Autoantibodies to Neuron-Specific Proteins S100, GFAP, MBP and NGF in the Serum of Rats with Streptozotocin-Induced Diabetes

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The appearance of autoantibodies to neuronal proteins (S100, GFAP, MBP, and NGF) in rat serum was analyzed by ELISA on days 5, 10, 17, 25, and 32 after streptozotocin injection. Simultaneously, blood glucose and insulin autoantibodies were assayed. Serum glucose level increased on the next day after streptozotocin injection and the level of autoantibodies to insulin significantly increased on day 5 indicating the development of diabetes. The levels of antibodies to specific neuronal proteins (S100, GFAP, MBP, and NGF) also increased at this term. It is concluded that diabetes with streptozotocin is associated with damage to the blood-brain barrier.

Key Words: type 1 diabetes mellitus; pancreas; autoantibodies to insulin; autoantibodies to neuronal proteins; central nervous system

Type 1 diabetes mellitus is an autoimmune disease of the pancreas characterized by death of β-cells under the action of activated or so-called diabetogenic T-lymphocytes leading to insulin deficiency. The cause of the autoimmune attack is unknown. In early discussions about the mechanisms of diabetes induction, an assumption was made that neuronal disorders probably contribute to the development of this disease [4,15].

Distal polyneuropathy (impairment of the structure and function of the peripheral nervous fibers) is the most common secondary pathology in type I diabetes mellitus. Distal polyneuropathy is with high frequency (65-90%) detected in newly diagnosed patients[12]. To explain these data, attention should be paid to the existence of common neuronal proteins specific both for nerve tissue and for endocrine pancreatic tissue [12]: glial fibrillary acidic protein (GFAP), MBP (myelin basic protein), nerve growth factor (NGF), and S100 [4,15]. It can be assumed that autoimmune T-cell attack is the result of a long chain of events initiated by impaired permeability of the blood-brain barrier (BBB) and is aimed at cells of the nervous system and pancreatic β-cells. In some studies, elevated serum level of autoantibodies (AAb) to some neurospecific proteins has been revealed in patients with diabetes mellitus type 1 [5,9], which could be caused by translocation of neurospecific proteins from the neuronal tissue into the bloodstream due to impaired BBB permeability [6].

Permeability of BBB can be affected by stroke, traumatic brain injury, nervous and mental diseases as well as heavy or prolonged stress. In this case, neurospecific proteins (S100, GFAP, MBP, and NGF) are released into the bloodstream. Increase in their blood level and the formation of neuronal AAb against these proteins were detected [6].

The structural and functional abnormalities in the central and peripheral nervous system in type I diabetes mellitus were studied using experimental rat model of diabetes induced by streptozotocin (STZ). CNS disorders, namely reduced GFAP in astrocytes...
of the spinal cord, hippocampus and cerebellum were revealed in this rat model at the early stages of diabetes (on days 3 to 7) [7,11,13]. At later terms (2 months or more), disorders in the peripheral nervous system developed, e.g., 2 months after diabetes modeling, decreased NGF in the sciatic nerve [8] and electrophysiological and morphometric signs of distal neuropathy were noted [10,14].

Here we studied neurogenic changes at the early stages of diabetes mellitus in vivo. To this end, we measured serum levels of AAb to neurospecific proteins (S100, GFAP, MBP, and NGF) and to insulin after STZ administration by ELISA.

MATERIALS AND METHODS

The work with animals was carried out in accordance with the Order of the USSR Ministry of 12.08.1977 No. 755 “On measures for further improvement of the organizational forms of work with experimental animals”.

The study included 20 male Wistar rats weighing 300 to 380 g. The animals in the experimental group (n=10) were administered once with 80 mg/kg STZ (Sigma-Aldrich) in saline intraperitoneally using the method of irrigation of the pancreas. The rats in the control group (n=10) were injected intraperitoneally with 0.5 ml saline. The glucose level in the blood from the tail vein was measured with One Touch control system (LifeScan Inc) 1 day before the administration of STZ and saline. Later, glucose levels in the experimental and control groups were measured on the next day and then on days 5, 10, 17, 25, and 32 after STZ injection. In both groups, the blood was taken from the caudal vein to determine the level of antibodies to insulin and to neuronal proteins. The blood was sampled in 1.5 ml polystyrene tubes without anticoagulants and centrifuged at 1000 g. The serum was collected and stored at -70°C.

The levels of AAb to insulin were evaluated by ELISA. The reaction was carried out on the surface of a polystyrene plate (Nunc MaxiSorp) using insulin as the antigen (JSC National Biotechnology) [6].

Levels of AAb to neuronal proteins S100, GFAP, MBP, and NGF were determined using commercial Neuro-AT ELISA kits (Biopharm-Test Ltd.) using rabbit anti-rat IgG/IgM/IgA secondary antibodies conjugated to horseradish peroxidase (Imtek Ltd.).

Optical density was measured with Anthos2010 spectrophotometer (Anthos) at 450 nm. Changes in AAb level for each rat were calculated using nonparametric Wilcoxon test for dependent groups. Nonparametric Mann–Whitney U test for independent groups was used for intergroup comparisons. Differences in AAb levels were considered statistically significant at a significance level p≤0.05.

RESULTS

On the next day after STZ injection, the animals in the experimental group developed hyperglycemia (25-30 mmol/liter of glucose), which persisted throughout the experiment (32 days), while glucose level in the control group remained at 5-7 mmol/liter (Fig. 1).

The level of AAb to insulin in the control group did not change. In the experimental group, it significantly increased (p<0.05) on day 5 after STZ administration and remained at that level until day 20 of the experiment. Persistent increase in anti-insulin AAb can be attributed to lysis of β-cells and accumulation of insulin in the bloodstream, which triggers the production of anti-insulin antibodies. Comparison of experimental and control group with Mann–Whitney U test revealed significant increase in AAb to insulin on days 5 and 10 (p=0.015 and p=0.019 respectively; Fig. 2, a).

The level of AAb to all four neuronal proteins significantly increased on days 5, 10, 17, 25, and 32 in the experimental group in comparison with the initial level (p<0.05 for every value, Wilcoxon test) and remained unchanged in the control group. Comparison of AAb levels in the experimental and control groups using Mann–Whitney U test showed significantly elevated AAb to GFAP and NGF proteins on the 5th day after diabetes induction in the experimental group (p=0.0041 and p=0.017, respectively; Fig. 2, b, c). The levels of AAb to S100 and MBP proteins in the experimental group did not significantly differ from the controls.

Fig. 1. Serum glucose levels in animals injected with STZ (1) and rats in the control group (2).