning at lower oxygen concentrations than other cells. At the same time, according to Malhi et al. [17], the liver is highly aerobic oxygen-dependent tissue, sensitive to anoxia and susceptible to ischemia, as playing the major role in metabolism, biosynthesis, excretion, secretion, and detoxification that require a large expenditure of energy.

Studies by Kayumov [18] showed a sharp expansion of blood vessels with slowing blood flow, and in some places stopping blood flow due to aggregation of formed elements and obstruction of lumen of microvessels by them, many focuses of

Table 2. Indicators of lipid peroxidation and enzyme activity of antioxidant protection in liver mitochondrial fractions of leukemic rats, M±m, n = 6 - 7

Groups and periods of investigation	The level of MDA, nmol/mg protein	Enzyme activity	
		SOD, CU/min*mg protein	Catalase, mcmolH ₂ O ₂ /min*mg protein
Intact	0.271±0.011	0.412±0.015	0.288±0.016
Leukemia, at:			
6 month	0.348±0.018	0.305±0.024	0.177±0.009
p-value	<0.01	<0.01	<0.001
7 month	0.314±0.021	0.329±0.017	0.215±0.020
p-value	<0.05	<0.01	<0.01
8 month	0.479±0.029	0.218±0.012	0.162±0.011
p-value	<0.001	<0.001	<0.001
Source: Authors			

diapedesis in the liver and kidneys after prolonged intoxication with benzyl. Along with this, Isroilov [19] reported increased levels of methemoglobin and 2,3-DPG, causing development of hemic hypoxia. Studies by Kopteva et al. [20] found decrease of pulse blood flow, increase of tone of blood vessels and difficulty of venous outflow in patients with hematological malignancies, causing development of hepatomegaly and hepatic cell hypoxia.

One of the mechanisms of reduction of enzyme activity of liver mitochondria in leukemic rats may be intensification of lipid peroxidation (LPO), contributing to destruction of mitochondrial membranes during hypoxia. To clarify this issue, we examined the levels of MDA and activities of enzymes of antioxidant protection (AOP).

In the dynamics of development of leukemia, in the mitochondrial fraction of liver of experimental animals, MDA levels were significantly increased in 1.28 (P<0.05) times, the enzyme activities of SOD and catalase were reduced in 1.35 (P<0.05) and 1.63 (P<0.01) times, respectively, indicating an imbalance in the system LPO/AOP (Table 2).

Further studies observed tendency to restore balance in the system LPO/AOP, probably, due to development of compensatory processes. By final deadline, MDA level is increased in 1.77 (P<0.001) times, activities of SOD and catalase are reduced in 1.89 (P<0.001) and 1.78 (P<0.001) times, respectively, in comparison with intact rats. Imbalance in the system LPO/AOP is confirmed by reduction of (SOD+catalase)/MDA to 1.38, 1.73 and 0.79 CU in leukemic rats after 6; 7 and 8 months, respectively, whereas in intact rats this parameter was 2.58 CU. However, these changes had compensatory nature, as after 7 months the existing imbalance tended to recover. This was evidenced by higher values of (SOD+catalase)/MDA by this period and further decline of this compensation, contributing to the processes of membranolysis in subcellular structures of the liver with formation of risk of liver failure. Such changes in the system LPO/AOP in liver mitochondrial fraction coincide with the dynamics of changes in the activities of enzymes inside the respiratory chain.

Our findings resonate with study by Pospelova et al. [5], which shows intensification of lipid peroxidation, decrease of glutathione peroxidase activity, contents of sulfhydryl groups, retinol, alpha-tocopherol in patients with hematological malignancies. In this case, Nemtsova et al. [21] and Frantsiyants et al. [22] reported that labilization of membranes, observed under these conditions, contributed to the out of toxic products of tumor metabolism, disruption of functional activity of the host cells [21,22] and development of endogenous toxicosis.

Conclusions

1. At 6 month, in rats with leukemia there are decline of activities of liver mitochondrial enzymes, compensatory activation of complexes II and III of the respiratory chain and transition to decompensation stage by final deadline. Activity of complex IV of the respiratory chain remains high.
2. The level of malondialdehyde in liver mitochondrial fraction of leukemic rats increases significantly at 6 months, subsequently tending to decrease, and re-increasing sharply by final deadline. The activities of enzymes of antioxidant protection are decreased. Adequately to this, there are changes in compensatory possibilities of antioxidant protection in the removal of active forms of radicals.

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