

Redox-Potential of Liver at Insulin Dependent Experimental Diabetes Mellitus

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Introduction

Diabetes Mellitus is one of the most important problems in modern Medicine. Its wide spread and the peculiarities of clinics make this disease as not only medical but also social problem. Thus is clearly understandable the huge attention to Diabetes Mellitus pathogenesis research and elaboration of new treatment media. In spite of that there are a lot of unclear points around it. Among them of importance are the cellular metabolic processes. The clinical study of these processes meets the serious problems with receiving the research material. So to decide this kind of problems the experimental study is used.

On this stage of research the aim of our study was to investigate liver oxidative processes on different stages of experimental, concretely, alloxan Diabetes Mellitus.

Materials and Methods

The study is performed on 120 male rats of 280-300 g weight. The experimental Diabetes Mellitus was caused by single injection of 12% alloxan solution under the skin of animal (dose - 180-200 mg/kg of weight). Diabetes was diagnosed by estimation of blood glucose (Johnson & Johnson Glucometer) in vein of tail within 48 hours, on 15th, 30th and 60th days after alloxan injection. For investigation of liver oxidative processes had been used electron-paramagnetic resonance (EPR) method. After devitalization of animal the tissue piece of liver was placed into the polyethylene tube of 20 mm length and freeze in liquid nitrogen ($t=-196^{\circ}\text{C}$). The EPR specters were measured on radiospectrometer @P3-1307# in liquid nitrogen by use of quartz doer placed in radiospectrometer resonator.

Results and Discussion

In norm the liver EPR specter revealed the EPR signals of free radicals, FeS and Mn^{2+} complexes, Mo^{5+}

xanthinoxidase, cytochrome P-450, NO and Fe^{3+} transferrin.

The results of investigation shows that on each stage of alloxan Diabetes Mellitus studied by us have been revealed changes of liver EPR specter. As the cause of this could be supposed as the alloxan direct influence on metabolic processes, so the damages of metabolism that have place in organism at Diabetes Mellitus. The literature data show that the alloxan is not more distinguished in blood after 5-10 minutes since injection, as there has a place its inactivation by SH- and NH-groups of aminoacids of blood and different tissue proteins [Baranova V.G., 1983]. Thus on the early stages of experiment as the cause of oxidative processes changes could be considered alloxan direct inclusion into metabolism, but on the later stages of investigation (15th, 30th, 60th days) this changes could be due to insulin deficiency and hyperglycemia. Meantime should be considered that any influence on organism (for instance, alloxan) causes stress reaction by stress hypercatecholaminemia, which also reflects on oxidative processes and causes its special changes. The above-mentioned could be supposed after the liver EPR specter changes that had been revealed on the early stages of experiment (hyperadrenalemia on the early stages of alloxan Diabetes Mellitus could be considered as one of the causing factors of hyperglycemia, as in this period had been revealed the highest level of blood glucose - 312%). Particularly, after 48 hours since alloxan injection in liver EPR specter was detected the increase of EPR signals of free radicals and FeS centers with unchanged (H). This change of EPR specter points to the activation of mitochondrial oxidation and increase of electron transporting chain-recovering ability in hepatocytes. All this could be due to both alloxan direct influence on NAD.H dehydrogenase system and stress hypercatecholaminemia, as literature data show that after hypercatecholamine stimulation of mitochondrial oxidation there has a place the activation of succinatdehydrogenase and increase of succinate use as oxidative substrate [Kuntsuvich A.K. 1987].

The above-pointed change of oxidation chain causes increase of superoxydradicals generation and, respectively, lipid superoxidative oxidation (LSO), that itself causes inactivation of antioxidant enzyme - superoxyddismutase (SOD), release of Mn^{2+} ions and marked increase of Mn^{2+} complexes EPR signals in EPR spectrum (71%).

The alloxan-caused insulin deficiency is followed by rough damage of all kinds of metabolism, and first of all, the damage of carbohydrates. The main point of pathogenesis of that is the slowing the hexokinase reaction due to decrease of cellular membrane transparence and glucose transportation through it. In hepatocytes whose membrane is free transparent for glucose should have a place not slowing of this reaction, but decrease of hexogenase activity and, accordingly, the damage of glucose-6-phosphate production from glucose. And this later will cause damage of all stages of this glucose-product cellular use, particularly, mitochondrial oxidation processes.

This is confirmed by changes of liver EPR specter that was revealed on the next stages of experiment (15th, 30th and 60th days) when was detected the increase of EPR signals of free radicals and FeS centers with decreased (H. This change of EPR specter points to the increase of semichinon part of ubichinon in free radical sum signal, damage of NAD.H-ubichinonoxidoreductase part of mitochondrial electron transport chain and reduction of NAD.H-dehydrogenase. The damage of enzyme system on this stage causes the over-accumulation of ubisemichinon and forced production of superoxydradicals.

The restriction of mitochondrial electron transport chain function in hepatocytes causes depression of macroergic substance synthesis, activation of hypoxanthine-xanthinoxidase system and transformation of xanthindehydrogenase into xanthinoxidase that is revealed by intensive EPR signal of Mo^{+5} containing xanthinoxidase in liver EPR specter. But xanthinoxidase, as well known, is the generator of superoxydradicals. Thus has been encircled the "Vicious Circle" of oxidative processes intensification.

Within 48 hours from alloxan injection the mentioned "explosion" of oxidative processes causes the activation of macrophage iNO-synthase in hepatocytes that causes the over-production of nitric oxide and rapid increase of spin-marked free NO EPR signal in liver EPR specter. The intensity of this signal decreases on 15th and 30th days but appears the EPR signal of NO complex with hemic iron containing proteins. In free

radicals exceed condition nitric oxide transforms into the peroxinitritis that also stimulates the generation of peroxidative processes.

On each stage of investigation, especially after 48 hours since alloxan injection, in liver EPR specter had been detected rapid increase of cytochrom P-450 EPR signal. This points to the damage of cytochrom P-450 binding to the substrate and depression of monooxygenase system. Should be pointed that increase of cytochrom P-450 EPR signals is maximal after 48 hours since alloxan injection, i.e. on the early stages of experiment. So surpassingly such an inactivation of cytochrom P-450 in this period of study could be caused, except of peroxidative processes, by direct influence of alloxan on monooxygenase reactions.

According to all above-mentioned could be concluded, that at alloxan Diabetes Mellitus there has a place the damage of liver oxidative processes, increase of free radicals generation, inactivation of cellular antioxidant enzymes and monooxygenase system. Due to all this increases the lipids (among them, membrane lipids) superoxidative oxidation, damage of membranes that could be considered as a molecular basis of tissue destruction and degeneration characteristic for Diabetes Mellitus.

Conclusions

1. In the early period of alloxan Diabetes Mellitus development on the phone of very high hyperglycemia (that, except of insulin deficiency, could be caused of stress hyperadrenalinemia) is revealed the increase of mitochondrial oxidation in liver, that changes later into the damage of electron transport on NAD.H ubichinon-oxidoreductase region;
2. In hepatocytes increases the generation of free radicals, inactivate the antioxidative enzymes, increases the synthesis of nitric oxide and decreases the production of macroergic substances;
3. In hepatocytes is revealed the inactivation of monooxygenase system that points to the decrease of desintoxication function of liver;
4. Together with increase of free radicals generation there has a place the activation of lipid superoxidative oxidation, destabilization of hepatocyte membranes that should be considered as a molecular basis of tissue destruction and degeneration characteristic for Diabetes Mellitus.

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Редокс-потенциал печени при инсулинзависимом экспериментальном сахарном диабете

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Р Е З Ю М Е

Методом электронно-парамагнитного резонанса в печени изучены окислительные процессы в динамике аллоксанового диабета у крыс. Установлено, что при аллоксановом диабете нарушаются окислительные процессы в печени: усиливается генерация свободных радикалов, инактивируются антиоксидантные ферменты и монооксигеназная система гепатоцитов. В результате усиливаются пероксидативные процессы, повреждаются мембраны, что является молекулярной основой характерных для диабета деструкции и дегенерации тканей.

Ключевые слова: аллоксановый диабет, окисление, антиоксиданты, монооксигеназная система гепатоцитов, свободные радикалы