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EFFECT OF TRANSPLANTATION OF PANCREAS CELL CULTURE IN ALLOXAN DIABETES MELLITUS IN RATS IN AN EXPERIMENTAL

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Abstract

Despite the significant advances achieved in modern diabetology, a number of questions remain open, which requires further study of this pathology and improvement of ways to correct it. Experimental diabetology is of great importance for identifying issues of pathogenesis, clinical picture, treatment and prevention of the disease. Experimental models of diabetes mellitus provide valuable information not only for understanding the pathophysiology of the disease, but also the mechanism of the antidiabetic action of various drugs for the purpose of their targeted use [1, 2, 4]. The alloxan model of diabetes mellitus in animals is optimal at the current level of development of experimental diabetology, because fully corresponds to the pathogenesis of diabetes mellitus in humans [2, 3, 5].

The studies were carried out on 40 white, sexually mature male laboratory rats weighing 200-250g, kept under normal vivarium conditions. Diabetes mellitus was induced by subcutaneous administration of a solution of alloxan tetrahydrate at a rate of 20 mg per 100 g of body weight, after starving the animals for 2 days. An alloxan solution was prepared by diluting the crystalline substrate Alloxan Tetrahydrate from Fluka-Sigma (Germany) in sterile distilled water. After dissolving the crystals of the substance, the sterility of the solution was achieved by passing it through a Millex-GV membrane with a 0.22 μm filter from MILLIPORE (France) and placed in sterile rolled bottles [7-10].

The material for preparing a culture of pancreatic cells was the pancreas of a 3-month-old chinchilla rabbit. In the sterile conditions of the experimental operating unit in a rabbit under intramuscular anesthesia Ketamine + Xylazine in a ratio of 40 + 7.5 mg/kg, a superomedian laparotomy was performed, the pancreas was

mobilized, the main pancreatic duct of the rabbit was punctured and a 0.25% trypsin solution was injected [11].

Biochemical blood parameters were determined using standard Lachema kits (Czech Republic). To study hormonal status, blood was taken in an amount of 5 ml under basal metabolic conditions in the morning on an empty stomach. Immediately after blood collection, it was transferred from a syringe into a 10-mL glass centrifuge tube. First, 0.5 ml of preservative solution was added to the test tube and cooled in an ice bath. The blood was centrifuged for 30 minutes with an acceleration of at least 3000g at a temperature of +40C on a K-23 refrigerated centrifuge (Germany). The resulting plasma was tubed into pre-labeled plastic Eppendorf tubes with a volume of 1.5 ml, in which they were stored (no more than 3-4 weeks) at a temperature of -700C. Determination of the content of insulin, cortisol, thyroxine and triiodothyronine, testosterone was carried out by radioimmunological method using standard commercial reagent kits from Immunotech (Czech Republic. Statistical processing of the obtained data was performed on a Pentium V computer using the Microsoft Excel 10, Statistica 6.0 programs [12-15].

After the administration of diabetogenic doses of alloxan, several phases of changes in the blood sugar curve were observed: the first phase is hyperglycemic, reaching a maximum during the first 2-4 hours; the second - hypoglycemic, which mainly manifested itself over 15-24 hours, and finally the third phase - the phase of persistent hyperglycemia.

The first signs of diabetes manifested themselves in the form of a sharp increase in daily water consumption (more than 120 ml), polyphagia, polyuria, hyperglycemia, sudden weight loss, and hair loss. At different times during the experiment, trophic ulcers of the lower leg and gangrene with self-amputation of the tail developed. About 15% of animals died as a result of hyperglycemic or hypoglycemic coma at different stages of the development of alloxan diabetes.

Changes in blood insulin concentration were determined using radioimmunoassay using standard commercial reagent kits from Immunotech (Czech Republic) - normal - 4.8 + 0.3; 1 day – 35.6+0.25; 3 days – 12.4+0.23; 21 days 2.2+0.27; Day 60 – 2.1+0.31 μIU/ml at $p<0.05$. On the first day of development of the disease, there is an increase in the concentration of contrainsular hormones: thyroxine and cortisol, a decrease in the content of triiodothyronine and testosterone. By days 7-

11, there is a decrease in the concentration of total triiodothyronine and total thyroxine, a decrease in cortisol and an increase in testosterone levels. At a later date (45-60 days), there is a significant increase in the concentration of testosterone and cortisol, as well as an imbalance of other hormones. Blood glucose was: normal 4.3+0.8; 1 day 35.8+0.12; 3 days 26.9+0.35; 21 days - 23+0.24; Day 60 – 22.6+0.22 mmol/l ($p<0.05$). When examining urine: specific gravity – 1.03; pH – 6; glucose – 17 mmol/l; protein – 100 mg/dL.

Insulin deficiency in alloxan diabetes leads to increased breakdown of tissue proteins, increased entry of amino acids into the blood, and an increase in total nitrogen. A characteristic disorder of lipid metabolism is an increase in the serum content of beta-lipoproteins (LDL) to 41.7% (normally up to 19.9%), triglycerides up to 1.03 mmol/l (normally up to 0.47 mmol/l), at $p<0.05$, as well as a decrease in alpha-cholesterol (HDL) from 38.1% (normal) to 12.8%. An increase in LDL in the blood with a decrease in the level of high-density lipoproteins leads to the development of atherosclerosis, which was observed in animals during histological examination of large vessels.

Histological examination of animals with alloxan diabetes primarily reveals damage to the pancreatic islets. Their number sharply decreases, the islands acquire a deformed shape with a clear decrease in cellular composition [11].

The animals were taken into the experiment 6 months after the administration of alloxan, and 3 degrees of severity of diabetes mellitus were conventionally distinguished: mild - the blood glucose concentration was within 10 mmol/l, moderate - 10-15 mmol/l, severe - 15 or more. For transplantation of pancreatic cell cultures, 2 groups of animals were used, 10 in each: group 1 - animals with moderate diabetes mellitus, group 2 - severe. Insulin and glucose levels were examined 12 hours later and on days 1, 5, 10, 30 and 60 after transplantation. Transplantation of cell-tissue cultures of the pancreas into rats was carried out into the anterior abdominal wall, with a thick needle into the upper quadrant of the abdomen.A decrease in glucose levels was noted 2-4 hours after transplantation, which was accompanied by an increase in blood insulin levels to 12.301 μIU/ml and C-peptide levels to 0.499 ng/ml. Subsequently, after 3 days, the insulin level increased to 23.117 μIU/ml, and C-peptide - to 0.52 ng/ml; by 60 days, the insulin level was at 2.69+0.2 μIU/ml, and the C-peptide level peptide 1.32+0.24 ng/ml. In parallel, there

was a decrease in the level of cortisol from 240.9 to 14.46 nmol/l, total triiodothyronine from 1.28 to 0.11 nmol/l, and an increase in total thyroxine from 21.09 to 128.85 nmol/l. Biochemical tests showed a decrease in glucose levels to 6.0 mmol/l in group 1 and to 7.3 mmol/l in group 2. The concentration of triglycerides also decreased to 0.48 mmol/l, the HDL content increased to 34.3%, although the LDL level remained practically unchanged. This effect was observed for up to 3 months, then gradually decreased and approached the initial values, which indicates graft rejection. Regeneration of pancreatic tissue is most likely due to the paracrine effect of the cell transplant, which occurs as a result of the release of biologically active substances aimed at stimulating the restoration of the islets of Langerhans.

Conclusions:

Alloxan diabetes is accompanied by changes in the animal's body characteristic of human type 1 diabetes mellitus, in particular hyperglycemia is accompanied by a decrease in the level of insular hormones and an increase in the concentration of contrainsular ones. Among other things, there is an imbalance in lipoprotein metabolism, which is reflected in an increase in the atherogenic index and the histological picture of blood vessels characteristic of diabetic angiopathy. In the pancreas, a large number of apoptotic bodies are observed in the area of the former pancreatic islets. Transplantation of rabbit pancreatic cell cultures makes it possible to adequately correct the level of glycemia in diabetes mellitus in rats. Transplantation of a cell culture is accompanied by the endocrine-correcting effect of contrainsular hormones, which allows not only by increasing the concentration of insulin and C-peptide to reduce the level of glycemia, but also by stabilizing biochemical parameters; histological examination revealed proliferation of the remaining pancreatic islets and the transformation of ductal cells into endocrine cells [10]. The use of a culture of pancreatic cells from a 3-month-old rabbit makes it possible to observe the effect of the graft within 90 days, and then rejection of the graft with its sclerosis is observed, which leads to the return of blood counts to the original values. Thus, transplantation of a culture of rabbit pancreas cells into rats with alloxan diabetes makes it possible to achieve stabilization of hormonal and biochemical parameters, however, this effect is short-lived and is most likely associated with sclerosis of the graft and a weakening of its endocrine-correcting

effect due to the production of insulin. The regeneration of the pancreas' own tissue is insignificant, which does not fully compensate for insulin metabolism.

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