

Content of Nitric Oxide in Organs and Tissues and its Importance in Pathogenesis of Alloxan Diabetes

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Abstract

Increasing of free radical oxidation and nitrogen oxide synthesis in pancreases, liver and walls of vessels characterize Alloxan diabetes. These processes are generalized and cause the disorders of organs and tissues.

Keywords: *alloxan diabetes mellitus, nitric Oxide. EPR*

Introduction

During the recent years of great importance is the function of multifunctional molecule - Nitric Oxide (NO) in the cellular processes of organism at norms and pathology (Y.Xia, J.Zweier, 1997; V.P.Reutov, 1995; N.Kipiani, 1999).

Nitric Oxide has wide biological area of activity. It is discussed as intra- and intercellular messenger, which may cause as the positive (therapeutical), the negative (toxic) effects as well (S.Moncala et al., 1991; P.E.Chabrier et al.1992; L.R.Odivanova et al., 1997). Dual activity can be described by wide biological function in the cellular activity.

By the recent opinion of the part of authors, NO has important role in the Diabetes Mellitus pathogenesis, but exact role and metabolism of this substance in different organs and tissues are still unclear (M.I.Balabolkin, 1998; S.Gupta et al., 2000, R.Solomonina 1999; T.Tabatabaie, et al 1997.). This partially is caused by limited number of studies of NO metabolism on tissue and cellular level. The real way of study of its role in organism is experimental investigation.

The aim of our study was to investigate the changes and importance of NO in Diabetes Mellitus during one of the most popular experimental model - Alloxan Diabetes Mellitus.

Material and Methods

120 male rats by 280-350gr weight were investigated. 20 rats were control group. Experimental Diabetes Mellitus was caused by injection of 12% Alloxan solution under the rat skin, dosage 180-200 mg/kg per weight. Control animals were divided into 4 groups, 25 animal in each. Devitalisation and tissue biopsy was carried out after 48 hours. 3 animals were dead after 7 days from Alloxan injection in II group. Experiment on remnant 22 rats was carried out at 15 day from Alloxan injection, on 30 day - III group animals and on 60 day - IV group.

Development of Diabetes Mellitus was estimated by measurement of glucose by glucometry in blood taken from animal tail vein. Devitalisation was performed by decapitation with special Geliotin. The unity of electronic transport chain in mitochondria and microcosms, generation of free radicals and intensity of lipids peroxidation, antioxidant ability of blood, hemolysis of

erythrocytes and detoxication process in organism was described by EPR method. The material for experiment (blood, liver, aorta, and pancreas) was immediately frozen in liquid Nitrogen, and aorta was frozen in liquid Nitrogen after washing it in physiological solution.

Results

The content of Nitric oxide in pancreas, liver, kidney, wall of aorta and blood in norm and during experimental Diabetes Mellitus is shown in Table. The dates show that content of nitric oxide changes at the beginning of the Diabetes Mellitus. NO content is increased by 62% at 48hr after Alloxan injection, almost by 100% - at 15 day and keeps the same level till the end of experiment.

Should be mentioned that complex of HbNO is revealed during the Alloxan diabetes in EPR specter, which shows the increased generation of NO and provides the partial decrease of toxic concentration of substance. Should be pointed that increase the NO production is caused by activation of NfkB by the expression of iNOS gene.

The revealed oxidation processes spread in liver could cause macrophage iNO activation in hepatocytes, that makes prevail of nitrogen oxide after 48hr after Alloxan injection and presents the increase level of spinpointed free nitrogen oxide in EPR specter of liver. At 15-30 day presents decrease rate of NO EPR signal intensity, but appears the EPR signal of HbNO complex.

After 48hr of Alloxan diabetes increased the intensity of free nitrogen oxide EPR signal by 30%. This signal is increased and shows the 260% of control data. Later

this signal decreased and is 200% at 60 day from Alloxan injection.

By influence of Alloxan the HbNO intensive EPR signal appears, which is not in EPR specter of intact vessels. This signal increased by 25% at 15-30 day and decreased at 60 day.

Free spinpointed nitrogen oxide EPR signal during the Alloxan diabetes was 177% of control data. NO signal is decreased and is equal of control data at 60 day. Signal intensity was 16,8 mm/mg, after 48hr in 6 animals from 20 rats. After 15 and 30 day the signal is not presented, but appeared at 60 day in blood of 9 animals (50%) from 18 animals. Intensity of signal was 21,3 mm/mg.

Thus, it's clear that content of nitric oxide in pancreases, vessels, blood is rapidly increased in the beginning of Alloxan Diabetes. The important role is activation of macrophages, next is expression of NO synthase and creation of NO from L-arginin, but of importance is to take into consideration increase of free radical oxidation by activated macrophages, what plays not a less, if not absolute, role in increased production of NO.

The study shows that increased rate of oxidation process of Alloxan injection is based on out of order of electron transport in mitochondria. Alloxan relation with NAD.H and monooxygenase systems of cytoqrome P-450 causes the increased level of superoxide and alloxan radicals.

Increase of production of free radicals and NO in organs and tissues is generalized process and causes the damage of these organs and tissues, which provokes the different complications of Diabetes Mellitus.

	<i>N</i>	<i>Pancreases</i>	<i>Liver</i>	<i>Vascular</i>	<i>Blood</i>
Control group	20	7.59 ± 0.08 1	19.6 ± 0.19 6	12.49 ± 0.15 11	- 16
After 48hr of Alloxan injection	22	- 2	30.63 ± 3.78 7	18.27 ± 2.18 12	26 ± 1.9 17
After 15 days of Alloxan injection	22	13.5 ± 0.12 3	21.13 ± 2.56 8	24 ± 0.92 13	26.45 ± 1.96 18
After 30 days of Alloxan injection	20	14.9 ± 2.17 4	18.2 ± 5.89 9	32.6 ± 3.56 14	27 ± 2.29 19
After 60 days of Alloxan injection	18	- 5	20.33 ± 3.18 10	25.33 ± 4 15	- 20

Tab.1 Electron-paramagnetic signal rate of nitric oxide in pancreases, liver, vessels and blood during the norm and Alloxan diabetes.

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Содержание оксида азота в различных органах и тканях и его значение в патогенезе аллоксанового диабета

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Р Е З Ю М Е

Методом электронно-парамагнитного резонанса с применением спин-ловушек изучены содержание оксида азота и окислительные процессы в панкреасе, печени, аорте и в крови крыс при аллоксановом диабете. Установлено неравномерное, но существенное увеличение содержания NO в тканях наряду с усилением перекисного окисления липидов. Эти взаимосвязанные процессы имеют генерализованный характер и вызывают повреждение тканей и органов.

Ключевые слова: *аллоксановый сахарный диабет, окись азота, ЭПР*