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Serum sialic acid concentration and glycoprotein sialosylation in relation to temporary development of experimental alloxan induced diabetes in rabbits

Surowicze stężenie kwasów sialowych i sialylacja glikoproteidów
podczas rozwoju doświadczalnej cukrzycy wywołanej alloxanem u królików

An elevated circulating sialic acid concentration is a risk factor for cardiovascular disease in the whole population (4) as well as in diabetic patients (6, 1). Many late complications such as microalbuminuria and clinical proteinuria, micro– and macroangiogenesis and retinopathy have been shown to be associated with elevated serum sialic levels in diabetic subjects (2).

However, no changes or even decrease in serum sialic acid concentration or their fractions were found in uncomplicated insulin dependent diabetes patients and experimental diabetic animals (5, 7). To determine metabolically the coincidence between sialic acid and changes of some parameters associated with diabetes, studies in experimental animal models of diabetes after streptozocin or alloxan treatment were undertaken (5, 3).

With respect to the effects of temporary development of experimental diabetes on plasma sialic acid levels, glycoprotein levels and their sialosylation, our study on rabbits with experimental alloxan induced diabetes was performed in time limits 3 and 6 weeks and 3 and 6 months.

MATERIAL AND METHODS

70 rabbits male, New Zealand rabbits, body weight about 3000 g were used. 10% Alloxan (Sigma) in dose 100 mg/kg body weight was administered via margin ear vein. Alloxan induces diabetes due to the destruction of B–cells of Langerhan's islets. Animals were divided into 4 groups according to the duration time of diabetes. Control group contained rabbits which lived in the same conditions as experimental animals. Glucose levels were measured in each animal before alloxan administration and after experimental diabetes duration time.

Blood samples for tests were taken after 3 and 6 weeks and after 3 and 6 months. After centrifugation, plasma samples were stored at –20°C until assay. Glucose and protein levels were measured by an automatic analyser using commercially available reagents. Glycoprotein levels were measured by means of Wintzler's method after their isolation from serum samples. Total serum sialic acid was measured by enzymatic method using reagents supplied in kit form (Boehringer Mannheim GmbH sialic acid test).

RESULTS

Alloxan administration to rabbits induced indeed the decrease in insulin production, thus increasing serum glucose concentrations were found in all the studied groups. The highest glucose concentration 656±145 mg/dl was found 3 weeks after the alloxan administration. Glucose concentrations in the remaining groups (6 weeks and 3 and 6 months) were at similar levels (379 ± 165, 442 ± 244, 404 ± 149 mg/dl), respectively.

Sialic acid concentration values were decreased in all diabetic rabbits in comparison to the control group. The most lowered values were noticed in the group obtained after 3 weeks of diabetes duration (67 ± 29 mg/dl vs. 102 ± 29 mg/dl in controls), p = 0.007. In rabbit diabetic groups obtained after 6 weeks, and after 3 and 6 months of diabetes duration values were significantly lower in com-

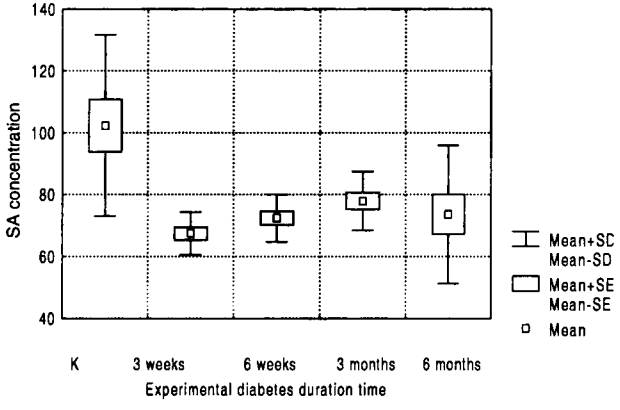

Fig. 1. Plasma sialic acid (SA) concentration in diabetes and control rabbits

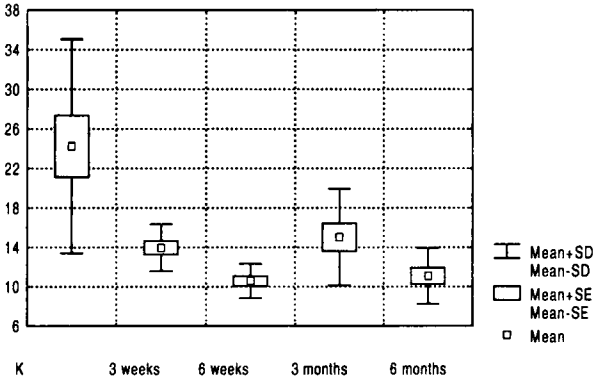

Fig. 2. Sialic acis/glicoprotein concentration index in diabetes and control rabbits

parison to controls, but there was some tendency to increase with the progress of diabetes. There was maximal differentiation of results, shown by standard deviation after 6 months of diabetes duration. Protein and glycoprotein concentration values in diabetic rabbits started to rise after 6 weeks of diabetes duration. The highest, but non–significant, protein values were obtained in 6–week–diabetes group (8.3 g/L vs. 7.3 g/L). Glycoprotein concentration values started to be elevated either after 6 weeks of diabetes duration (6.9 ± 1.3 mg/dl vs. 4.4 ± 1 mg/dl) $p = 0.0001$ and results obtained after 3 and 6 months of diabetes duration remained still on the similar level (5.6 ± 1.4 mg/dl and 6.6 ± 1 mg/dl) $p = 0.04$ and $p = 0.0002$, respectively in comparison to controls. The increased glycoprotein concentration and decreases in sialic acid levels made us investigate glycoprotein sialilation levels in control group and diabetic rabbits. The index of plasma glycoprotein sialosylation of the control group was 24.3 ± 10. This index for diabetic rabbits was decreased to the values (13.9 ± 2.3 $p = 0.02$), (10.5 ± 1.7 $p = 0.0009$), (15.0 ± 4.8 $p = 0.01$), (11.0 ± 2.8 $p = 0.0009$) in the consecutive 3 and 6 weeks and 3 and 6 months, the obtained diabetes groups.

DISCUSSION

Serious late complication in diabetic non–insulin dependent patients has been shown to be associated with wide disturbances of sialic acid distribution in the plasma and cell glycoproteins and lipoproteins. Elevated serum sialic acid levels in these patients have been found in spite of the fact that tissue sialic acid concentration was reduced (8). Some investigations have been made to find out the coincidence between sialic acid levels and insulin concentration in human and experimental diabetes in animal models but results are not clarified yet. In human non–insulin–dependent diabetes mellitus (NIDDM) elevated circulating sialic acid concentration (5, 2, 1) is suggested to be associated more with insulin deficiency state than hyperglycaemia. So, insulin seems to be more likely a mediator of sialic acid changes than any other alternations in plasma glucose levels. However, in human insulin dependent diabetic patients no changed or decreased plasma sialic acid concentrations have been

found and elevated sialic acid levels in some patients might be predictor as a late complication risk factor (6, 1).

Sialosylation of biologically important glycoprotein in diabetic patients were taken into consideration as involved in serious diabetes complications. Some studies showed that the percentage of sialosylation of the acute phase protein was reduced in diabetes (3). In our study similar results of reduced proportion of sialic acid to the whole serum glycoprotein contents were obtained in experimental alloxan induced diabetes in rats.

Decrease in serum sialic acid concentration and elevated glycoprotein concentration caused a significant downfall in sialic acid/glycoproteins index in our study with experimental diabetic rabbits. The possibility of a general decrease in the sialosylation of proteins in diabetes is found together with other results including the decreased activity of liver enzymes associated with sialic acid synthesis in diabetes rats, reduced sialosylation of the erythrocyte membrane protein, glycophorin, in human diabetes, reduced sialylation of a glycoprotein associated with insulin receptor or insulin action in diabetic rats and reduced glomerular sialic acid in human and experimental diabetes (9, 10). Significantly elevated increases in cholesterol content LDL, producing atherogenic effect in diabetic patients were characterised by significantly lowered sialic acid content, too (7).

In conclusion, we can state that the decreased sialic acid concentration and elevated serum glycoproteins contribute together to a lowered sialic acid/glycoprotein index and their possible structural alternations in alloxan developed diabetes in rabbits. They could be involved in a wide range of disorders in diabetes patients observed previously and thought as associated with disturbed sialic acid concentration.

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STRESZCZENIE

Niektóre późne powikłania występujące u chorych z cukrzycą wykazują związek ze zmianą stężeń kwasów sialowych w surowicy i glikoproteidach błon komórkowych. Przeprowadzono badania kwasów sialowych w surowicy królików z wywołaną alloxanem cukrzycą i uzyskane wyniki odniesiono do zmian w stężeniach białek surowicy oraz glikoproteidów, w których spełniają ważne funkcje określające ich właściwości, funkcje i antygenowość. Stwierdzono istotny spadek stężeń kwasów sialowych już po trzech tygodniach od wywołania cukrzycy, a zbliżone stężenia utrzymywały się do końca doświadczenia. Pomimo że stężenia glikoproteidów w odniesieniu do całkowitego stężenia białek były wyższe u królików z wywołaną cukrzycą, indeks sialilacji glikoproteidów był znacznie obniżony u królików z cukrzycą.