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PI3-kinase signaling pathway mediates changes in the structural and functional state of leukocyte membranes under type 1 diabetes mellitus

Szlak sygnałowy PI3-kinazy jako mediator stanu strukturalnego i funkcjonalnego błon leukocytarnych w cukrzycy typu I

INTRODUCTION

Surface leukocyte glycoproteins play a decisive role in the pathogenesis of angiopathies under type 1 diabetes mellitus (DM) since they participate in the molecular cell mechanisms responsible for blood cell aggregation and adhesion. Cell aggregation is a complex phenomen requiring the whole range of necessary conditions for its realization. Apart from the presence of bivalent cations, certain cytoskeletal elements and the defined quantity of electrostatic charge, surface cell receptors receiving extracell stimul are of paramount importance as well. The ability of leukocytes to aggregate caused by lectins taken as molecular probes can be used as the model of premigration response to the chemoattractant which provides the chemotaxis for an increase in neutrophils in inflamed places. Leucocyte activation is directly linked with the condition of surface receptors and adhesion molecules on these cells, as well as with the reception apparatus of vessel epithelium plasma membranes internalization, expression of masking of those or other receptors considerably effects the functional activity of leukocytic series cells.

The specific aims of the research work are to dissect the leukocyte functional pecularities under type 1 D.M. through research into lectin-induced aggregation of the cells, and determine whether phosphatidylinositol (PI)-3 – kinase signal pathways (PI-3'K) signal pathway participates in leukocytic aggregation.

MATERIAL AND METHODS

The object of the research are neutrophilic granulocytes and lymphocytes taken from peripheral blood of healthy donors and patients with type 1 D.M. The following lectins were used to induce

leukocyte aggregation: **WGA, SNA** ("Lectinotest", Ukraine), **MAA** ("Sigma", USA): **WGA –** wheat germ lectin, which is specifically linked with **N –** acetyl-β, D-glucoseamin **(β,DglcNAc)** and N-acetylneuraminic (sialic) acid **(NeuNAc)**; **MAA** – maackia amurensis lectin, which displays affinity to wards the sequence of N-acetylneuraminic acid - (α2→3) - D-galactose / N-acetyl-D-galactose (**NeuNAc(α2→3)DGal/DgalNAc**), without linking disaccharide fragments that have (α2→6) glycosydic bonds; **SNA** – ricinus communis lectin, which is specific to the sequence of **NeuNAc(α2→6)DGal/DgalNAc**, without linking the sequence of **NeuNAc(α2→3)DGal/DgalNAc** in oligosaccharides.

Neutrophilic granulocytes and lymphocytes were isolated from venous hepareninized blood (the final dilution of heparin to whole blood being 1:1000) in the density gradient by means of Gradisol – G ("Aqua-medica", Poland) according to the manufacturer's instructions. After centrifuging, the cells were twice washed in the buffer solution (pH 7.4). The viability of cells in the test conducted with trypan blue was not less than 98%. After washing off the Gradisol – G, the cells were calculated in Horiayev camera, and neutrophilic and lymphocytes suspension were prepared with the concentration of 2.5 x 10^6 cells per 1 ml.

The aggregation was determined by the standard turbidimetric method with the help of bichannel laser aggragation analyzer "230 LA Biola" ("NPF Biola" Russia) in a suspension of washed leukocytes at the concentration of 2.5 x 10^6 cells per 1 ml at 37°C mixed at the velocity of 800 rpm by the change in light capacity.

To study the aggregation, 10 µl of lectin at the concentration of 32 µg/ml was added to 300 µl of cell suspension after its thermostatting for 1 min at 37°C. The aggregation was registered during 12–15 min by the change in light capacity indices of the cell suspension. The aggregation indices were measured by the aggregation curve. The aggregation level, i.e. the maximum increase in light capacity after the addition of an inductor, was given in percents. The aggregation velocity, i.e. the maximum light capacity curve slope after the addition of an inductor, was given in percents per minute.

RESULTS

The indices of lectin-induced aggregation under type 1 D.M. were the most markedly increased while using WGA (Fig. 1A) Under the pathology, the maximum aggregation level is higher than the analogous index in the control group by 21% (Fig. 1).

While using SNA (Fig. 1C) the neutrophilic granulocyte aggregation index in patient increased by 20% in comparison with that in control donors. In case of lymphocyte aggregation, the index in patient decreased in comparison with that in control donors (Fig. 1F). The neutrophilic granulocyte aggregation level decreased by 27% in diabetic patients while using MAA (Fig. 1B).

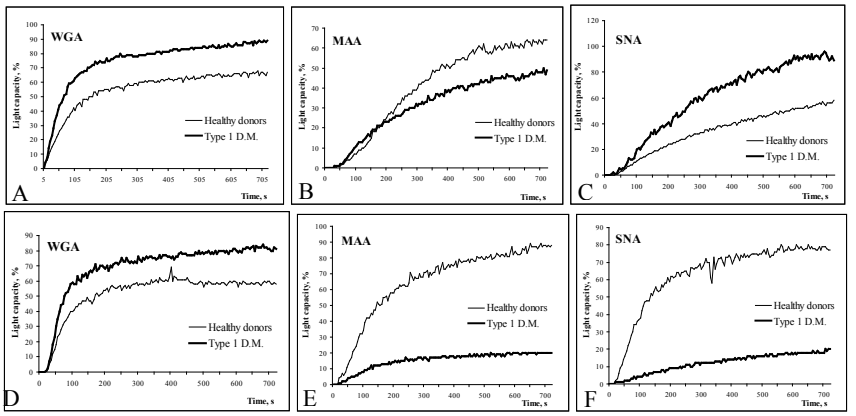

Fig. 1. Typical curves of leukocyte aggregation in healthy donors and type 1 diabetics induced by WGA, SNA, and MAA; A,B,C – typical curves of neutrophylic granulocyte aggregation, D,E,F – typical curves of lymphocytes aggregation

Immunoreactiv bands in membrane and cytosolic fractions of polymorphonuclear and mononuclear leukocytes both in control and diabetic sample were found by means of Western blot analysis (Fig. 2).

	Mononuclear leukocytes		**Polymorphonuclear leukocytes**	
	Healthy donors	Type 1 diabetes mellitus	Healthy donors	Type 1 diabetes mellitus
p85-α cytosolic fractions				
β-actin				
p85-α membrane fractions				
β-actin				

Fig. 2. Western blotting analyses of membrane and cytosolic fraction of leucocytes

The distribution of p85α regulatory subunit of PI-3'-kinase in membrane (80–90%) and cytosolic (10–20%) lisate fraction of mononuclear leukocytes in healthy donors was found. Under type 1 D.M., the distribution of that form of PI-3'-kinase was the following : 40–50% in membrane and 50–60% in cytosolic fractions. Among neutrophil granulocyte lisates in healthy donors, p85α regulatory subunit of PI-3'-kinase was evenly distributed between cytosolic and membrane fractions. In type 1 diabetic patients, p85α regulatory subunit of PI-3'-kinase was located mainly in the cytosolic fraction.

DISCUSSION

An increase in lectin-induced aggregation indicies under type 1 D.M. while using WGA may testify to an increase in expression levels on the surface of glycoconjugate cell complementary to that lectin. Among WGA-binding glycoproteins, there is a receptor to N-formyl-methionyl-leucyl-phenylalanine (fMLP), C_{5a}-component of the complement system, IL-8, the receptor of granulocyte – macrophage colony-stimulating factor (GM-CSF- receptor), and cell receptor 3 (Mac-1). It is through Mac-1 receptor that neutrophilic granulocytes interact with the molecule of intercell adhession 1 (ICAM-1, CD 54), which participates in the adhesion of leukocytes to vessel epithelia during inflammation.

Strong and fast neutrophilic aggregation induced by WGA and SNA is caused by the increased content of carbohydrate determinants in the structure of glycoprotein receptors of neutrophil granulocyte membranes in type 1 diabetics compared with healthy donors, which mostly contain NeuNAc(**α2→6**) DGal/DgalNAc instead of NeuNAc(**α2→3**)DGal/DgalNAc – disaccharide fragments.

Such peculiarities of terminal residues of neutrophil granulocyte plasma membrane glycoconjugates under the pathology may affect both dynamic and kinetic indices of cell aggregation. The detected changes in dynamic and kinetic indicies of leukocyte aggregation under type 1 D.M. may testify to changes in the quantity or rearrangement of carbohydrate determinant structures of sialocontaining receptor complementary to WGA, SNA, and MAA.

Changes in the distribution of p85α regulatory subunit of PI-3'-kinase in leukocytes correlate with changes in leukocyte aggregation activity, which can be explained by the participation of the enzyme in signal pathways mediating changes in cell receptor apparatus, which in its turn regulates the ability of cells to aggregate.

CONCLUSIONS

Changes in carbohydrate determinant qauntity or structure may increase cell aggregation and adhesion, and as result operate as a pathogenetic factor contributing to blood microcirculation disturbance and angiopathy development. It was shown that PI-3'-kinase participates in signal pathways mediating changes in molecular mechanisms responsible for aggregation and adhesion of leukocytic series blood cell. Differencies in leukocyte aggregation abilities in both healthy donors and type 1 diabetics caused by sialo-specific lectins may provide a basis for the test system created for the detection of disturbances in the functional state of leukocytes under the studied pathology.

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SUMMARY

The research reveals the lectin-induced leukocyte aggregation abilities in healthy donors and type 1 diabetics. It is shown that PI-3'-kinase participates in signal pathways mediating changes in molecular mechanisms responsible for aggregation and adhesion of leukocytic series blood cells. The obtained results may be used in the development of a test system for diabetic diagnosis.

Key words : type 1 diabetes mellitus, leukocyte, aggregation activity, lectins

STRESZCZENIE

W pracy oceniono zdolność leukocytów do agregacji indukowanej lektynami u osób zdrowych i z cukrzycą typu I. Wykazano, że PI-3'-kinaza uczestniczy w szlaku sygnałowym, pośrednicząc w wystąpieniu zmian w mechanizmach molekularnych odpowiedzialnych za agregację i adhezję komórek leukocytarnych krwi. Uzyskane wyniki mogą być podstawą do opracowania testów diagnostycznych stanów funkcjonalnych leukocytów w przebiegu cukrzycy typu I.

Słowa kluczowe: cukrzyca typu I, leukocyt, aktywność agregacyjna, lektyny