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Changes in the antioxidant system and level of proinflammatory cytokin IL-1β in the blood patients of sufferi

Diabetes mellitus type 1 provokes the development of the oxidative stress, accompanied by the increased level of TBA-active products, activation of NO-synthases and increased production of nitric oxide. Activation of glutathione reductase, decrease of the glutathione peroxidase and superoxide dismutases activity was observed. This shows a specific disturbance in the functioning of the antioxidant defense system and the augmentation in the concentration of one of the major first line reacting cytokin (IL-1β).

Key words: antioxidant system, pro-inflammatory cytokin (IL-1β), type 1 diabetes mellitus, blood.

INTRODUCTION

Type 1 diabetes mellitus is a chronic autoimmune disease, caused by the selective organospecific destruction occurring in insulin producing β-cells of Langerhans islets cells of pancreas [18]. As a result of the gradual destruction or decrease in the functional activity of about 80-90 % of β-cells insulin insufficiency occurs, causing alterations of glucose metabolism and occurrence of clinical symptoms specific to type 1 diabetes mellitus.

Although the actual trigger of the β-cell destruction is still unknown, various external (chemicals, viruses) or internal factors (free radicals, cytokines) have been proposed to initiate a deleterious chain of events leading to immune cell infiltration, release of free radicals and immune mediators such as cytokines, and eventually the destruction of β-cells. It appears that NO and various related free radicals and oxidant species are major effectors of β-cell death. The increased production of NO is due to the infiltrating macrophages and also various cell types of the islets overexpressing iNOS as a result of exposure to inflammatory cytokines such as IL-1β, TNF-α, and IFN-γ [12].

Exposure of isolated rat, mouse, and human islets of Langerhans (in vitro models of type 1 diabetes) to various combinations of pro-inflammatory cytokines leads to inhibition of insulin secretion via NOS induction and subsequent free radical formation [6] and consequent poly (ADP – ribose) polymerase (PARP) – receptors activation and depletion of islet ATP and NAD sources, eventually culminating in apoptosis or necrosis [14].

Nowadays, this process is considered as the universal mechanism uniting the basic biochemical pathway of toxic effect of hyperglycemia on the organism. As a result structural changes of the membranes, permeability disturbances and loss of elastic properties up to their destruction occur.

For the protection of Langerhans' islets of pancreas from the damage caused by the superfluous quantity of free radicals of oxygen species, a powerful antioxidant protection exists. Damage and destruction of β-cells is observed only in cases of oxidative stress. Oxidative stress is supposed to be a disbalance between prooxidant and the components of antioxidant protection system and if it is not restored insufficiency of protective antioxi-

dant enzyme complex develops, leading to the development of diabetes mellitus.

Aim: to investigate the changes in antioxidant system and the level of interleukin (IL-1β) in type 1 diabetes mellitus.

METHODS

Hemolysate of erythrocytes and plasma fro type 1 diabetes mellitus patients were used. The research group included 25 patients with insulin dependent diabetes. Middle age of all patients was between 30-40 years. Among them there were 10 (40 %) men and 15 (60 %) women. Glicemic level (sugar in blood) ranged in 8.5 – 10.7 mmol/l. The control group included 10 healthy persons. Blood for biochemical research was taken from the ulnar vein at the first day of the hospital stay, 12 hours after last meal.

Intensity of lipid peroxidation processes was evaluated by measuring the level of malonyl dialdehyde (MDA), TBA (Thiobarbituric acid)-active substances on Beruheim F. method (modified by Timirbulatov R.A. and Selezneva's E.I.) [1]. Maintenance of the stability of metabolite $NO_2^-$was defined by the method of Green L.C., David A.V.[7]. Activity of NO-synthase determined by the method of Sumbaeva V.V., Jasinska I.M. [13]. Activity of superoxidedismutase (SOD) was measured by means of restoration reaction of Nitrotetrazoline blue (NTB) to Nitroformazane [11]. Activity of glutathionreductase (GR) was defined by the level of decrease in the level of $NADH+H^+$ in the course of glutathionreductase reactions, activity of glutathionperoxidase (GP) by the method [16] modified by Pereslegina I.A. Level of pro-inflammatory cytokine IL-1β was defined by means of ELISA method (Enzyme Linked Immunosorbent Assay). The received results were processed statistically based on the Student's criteria with the help of software program Microsoft Excel 8.0.

RESULTS AND DISCUSSION

Type 1 diabetes mellitus (or insulin-dependent diabetes mellitus) is characterized by autoimmune destruction of the pancreatic islets insulin producing β-cells resulting in prolonged periods of hyperglycaemia via reduced uptake of glucose and relative increase in glucagon secretion and gluconeogenesis [3].

Results shows that patients with type 1 diabetes mellitus had increased activity of peroxidative processes which was confirmed by 1,6-fold rise of the TBA-active products ($P<0.05$; table).

Such a considerable growth is an indicator of finishing process of free radical damage caused by polyunsaturated fatty acids (PUFA) and their elimination from the lipid bilayer of cell membrane [11]. Simultaneously there is rising of the maintenance a nitrite-

Indicators of the levels pro- and antioxidant system type 1 diabetes mellitus in blood patients

Investigated indicators	Control	Research
TBA-reacting substances, мmol MDA/ml, plasma blood	12.01±0.09	19.82±0.56*
SOD μmol NTB/ml · min, hemolysate of erythrocytes	3.21±0.69	1.32±0.88*
GR, μmol $NADPH+H^+$/ml · min, hemolysate of erythrocytes	6.02±0.60	10.19±1.83*
GP, μmol GSH/ml · min, hemolysate of erythrocytes	27.81±2.50	11.93±2.43*
NO_2^-, μmol/l, plasma blood	5.84±0.36	10.59±0.78*
INOS, nmol $NADPH+H^+$/min · mg Hb, hemolysate of erythrocytes	78.8±0.42	97.07±0.98*
IL-1β, pg/ml, plasma blood	3. ±0.62	11.71±0.69*

Note: * $P<0.05$ – is significant with the changes that are probable concerning the value from the control group.

anion in 1,8 times ($P<0.05$).

It leads to a condition in which NO losses its protective functions and renders vasodepressor and cytotoxic action. According to the changes of NO_2^- there was an increasement activity of iNOS in 1,2 times ($P<0.05$) for the account of inducible form which is capable to produce NO in increased activity. Activity of the rising iNOS can be a result of an gene expression of the enzyme that proves to be a true augmentation of intracellular level of mRNA enzyme at hyperglycemia [12].

In conditions of oxidative stress which are elucidated by excessive generation of free radicals, especially-active forms of oxygen, oppression antioxidant protection system activity which is related to respective alterations of its components. Enzymatic protection is possible with the help of SOD, catalase and enzymes of glutathione systems-glutathionperoxidase, glutathionreductase and glutathiontransferase. These enzymes consistently reduce superoxide-radicals, H_2O_2 and organic hydroperoxides.

The primary form of active oxygen is superoxide anion that is detoxicated by superoxidedismutase. Activity of the last one was decreased 2,4-fold ($P<0.05$). This may be a result of its modification both as active oxygen and its metabolites (peroxynitrite-anion) and glucose [13]. As a result of SOD inactivation the significant amount of superoxide anion is produced. Through the non - enzymatic pathway superoxid anion transforms into hydrogen peroxide which in turn activates the process of lipid peroxidation.

As a result of accumulation of these products and hydrogen peroxide, activity of glutathionperoxidase is decreased 2.3-fold as well, using the substrate which is reduced by glutathione, transforming it into the oxidized form. The glutathionreductase activity increased 1,7-fold and is referred on maintenance of glutathionperoxidase with reduced glutathione. This enzyme is plays a central role in protection against negative influence of oxidative stress at development of type 1 diabetes mellitus.

Increasing levels of NO considerably enhances the effect of free radicals oxidation. NO in such situation shows cytotoxic action, co-operating with O_2 and forming a powerful oxidant nitro peroxide ($ONOO^-$) which induces the damage of DNA, mutations and inhibition of functions different enzymes. Therefore NO in high concentration can be the factor of endogenous intoxication which plays an essential role in the course of pathogenesis.

Type 1 diabetes mellitus is always connected with hyperglycemia witch triggers oxidative stress via multiple mechanisms including activation of the polyol pathway, glucose autooxidation, alterations of cellular redox state. Superoxide anion appears to play a particularly important role in the pathogenesis of diabetic cardiovascular dysfunction, and this reactive oxidant was reported to activate many of the above-mentioned pathways [14].

The cellular sources of superoxide anion are multiple and include NAD(P)H and xanthine oxidases, the arachidonic acid cascade (including cyclooxygenase and lipoxygenase), microsomal enzymes, and the mitochondrial respiratory chain, with the latter being the most important source in diabetes [15].

Hyperglycemia-induced superoxide production leads to increased expression of NAD(P)H oxidases, which in turn generate more superoxide anion. Through the activation of NF-κB, hyperglycemia favors increased expression of iNOS which can increase the generation of NO [16]. Multiple lines of evidence support the pathogenetic role of endogenous peroxynitrite formation in diabetic cardiovascular complications both in experimental animals and in humans [17].

Superoxide activates protein kinase C, polyol (sorbitol), hexosamine, and stress-signaling pathways leading to increased expression of inflammatory cytokines, angiotensin II, endothelin-1, and NAD(P)H oxidases, which in turn generate more ($ONOO^-$), which induces cell damage via lipid peroxidation, inactivation of enzymes and other proteins by oxidation and nitration.

Peroxynitrite also acts on mitochondria, triggering the release of proapoptotic factors such as pathways. Peroxynitrite, in concert with other oxidants (H_2O_2), causes strand breaks in DNA, activating the nuclear enzyme poly (ADP-ribose) [15].

According to the experimental researches IL-1β is considered as a key mediator which causes inhibition of insulin secretion and stimulates the expression of gene, responsible for coding the inducible nitric oxide synthase and NOS. The last one synthesizes NO and thus initiates necrosis and apoptosis of β-cells in rodents with spontaneous autoimmune diabetes [18].

Researches of pro-inflammatory cytokines in type 1 Diabetes mellitus patients showed compensation of the system inflammatory response due to hyper activation of cytokine's cascade and a condition of immunosuppression status that proves to be true with the authentic rising of concentration of IL-1β in 3.2 times.

CONCLUSION

1. Diabetes mellitus results in the development of oxidative stress as a course of 1.6-fold increase of TBA active products, 1.8-fold increase of nitric anion content and 1.2-fold increase of iNOS activity.

2. We observed 1.7-fold increase of glutathionreductase activity, 2.3-fold decrease of glutathionperoxidase activity and 2.4-fold decrease of activity that is a result of damage of antioxidant system.

3. Increase in the content of one of the main cytokines IL-1β helps to in secretion of secondary cytokines and activation of inflammatory process in pancreas.

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ЗМІНИ В АНТИОКСИДАНТНІЙ СИСТЕМІ ТА ВМІСТ ПРОЗАПАЛЬНОГО ЦИТОКІНУ (IL-1β) У КРОВІ ХВОРИХ НА ЦУКРОВИЙ ДІАБЕТ 1-ГО ТИПУ

За умов цукрового діабету 1-го типу відбувається окиснювальний стрес, що підтверджується збільшенням рівня ТБК-реагуючих продуктів, оксиду азоту та активації синтезу NO-синтази. Це підтверджується специфічними змінами у функціонуванні антиоксидантної системи захисту та збільшенням концентрації цитокіну (IL-1β) як першої головної лінії реагування.

Ключові слова: антиоксидантна система, прозапальний цитокін (IL-1β), цукровий діабет 1 типу, кров.

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ИЗМЕНЕНИЯ В АНТИОКСИДАНТНОЙ СИСТЕМЕ И УРОВЕНЬ ПРОВОСПАЛИТЕЛЬНОГО ЦИТОКИНА (IL-1β) В КРОВИ БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ 1-ГО ТИПА

Сахарный диабет 1-го типа сопровождается окислительным стрессом, что подтверждается увеличением содержания ТБК-реагирующих продуктов, оксида азота и активацией синтеза NO-синтазы. Это проявляется специфическими изменениями в функционировании антиоксидантной системы защиты и увеличением концентрации цитокина (IL-1β) как первой главной линии реагирования.

Ключевые слова: антиоксидантная система, провоспалительный цитокин (IL-1β), сахарный диабет 1-го типа, кровь.

REFERENCES

1. Beruheim F., Mary L.C. Beruheim, Karl M. Wilbur: The reaction between thiobarbituric acid and the oxidation products of certain lipids // J. Biol. Chem. – 1948. – **174**. – P.257–264.
2. Brownlee M. Biochemistry and molecular cell biology of diabetic complications // Nature. – 2001. – **414**. – P.813–820.
3. Ceriello A., Quagliaro L., DrAmico M., Di Filippo C., Marfella R.,Nappo F.,Beriino L., Rossi F., Giugliano D. Acute hyperglicemia induces nitrotyrosine formation and apoptosis in perfused heart from rat // Diabetes. – 2002. – **51**. – P.1076–1082.
4. Ceriello A. New insights on oxidative stress and diabetsc complications may lead to a "causa" antioxidant therapy // Diabetes Care. – 2003. – **26**. – P. 1589–1596.
5. Ceriello A., Piconi L., Quagliaro L., Wang Y., Schnabel C.A., Ruggles J.A., Gloster M.A., Maggs D.G., Weyer C. Effects of pramlintide on postprandial glucose excursions and measures of oxidative stress in patients with type 1 diabetes // Diabetes Care. – 2005. – **28**. – P.632–637.
6. Corbett J.A., Wang J.L., Sweetland M.A., Lancaster J.R., Mc-Daniel M.L. Interleukin 1 beta induces the formation of nitric oxide by beta-cells purified from

rodent islets of Langerhans. Evidence for the beta-cell as a source and site of action of nitric oxide // J. Clin. Invest. – 1992. – **90**. – P. 2384–2391.
7. Green L.C., David A.W. Analysis of nitrate, nitrite and (15N) nitrate in biological fluids // Anal. Biochem. – 1982. – **126**. – P. 131–138.
8. Mazzanti L., Rabini R.A. Altered platelet membrane dynamic properties in type 1 diabetes // Diabetes. – 2005. – **46**, № 12. – P. 2069–2074.
9. Pacher P., Beckman J.S., Liaudet L. Nitric oxide and peroxynitrite in health and disease Phisiol // Phisid. Rev. – 2007. – **87**. – P. 315–424.
10. Pacher P., Szabo C. Role of preoxynitrite in the pathogenesis of cardiovascular complications of diabetes // Curr. Opin Pharmacol. – 2006. –**6**. – P.136–141.
11. Sandstrom J., Nilsson P., Karlsson R. 10-Told increase in human plasma extracellular superoxide dismutase content caused by amutation in heparin-binding domain // J. Biol. Chem. – 1994. – **269**, №29. – P. 19163–19166.
12. Suchek C., Fehsel K., Kroncke K.D., Sommer A., Kolb-Bachofen V. Primary cultures of rat islet capillary endothelial cells. Constitutive and cytokine-inducible macrophagelike nitric oxide synthases are expressed and activities regulared by glucose concentration // Amer. J. Pathol.1994. – **145**. – P. 685–695.
13. Sumbajev V.V. Jasinskaja I.M. Vlijanije DDT na aktivnost' sintazy oksida azota v pecheni, ljohkih i golovnom mozge krys // Sovr. probl. toksikologii. – 2000.– **3**. – P. 3–7.
14. Szabo C. Roles of poly (ADP-ribose) polymerase activation in the pathogenesis of diabetes mellitus and its complications // Pharmacol Res. – 2005. – **52**. – P. 60–71.
15. Spitaler M.M., Graier W.F. Vasular targets of redox signalling in diabete mellitus // Diabetologia. – 2002. – **45**. – P.476–494.
16. Ursini E., Maiorino M., Gregolin C. The selenoenzyme phospholipids hydroperoxide glutathione peroxidase // Biochem. and Biophys. Acta. – 1985. – **839**. – P.62–70.
17. Wu G., Bazer F.W., Davis T.A., Kim S.W., Li P., Rhoads J.M., Satterfield M.C., Smith S.B., Spencer T.E., Yin Y. Arginine metabolism and nutrition in growth, health and disease // Amino Acids. – 2009. – 37. – P. 153–168.
18. Zak K.P., Popova W.W. Cytokin and Diabetes type 1 in human body // Ukr. Med. Newspaper. – 2006. – 1, № 51, I/II. – P. 78–88.

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