Experimental Cell Therapy in Type I Diabetes

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***Abstract — Background*: Type 1 diabetes is a multisystem disease with both biochemical and anatomical consequences. It is a chronic disease of carbohydrate, fat and protein metabolism caused by the lack of insulin, resulting in marked and progressive inability of the pancreas to secrete it because of the autoimmune destruction of beta cells. Diabetes is a widespread disease in the world, reaching a population of 371 million in 2012, of which 5-15% are type I diabetics, most of them children and young people. Cell therapy is a new direction in the treatment of this type of diabetes.**

***Materials and Methods*: This experimental study tried using umbilical stem cells and pancreatic cells for the treatment of alloxan induced diabetes in rats. The research was conducted in the Laboratory of Tissue Engineering and Cellular Culture at the State University of Medicine and Pharmacy "Nicolae Testemitanu". The method of extraction of the pancreatic beta cells was developed, then the pancreatic cells were grown, and their ability to produce insulin was rated. The cryopreserved umbilical cells had a viability, which was 91.2 ± 1.8%. The suspension of pancreatic cells and umbilical cells was inoculated intraperitonealy in rats with alloxanic diabetes.**

***Results*: It was determined that the in vivo inoculation of umbilical cord blood stem cells and allogeneic pancreatic beta cells normalizes the glucose level in animals with experimental induced type I diabetes, and the animals treated with pancreatic cells had lower blood glucose levels than those treated with umbilical cord blood cells..**

***Index Terms* — five key words or phrases arranged alphabetically and separated by commas.**

I. INTRODUCTION

Diabetes is a widespread disease in the world, reaching a population of 371 million in 2012 [1], of which 5-15% are type I diabetics [2], 18-37 million worldwide. Type 1 diabetes is the most common metabolic disease of childhood. Approximately 1 of 400-600 children and adolescents has type 1 diabetes. [3] The incidence of this disease increases with age and is highest among children aged 10-14 years. [4]

Type 1 diabetes is a multisystem disease with both biochemical and anatomical consequences. It is a chronic disease of carbohydrate, fat and protein metabolism caused by the lack of insulin, resulting in marked and progressive inability of the pancreas to secrete it because of the autoimmune destruction of beta cells. [5]

Adult human pancreas contains approximately 10^9 β cells. In a healthy pancreas, aging cells are constantly replaced with new cells generated by the pancreas. In patients with type I diabetes, the amount of dead β cells exceeds the regenerative capacity of the pancreas, and when the destruction reaches more than 80-90% of islets [5], the insulin deficiency becomes clinically expressed. Currently type I diabetes cannot be treated completely with traditional medication or exogenous insulin injection.

Another type of treatment for type I diabetes aims at preserving or replacing a sufficient number of β cells. This could be achieved in three ways: prevention of β cell death, regeneration of β cells and β cell transplantation. [6].

For pancreatic islet transplantation, the islets are taken from a deceased donor. The islets will then be purified, processed and then transplanted to the patient. Once implanted, the beta cells of the islets will begin to produce and secrete insulin.

In recent years scientists have made great progress in pancreatic islet transplantation. Beginning with the scientific communication in June 2000 in the New England Journal of Medicine, researchers at the University of Alberta in Edmonton, Canada, have continued to use and refine the procedure called "Edmonton Protocol" for pancreatic islet transplantation in patients with type I diabetes. In 2005, researchers have published the results of a 5-year follow up of the 65 patients who received islet transplant, reporting that 10% of patients no longer required insulin injections to normalize glucose levels. Others, returned to using insulin due to loss of function of transplanted islets. But in most transplant recipients there could be seen a decrease in the need for insulin and a greater stability of glucose levels [7]. The treatment of type I diabetes mellitus with embryonic stem cells has gained attention in recent years [8], [9], [10].

Stem cells differentiate into insulin-producing cells [11], [12], [13], [14], [15], [16], [17], [18], [19], [20] and decreases hyperglycemia in animal models with experimental diabetes. Soria [21] implanted a million insulin-secreting cells derived from mouse embryonic stem cells in the spleen of mice with streptozocinic

diabetes and caused a normalization of blood glucose levels within one week. Subsequently other researchers have reported an improvement or even normoglycemia after stem cell therapy [22], [23], [24], [25].

II. MATERIALS & METHODS

Animals: The research was conducted in the Laboratory of Tissue Engineering and Cellular Culture at the State University of Medicine and Pharmacy "Nicolae Testemitanu", Chisinau, Moldova. The experiments were performed on 100 white laboratory rats of both sexes, body weight 180-220g and aged between 9-12 months. From 100 rats, 20 were used as pancreas donors to obtain β cells.

The animals were divided into four experimental groups of 20 rats: I – the control group; II – the alloxanic group; III – the group treated pancreatic cells; IV – the group treated with umbilical cells.

Modelling alloxanic diabetes: Alloxan is a toxic glucose analogue which, administered to rodents and many other species of animals, selectively destroys insulin-producing cells in the pancreas. Thus causing insulin-dependent diabetes in animals, called alloxanic diabetes, similar to type I diabetes in humans. Alloxan is selectively toxic to insulin-producing cells as it accumulates preferably in β cells through glucose transporters GLUT 2. In the presence of intracellular thiol, alloxan generate various forms of active oxygen. Alloxan's toxic action on β cells is initiated by free radicals formed in the redox reaction. [26]

To the control group rats was administered 1 ml 0.9% NaCl solution intraperitoneally. To the rats from the other 3 groups, to model diabetes, was administered intraperitoneally a single dose 200mg/kg of alloxan [27], [28]. Glucose was measured with GM 110 glucometer Bionime Switzerland. Blood was drawn from the dorsal caudal artery. After 7 days of hyperglycemia, alloxan induced diabetes is considered stable. Subsequently the animals were treated: the alloxanic group (group II) was treated with 1ml of 0.9% NaCl intraperitoneally three times with an interval of three days between injections. The III group was treated with pancreatic cells in suspension, 1 ml intraperitoneally injected three times with an interval of three days between injections. The IV group was treated with umbilical cells in suspension, the number of cells per ml: 9.8 x 106, 1 ml intraperitoneally three times with an interval of three days between injections.

Collection and cultivation of pancreatic cells: In order to extract the pancreases from donor rats, the animals were euthanized in a CO2 chamber. The operatory field was prepared with antiseptic solution, the abdominal cavity was opened through a midline incision from sternum to the pubic symphysis. The pancreas is placed in the region of the greater curvature of the stomach. The pancreas is then taken off from adjacent tissues, is extracted and then placed in 10 ml of cold HBSS medium. In a small clean Petri dish, the pancreas is cleaned from adipose tissue, lymph nodes and visible blood clots so it's ready for digestion and isolation. The pancreas is cut into small pieces of $1mm^3$ and washed 2-3 times with HBSS solution and then digested with 0.5 g / l collagenase (Sigma Type V 663 U / mg). The pancreatic fragments are then incubated in ferment solution for 1 hour and after the ferment is inactivated with Hanks solution then is centrifuged at 1000 rpm and then the upper portion of supernatant is extracted and then cultivated. The digestion of the pancreatic will be performed three times in total, and every time the upper portion of the supernatant will be extracted. Cells were cultured in DMEM/F12 special nutrient medium (glucose 8mm) with 1 g / l ITS supplement (5mg / l insulin, 5 mg / l transferrin, 5 mg / l selenium, Sigma), 100 UN / ml penicillin, 100μg/ml streptomycin , 2 g / l BSA, 10 mM nicotinamide and keratinocid growth factor (KGF) in incubator "Binder", with the CO2 concentration of 5% and 95% humidity at 37 ° C temperature in Petri dishes with a diameter of 6 cm3. In the process of cultivation, the growth of cells was examined with inversion microscope. The cells were cultured for 3, 5, 7, 9 and 13 days respectively and were examined by transmitted electron microscopy. The nutrient medium was initially changed after 3 days and then every 2 days. During cultivation some cells were taken and were stained with dithizone (difeniltiocarbozon). Dithizone binds zinc ions present in pancreatic beta cells and therefore insulin secreting cells stain red. The exocrine tissue doesn't bind dithizone and therefore it will not be stained [29]. The karyotyping of the cells has been made.

Collection and preparation of umbilical cord blood cells: The cord blood was obtained from rat embryos. Blood was collected in sterile recipients which contained anticoagulant. Frozen cells were defrosted, the content

Table 1. Shows the blood glucose level in treated and untreated rats at 24 hours, 7, 15 and 30 days after the administration of alloxan.

Groups	**After the administration of alloxan**			
	24 hours	**7 days**	**15 days**	**30 days**
I Grpup-control	5,1 ± 0,2	5,3 ± 0,2	5,1 ± 0,1	5,2 ± 0,3
II Group- alloxan	27,8 ± 2,7	19,3 ± 8,9	16,1 ± 8,1	15,5 ± 0,8
III Group - alloxan + pancreatic cells	25,6 ± 0,5	12,9 ± 7,2	12,1 ± 6,6	5,4 ± 0,2
IV Group alloxan + umbilical cells	25,3 ± 1,7	20,3 ± 8,4	18,3 ± 7,7	5,9 ± 0,8

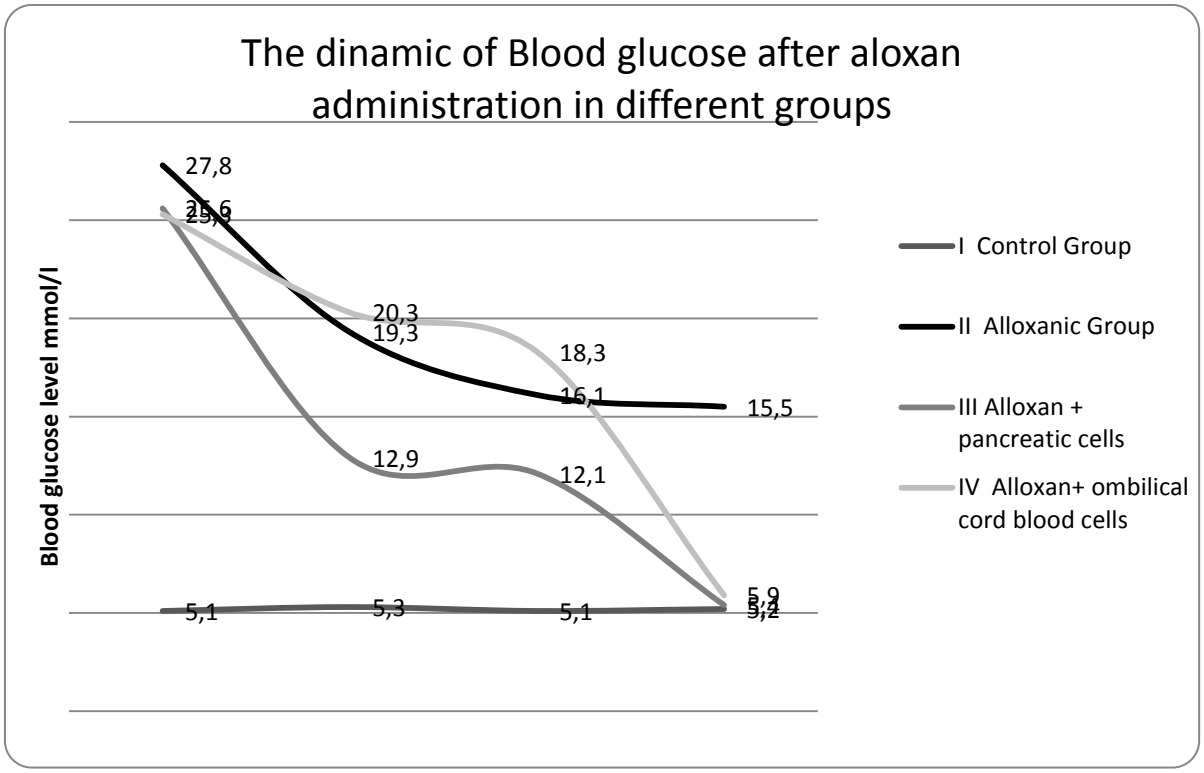

Fig. 1. The blood glucose level in untreated rats and those treated with umbilical cord blood nucleated cells and alogenous pancreatic cells. Thus glucose level is significantly decreased in treated rats compared with those untreated, and those treated with pancreatic cells had lower blood glucose levels than those treated with umbilical cells.

was placed in a tube with nutrient medium and was centrifuged. The obtained supernatant was discarded, and to the suspension was added 5 ml of warmed medium. In one ml of cell suspension were 10.2 x 10^6 cells.

When the animals were sacrificed (in the CO_2 chamber), were taken pieces of skin, pancreas, kidney, heart, liver and eye, which were fixed in 10% formalin.

From the organs were made 5μm thick histological sections stained with hematoxylin-eosin and then examined under the optical microscope.

III. RESULTS

The body weight of rats at the beginning of the experiment was 213 ± 45g. When removing animals from experience the body weight remained unchanged for the rats in the control group. The weight of those in the experimental groups, 7 days after the alloxan injection, was 185 ± 41gr. Thus it was observed that rats in aloxanic groups decreased weight compared with the control group ($p < 0.05$).

The blood glucose level in rats before the treatment with pancreatic umbilical cord blood cells was 21.8 ± 4.9 mmol/l

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Histology:

- On histological examination of the control group there were no significant changes.
- The histological examination of the untreated alloxanic group shows kidney tubular necrosis, cardiomyocyte and hepatocyte necrosis, atrophy of the islands of Langherhans.
- The histological examination of the group treated from the 7th day with pancreatic cells shows regenerative changes in the pancreas with the formation of new islets of Langherhans.
- The histological examination of the group treated from the 7th day with umbilical cells shows liver, kidney and cardiomyocyte dystrophy, some rats have chronic hepatitis with perilobular sclerosis and lymphocytic infiltration, parenchymal jaundice.

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