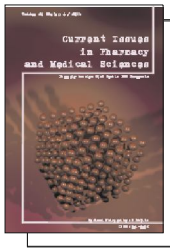

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Sialylation status of leukocyte cell-surface glycoconjugates in streptozotocin-induced diabetic rats and after treatment with agmatine

IRYNA FERENTS, IRYNA BRODYAK, MARYANA LYUTA, NATALIA KLYMYSHYN, VOLODYMYRA BURDA, NATALIA SYBIRNA*

Ivan Franko National University of Lviv, Department of Biochemistry of Biology Faculty, Ukraine

ABSTRACT

Development of experimental diabetes mellitus in rats was accompanied by increase of desialylation of carbohydrate determinants of leukocyte membrane glycoproteins. A decreased level of both α(2,3)- and α(2,6)-linked sialic acid residues and uncovering the penultimate galactose residues was shown. Our findings shown increase in sialation (in particular α(2,3) sialation) of oligosaccharide sequences of leukocytes membrane glycoconjugates after treatment diabetic rats with agmatine. Detected changes in configuration of leukocyte membrane components in diabetic animals after agmatine administration indicate the positively effects of this polyamine due to its hypoglycemic effect.

Keywords: experimental diabetes mellitus, leukocyte, membrane glycoconjugates, sialic acid, agmatine

INTRODUCTION

Sialic acid, as a terminal saccharide residue in cell surface glycoconjugates, plays an important role in a variety of biological processes. Sialic acid residues on the leukocyte surface reduce cell-to-cell adhesion, prevent the deposition of complement on the cell surface, permit evasion of immune recognition, and regulate the binding of ligands to their membrane receptors. Modification of the sialylation status of membrane glycoconjugates may provide an additional layer of regulation of leukocytes interactions with other cells or informational molecules (e.g., cytokines, hormones) [1, 6].

The sugar chains in cellular glycoconjugates with sialic acid in terminal position are key elements in the initial recruitment of leukocytes to the site of inflammation, serve as ligands for the selectins that mediate tethering and rolling of leukocytes on activated endothelial cell [9]. There is now growing evidence that increased leukocyte adhesion to the endothelial wall and entrapment (leukostasis) may play an important role in the development of diabetic microangiopathy. The pathogenic mechanisms mediating abnormal leukocyte-endothelial cell adhesion include modification of cell surface carbohydrates expressed on leukocyte surface and control cell adhesion events [1, 2, 7].

Therefore, the aims of the present study were to evaluate changes in total sialic acid content on the surface of leukocytes and determine amount and type of saccharides found at the penultimate position (relative to sialic acid) in oligosaccharide chains of leukocyte glycoconjugates in streptozotocin-induced diabetic rats and after treatment with agmatine (AGM), blood sugar-lowering agent.

MATERIAL AND METHODS

Animal preparation. The male rats weighing between 160 and 200g were used in this research. The animals were divided into four experimental groups: control group, AGM-treated control group (20 mg × kg^{-1} × day^{-1} i.m. AGM for 14 days), streptozotocin (STZ)-diabetic group (60 mg × kg^{-1} STZ i.p.), AGM-treated diabetic group (20 mg × kg^{-1} × day^{-1} i.m. AGM for 14 days). All experiments were carried out according to the National Institute of Health Guidelines for the care and use of laboratory animals and the European Council Directive on 24 November 1986 for Care and Use of Laboratory Animals (86/609/EEC), and approved by the Local Ethics Committee.

Corresponding author
* Department of Biochemistry, Ivan Franko National University of Lviv 4, Hrushevsky Street, Lviv 79005 Ukraine
e-mail: sybirna_natalia@yahoo.com

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Blood collection. After the rats were lightly anaesthetized with diethylether, blood was collected. Heparin was added beforehand to prevent coagulation (the final solution being 1:100).

Isolation of blood leukocytes. Leukocytes were isolated from blood by centrifugation in gradient of ficoll-triombrast density (ρ=1.076–1.078). Afterwards, the cells were washed twice in phosphate buffered saline (PBS, pH 7.4). Cell viability will be controlled by trypan-blue (0.1% w/v solution) exclusion test.

Measurement of sialic acid content. Total cell-associated sialic acid content was measured by the Warren thiobarbituric acid assay as previously described [8].

Lectin-induced leukocyte aggregation. Leukocyte aggregation was measured using aggregometer «Biola» (Russia). For this assay 0.3 ml of cell suspension (2.5×10^6 cells in 1 ml), prewarmed to 37°C, were incubated with 0.01 ml lectin solution prepared in PBS (1.0 mg/ml), which was added to stirred leukocyte suspensions at 15 second after the beginning of registration. The mean radius of formed leukocyte aggregates was then obtained. The lectins used were SNA (lectin from Sambucus nigra) and MAA (lectin from Maackia amurensis), specific for α(2,6)- and α(2,3)-bound sialic acids, respectively.

Enzyme-linked lectin assays (ELLA) of leukocyte. ELLA is based on binding of biotinyled lectins to glycoconjugates of cells. Leukocytes (2.0×10^6 cells in 1 ml) were immobilized on a microtiter plate using poly-L-lysine (mol wt 15.000–30.000). Then plate was incubated for 2 h at 37°C, whereafter cells were fixed with glutaraldehyde and washed very gently five times using PBS-tween and a multichannel pipetor. Then to each well there were added in turn biotinilated lectins, avidin-alkaline phosphatase conjugate and p-nitrophenyl phosphate (substrate for alkaline phosphatase). At each stage plate was washed three times using PBS-tween. The optical density (OD) of each well was measured at 405 nm with a microplate spectrophotometer «EPOCH» (USA). ELLA was performed using lectin RCA (for the identification of terminal Gal in the disaccharide Galß(1,4)GlcNAc), PNA (terminal Gal in the disaccharide Galß(1,3)GalNAc) and SBA (terminal α/ßGalNAc).

RESULTS

Change in sialic acid level in cell surfaces glycoconjugates has been associated with the initiation or modification of diverse cellular functions. The results of this study indicated a decrease in total sialic acids content in diabetic rats leukocytes in comparison to controls (Fig. 1). In order to determine the change in expression of α(2,3)- and α(2,6)-linked sialic acid we performed lectin-induced leukocyte aggregation analysis with lectins MAA and SNA. As illustrated in Fig. 2, mean radius of leukocyte aggregates was decreased using both lectins SNA and MAA. To further define the change in sialylation status of cell surface glycoconjugates the amount and type of saccharides found at the penultimate position (relative to sialic acid) of oligosaccharide chains was investigated. Anincrease in level of RCA-binding determinants on the leukocyte surface in diabetic rats was shown whereas the binding of lectins SBA and PNA did not show any statistically significant difference as compared to control (Table 1).

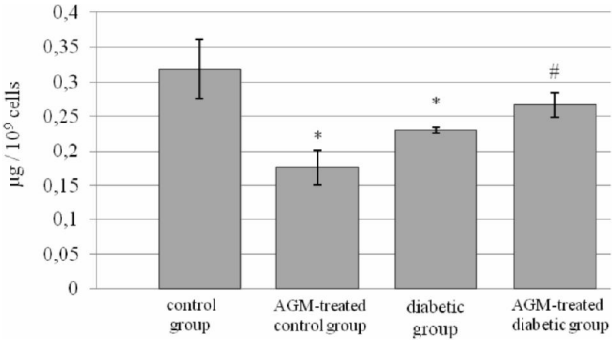

Fig. 1. Sialic acid content in leukocyte cell-surface glycoconjugates in control and diabetic group of rats after treatment with agmatine (M±m, n = 8?10). * – p<0.05, as compared with the control group; # – p<0.05, as compared with the diabetic group

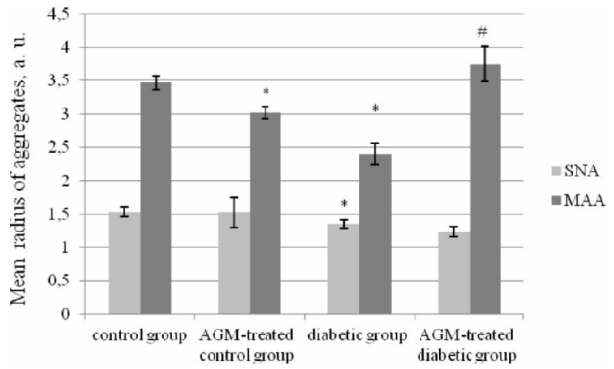

Fig. 2. Index of lectin induced aggregation of leukocyte in normal and diabetic rats after treatment with agmatine (Ĕ±m, n = 8?10). * – p<0.05, as compared with the control group; # – p<0.05, as compared with the diabetic grou

After treatment with AGM, the content of sialic acids in leukocyte of control group of rats was significantly lower and MAA-induced aggregation of cells was decreased. Our findings show also a decreased binding of lectin RCA to the leukocyte glycoconjugates in AGM-treated control group of rats. In contrast, sialic acid content in leukocyte of diabetic rats after administration of agmatine was significantly higher than those in the diabetes (Fig. 1). An increase of MAA-indused aggregation index of leukocytes was also found (Fig. 2). Binding of RCA by cells decreased and the level of PNA-binding oligosaccharides on the leukocyte surface increased in

AGM-treated diabetic group when compared with the diabet (Table 1).

Table 1. Result of ELLA using lectins RCA, PNA and SBA (M±m, n = 8?10)

Lectins	$OD_{405\ HM}$			
	control group	AGM-treated control group	diabetic group	AGM-treated diabetic group
RCA	0.708±0.035	0.303±0,017*	0.861 ±0.037*	0.790±0.023#
PNA	0.026± 0.004	0.029±0.002	0.016±0.004	0.033±0.003#
SBA	0.079 ±0.017	0.044±0.009	0.050±0.004	0.061±0.005

* – p<0.05, as compared with the control group; # – p<0.05, as compared with the diabetic group

DISCUSSION

The obtained data demonstrate increase in desialylation of carbohydrate determinants of leukocyte membrane glycoconjugates in diabetic rats. Adecrease in both α(2,3)- and α(2,6)-linked sialic acid residues was shown. This was associated with detecting of the penultimate galactose residues in the disaccharide Galß(1,4)GlcNAc, often found in structure of N-glycans of glycoproteins. Such changes in sialylation status of leukocyte cell-surface glycoconjugates may be due to increase in activity of endogenous sialidases in cells. These enzymes influence the cellular activity by removing terminal sialic acid residues from glycolipids and glycoproteins. Loss of sialic acid residues has been associated with changes in various cell functions: activation of neutrophil (e.g., increased adhesiveness and decreased surface charge), enhanced cytokine production by lymphocytes and enhanced interaction of monocytes with hyaluronic acid, a component of the extracellular matrix [4, 5, 7]. This process may be involved in the development of diabetic complications. Decrease of sialic acid content (in particular α(2,3)-linked residues) was also shown in control group of rats after i.m. injection of AGM. But in this case a decrease in RCA-binding oligosaccharide determinants on the leukocyte surface was found. Thus, the pathogenic mechanisms mediating decrease of sialic acid in this experimental group of rats include among other shedding of glycoprotein from the leukocyte surface. These changes may be associated with the modification of diverse cellular functions and change of signaling pathways in leukocyte [4]. Our findings show increased sialation (in particular α(2,3)-sialation) of oligosaccharide sequences in leukocyte membrane glycoconjugates after treatment of diabetic rats with AGM. Also a decrease in the exposure of terminal galactose residues in the disaccharide Galß(1,3)GalNAc of glycoconjugates and increase in galactose level, α(1,3)-linked to GalNAc was shown. Detected changes in configuration of leukocyte membrane components indicate the positive effect of AGM on white blood cells in diabetic animals. Treatment with this polyamine is known to lead to normalization of blood glucose levels under diabetes mellitus [3]. Thus, AGM causes the recovery of functional state of leukocyte in diabetic rats and consequently positively affects the glycoconjugate sialation process in these cells.

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