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Treatment of Streptozotocine Induced Diabetes Mellitus in Male Rats by Immunoisolated Transplantation of Purified Langerhans Islet Cells

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Abstract: Induction of experimental diabetes mellitus is indeed, the first step in the process of purification of pancreatic Langerhans islet cells of normal rats for transplantation under the testis subcutaneous of experimentally induced diabetic rats. For induction of experimental diabetes in male adult rats weighting 250-300 g (75-90 days), 60 mg kg^{-1} of streptozotocin was injected intravenously. Biopsy of pancreas tissue of diabetic and normal rats showed that the Langerhans islet beta cells of diabetic rats have been clearly degenerated. In the process of purification of islet cells, after collagenase digestion of pancreases, islets were isolated and dissociated; employing enzymes like DNase and trypsine, so the islet cells were changed into single cells and these cells were assayed by flow cytometry. Flow cytometry of these cells indicated that there were 91% of beta cells in cell suspension. Donor tissue in each step of this research was prepared from 38 adult wistar male rats. Transplantation was done in rats after 2-4 weeks induction of diabetes. Encapsulation of pancreatic islet cells allows for transplantation in the absence of immunosuppression. This technique which is called immunoisolation is based on the principle that transplanted tissue is protected for the host immune system by an artificial or natural membrane. The diabetic, treated and normal animals were kept in the metabolic cages separately and their body weight, consumption of food and water, urine volume, the levels of serum glucose, insulin and C-peptide quantities in all animals were measured. Analysis of variance showed a high significant difference between them.

Key words: Streptozotocin, diabetes induction, purification, immunoisolated-transplantation, islet cells

Introduction

Diabetes is a chronic disease that is relatively common throughout the world. In recent decades, various epidemiological studies have been carried out on prevalence of diabetes mellitus in Iran, according to which the population of diabetics was estimated to exceed 1.5 million. In 2004, according to the World Health Organization reports, more than 150 million people throughout the world suffered from diabetes (World Health Organization; htt:/www.who.int/medacenter/factsheets/fs/138/en/Page1-3). The only simple, inexpensive, easy and available way is to refine the Langerhans islets and to graft them under the testis subcutaneous, which we hope to present the progressive stages of this method. Experimental diabetes mellitus has been induced in laboratory animals by several methods. The generally effective method is to take the pancreas out of the body. However, to induce a notable form

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of diabetes, at least 90-95% of the pancreas has to be removed. Otherwise, the Langerhans islets cells in the remaining pancreas may undergo hypertrophy and secrete sufficient amount of insulin for fulfilling the natural metabolic needs. The second method for creating diabetes in animals is injection of drugs such as alloxan or streptozotocin. These materials inflate and ultimately, degenerate the Langerhans islets beta cells (Ikebukuro *et al*., 2002; Weiss, 1982). A less reliable method for creating diabetes is the injection of the anterior hypophysis extract (Gray and Morris, 1987; Sutherland *et al*., 2001). The final symptoms of insulin deficiency are clearly seen in rats afflicted with diabetes chemically by streptozotocin (Pipeleers *et al*., 1991a, 1985). Injection of 60 mg kg^{-1} of body weight of streptozotocin results in the toxicity of beta cells and emergence of clinical diabetes, within 2-4 days. Therefore, the study made us, first, to induce experimental diabetes mellitus in order to study the effect of transplantation of the Langerhans islets beta cells in diabetic rats with streptozotocin so as to be able to study the clinical parameters before and after the pancreas islet cells transplantation (Lacy and Kostianovsky, 1967; Olack *et al*., 1999). Transplantation of pancreas components can be done in one of the following forms: i) Transplantation of dissociated Langerhans islets beta cells; ii) Transplantation of mass of the Langerhans islets cells; iii) Transplantation of embryonic tissues; iv) Transplantation of neonatal tissues; v) Transplantation of pancreas for treatment of diabetes mellitus, which has been carried out successfully in different areas such as liver, kidneys, spleen, testis. However, transplantation of the Langerhans islets beta cells as a logical solution for the treatment of these patients is still argued (Winkel, 1982; Rabinovitch *et al*., 1982). Transplantation of the Langerhans islets cells is a new method for the treatment of diabetes. The standardized and optimized separation and purification conditions of Langerhans islets cells is one of the most important phases of the transplantation (Boker *et al*., 2001; Yasumizu *et al*., 1987). It is inexpensive, simple, safe and practical treatment method for all diabetic patients. Factors such as the number of implanted cells, capacity of performance of the new medium and the size of cell groups are effective in the relative control of the metabolism after transplantation which is needed to be considered in such studies (Rastellini and Shapiro, 1997; Holemans *et al*., 1997).

Materials and Methods

Materials

Collagenase, Crystalline Trypsin, Bovine Pancreatic DNase, 2-[4-(2-Hydroxyethyl)-1-piperazinyl]-Ethan-sulfonic acid (HEPES), silicon dicholorodimethylsilan and bovine serum albumin fraction V were supplied from the German Company, Merck. Percol is a commercial solution from silicon particles, coated with polyvinyl pyrolidone. Silicon coated with polyvinyl pyrolidone is used for sterilized cellular separation. Streptozotocin was supplied from the Swedish company Pharmacia. Ethylene glycol-bis (β-amino ethyl ether)-N, N, N', N',-tetra acetic acid are products of Sigma.

Mediums

All mediums were sterilized by 0.22 micrometer filters and the materials were autoclaved or were purchased as sterilized single use materials. The glass containers used for collecting Langerhans islets cells were siliconized with silicon. Silicon-coating consists of 30 min incubation of the containers to be used with sterilized solution of 10 μg mL^{-1} silicon after washing with distilled water. Separation of the islet and cellular purification were carried out in an Embryo Handling buffer (EH buffer) medium consists of the following components: 123 mM NaCl, 1.8 mM $CaCl_2$, 0.8 mM $MgSO_4$, 5.4 mM KCl, 1.0 mM NaH_2PO_4, 4.2 mM $NaHCO_3$, 2.8 mM Glucose, 10 mM HEPES. The medium was completed with 2.5 and 5% solutions from fraction V Bovine Serum Albumin (BSA), its pH was kept at 7.3 with 5% CO_2 in room temperature and the final volume was reached to 1 L.

Animals Used

Six adult Wistar rats weighting 250-300 g (75-90 days old) were used for inducing diabetes and 6 rats were taken as control. The donor tissues were taken from 38 male adult Wistar rats weighting 250-300 g (75-90 days old). The Islet beta cells were purified from pancreas of 38 rats and transplanted to 19 rats and the other 19 rats were taken as control.

Induction of Diabetes in Rats

The animals were injected with streptozotocin at the dose of 60 mg kg^{-1} of the body weight intravenously. Streptozotocin induces diabetes within 3 days by destroying the beta cells (Ikebukuro *et al*., 2002). Diabetic animals and non-diabetic control group were kept in metabolic cages individually and separately and under feeding and metabolism control (Elias *et al*., 1994). Glucose in the blood of diabetic rats exceeded that of the non-diabetic control ones (Weiss, 1982). Food consumption was measured in terms of (g), water consumption was measured in terms of (mL) and urine volume was measured in terms of (mL) on a daily basis while every 2-4 weeks in 80 days the levels of C-peptide, insulin and glucose in blood serum were also measured, so that chemical diabetes was verified in rats injected with streptozotocine (Pipeleers *et al*., 1985; Holemans *et al*., 1997).

Pancreatic and Testis Biopsy of Normal and Diabetic Rats

For the study and comparison of pancreas Langerhans islet beta cells testis tissue in diabetic rats induced by streptozotocine and normal rats, pancreatic and testis biopsy of normal and diabetic rats were done and samples were fixed in 10% formalin and were given to the department of electronic microscope for light microscopic examination. After framing in paraffin, thin 3-micron tissue cuts were created. Staining was carried out by Hematoxylene and Eosin stain in order to recognize the normal and diabetic rats' pancreas tissue. Figure 1 and 2 show pancreatic biopsy of normal and diabetic rats with Leitz microscope by 4000 times enlargement. The comparison of these pictures shows that the tissue of Pancreas and Langerhans and the beta cells of diabetic rats have been degenerated irreversibly (Fig. 1 and 2), while no changes are observed in the testis tissue, under the subcutaneous of the normal and diabetic rats (picture has not been shown).

Separation of the Langerhans Islets

The pancreatic Langerhans islets cells were separated from the rats with the modified collagenase digestion method (Ikebukuro *et al*., 2002; Sutherland *et al*., 2001). Two hours prior to dissection for identifying the pancreas, pilocarpine (0.2 mL from 0.2% solution) was injected to the animals intraperitoneally (Pipeleers, 1981; Gray and Morris, 1987). To carry out dissection, first the animals were anesthetized in an appropriate desiccator. Then, by opening the rat's abdomen and closing the pancreatic canal, 10 mL of Embryo Handling (EH) buffer containing 1.5 mg mL^{-1} of collagenase was injected into the pancreas to expand it and to make the lymphatic nodes and fatty tissues of the pancreas separable. Then, it was cut into pieces (Pipeleers *et al*., 1991a). After 15 sec sedimentation, the upper solution was disposed off and the tissue suspension was diluted with an equal volume of Embryo Handling (EH) buffer, including 4 mg mL^{-1} of collagenase (Lacy and Kostianovsky, 1967). The tissue was shaken at 37°C for 10 min at 300 rpm so as to be digested. Then, the Langerhans islet beta cells were separated in room temperature for 3 min by mildly pipetting (Olack *et al*., 1999). What is obtained from digestion is then passed through a nylon sheet of 500 μm diameter. The filtered part was centrifuged for 3 times and each time it was washed with Embryo Handling (EH) buffer and further made into a suspension in Embryo Handling (EH) solution (Boker *et al*., 2001). What remained on the filter was further made into suspension in Embryo Handling (EH) solution without collagenase and then shaken in the shaker incubator for 4 min at 300 rpm at 37°C and filtered as mentioned above. The product of the second digestion was finally washed in EH solution. The Langerhans islets were filtered and washed and the remained parts were carefully examined by light microscope and the cleaned islet beta cells were collected (Fig. 3). This method should be carried out appropriately,

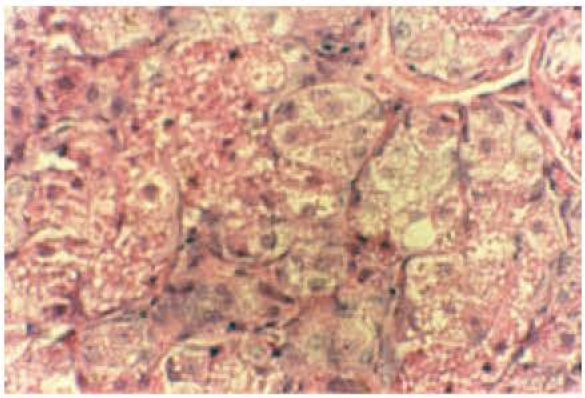

Fig. 1: Pancreatic biopsy of normal rats

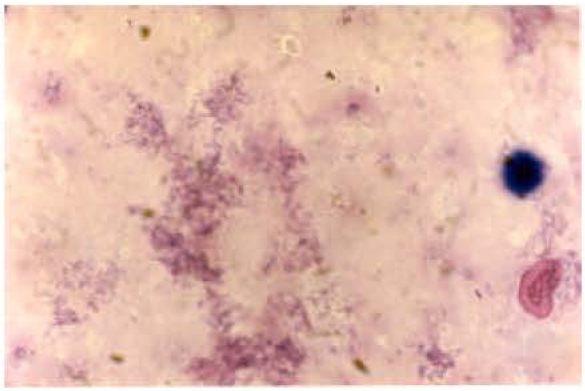

Fig. 2: Pancreatic biopsy of diabetic rats that confirms the destruction of islets and cells due to the effect of streptozotocin

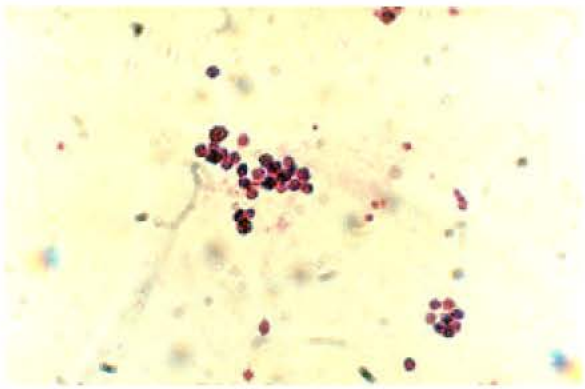

Fig. 3: The existing cells of Langerhans islets at the cell suspension colored by the Gimsa and photographed by Litz microscope, by 1000 time magnification

carefully and rapidly so that the islets would be less damaged (Gray and Morris, 1987). The Langerhans islets suspension was first kept in room temperature for 8 min and then aspired by a 9 inch siliconized Pasteur pipette. Then, trypsin with final concentration of 25 μg mL^{-1} and DNase with final concentration of 1.5 μg mL^{-1} were added to it (Pipeleers *et al*., 1991b). The degree of enzymatic differentiation and dissociation were regularly examined with contrast-phase microscope and when 50-60% of the cells converted into single units, the work was stopped (Yasumizu *et al*., 1987; Rastellini and Shapiro, 1997). This condition often occurs after 10 min. The suspension of the Langerhans islets cells was diluted immediately with 40 mL of Embryo Handling buffer and the whole collection was put in ice and filtered by passing through a 63 μm diameter nylon sheet. Thus, the undigested materials and the big cell masses were eliminated. The resulting product, which contained single cells, was centrifuged for 6 min in 300 g. (Titus *et al*., 2000; Thomas *et al*., 1999). The sediment was further changed to suspension and centrifuged. In this stage, the cellular sediment was suspended in isotonic percol solution with density of 1.040 g mL^{-1} and was put in ice for 10 min so that the cellular suspension was layered and thus, the dead and destroyed cells and cell pieces obtained in consecutive centrifugation were eliminated. Finally, in the cellular suspension layer, the healthy cells were dissolved in the physiological serum (Holemans *et al*., 1997).

Flow Cytometery

This system is a new technique, through which the physicochemical specifications of the cells or any biological component are recorded individually when they pass against laser beam. The individuality and solution nature of the cells are important in flow cytometry. The sample must be a solution from the outset or be made into a solution with enzymatic methods, in which each tissue is prepared with special methods of its own. Measurement of parameters such as size, form, DNA content, surface cell receptors, enzymatic activity, membrane permeability and calcium pump are possible with this method (Nielsen *et al*., 1982). Our goal in flow cytometry is to obtain information on the homogeneity of beta cells and the percentage of homogeneity of these cells in cellular suspension obtained at the end of purification of the Langerhans islets cells so as to determine the percentage of beta cells in the transplanted suspension. In view of the considerable difference in the sizes of types of Langerhans islets cells, a sample of cellular suspension solution can be injected into the flow cytometry system and prepare the appropriate graph, which indicates the types of cells and their percentage in the suspension. The Langerhans islets cells purified by the collagenase method were 91% of beta cells in the cellular suspension (Winkel *et al*., 1982; Rabinovitch *et al*., 1982) (Fig. 4).

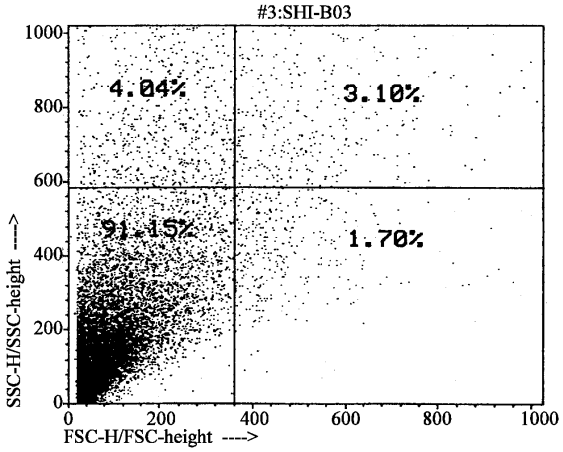

Fig. 4: Superficial distribution curve of Langerhans islets cells suspension obtained from flow cytometry, in the flow cytogram of a homogenous bulk of cells with purity of 91% which belongs to the cells with fewer granularities among the langerhans islets cells, i.e., beta cells

Islet Identification

Islets were specifically stained by dithizone. 10 mg dithizone was dissolved in absolute ethyl alcohol (50 mL concentrated NH_4OH), supplemented with 12 mL Hank's solution Sigma [45 mM Na_2HPO_4, 2.5 mM citric acid, 0.1% Triton X-100]. Just before using, the preceding solution was diluted with Hank's solution (pH 7.8) by 1 to 100, passed through a 0.22 mm filter membrane. Islet suspension was mixed with dithizone and placed 10 min and identified by light microscope (Pipeleers and Pipeleers-Marichal, 1981).

β-cells Immunofluorescence

β-cells were fixed using Bruin's solution [71.4% picric acid solution (1.2% w/v), 23.8% formalin and 4.8% glacial acetic acid]. After 24 h, cells were rinsed three times with PBS, dehydrated and permeabilized with graded concentrations of ethanol and incubated for 2 h at room temperature with an anti-insulin antibody (Sigma-Aldrich. Com) diluted 1:1,000 in PBS. After rinsing, slides were incubated for 1 h at room temperature with a fluorescein-labeled goat anti-guinea pig second antibody (1:400). After rinsing in PBS, slides were covered with 0.02% p-phenylenediamine in PBS-glycerol (1:2, v/v) and screened by fluorescence microscopy (Pipeleers *et al*., 1991b).

Treatment of Diabetic Rats by Transplantation of Langerhans Islets Cells

The purified cells of the Langerhans islets were transplanted to diabetic rats stimulated with streptozotocin in a group of diabetic samples inside the testis subcutaneously (Ikebnkuro *et al*., 2002; Boker *et al*., 2001; Gray and Morris, 1987; Pipeleers *et al*., 1991a; Sutherland *et al*., 2001). Transplantation of the cellular suspension in the physiological serum was carried out by using needle No. 20, inside the testis, subcutaneously in each injected rat. Figure 4 successfully shows the transplanted Langerhans islets in diabetic rat (Yasumizu *et al*., 1987; Lanza and Ecker, 1999).

Measurement of Glucose, Insulin and C-Peptide in Blood

Normal, diabetic and donor rats were anesthetized with ether (two minutes contact with ether does not affect blood glucose, insulin or C-peptide concentrations). Each time 0.5 mL of blood was taken from them in order to measure sugar, insulin and C-peptide (Pipeleers *et al*., 1985). Blood was taken from the heart. The samples were collected in sterilized tubes and kept at 4°C and after separating the clot, the serum was separated by centrifuging (Van De Winkel and Pipeleers, 1983). Blood glucose was measured by glucose oxidase method and serum C-peptide by radioimmunoassay method. This phase of the study was carried out once every 2-4 weeks for 80 days in diabetic and donor rats as well as in their control counterparts (Lanza and Ecker, 1999).

Biopsy and Histology of Langerhans Islets Cells Growing

Two months after transplantation, the transplanted areas were vivisected in order to identify the Langerhans islets cells grown in the transplant receptor. For this purpose, the testis of the recipient rats were removed, stabilized in 10% formalin and given to the Electronic Microscope Department for light microscopic examination. After framing in paraffin, thin 3-micron tissue cuts were created. Staining was carried out by Hematoxylene and Eosin stain in order to recognize the transplanted islets cells. Figure 4 shows transplanted Langerhans islets cells normal growing with Leitz microscope by 4000 times enlargement under the testis subcutaneous of treated rat.

Results

Normal levels of glucose, insulin and C-peptide in healthy adult rats were measured to be 135±5 mg dL^{-1}, 2.1±0.2 m IU L^{-1} and 0.056±0.001 ng mL^{-1}, respectively. Daily consumption of water and food in healthy adult rats were found to be 30±5 mL and 10±2 g, respectively. Daily urine

Table 1: Shows data of number, weight, age, amount of streptozotocine injection, glucose, insulin, C-peptide of blood, consumption of water, food and volume of urine in normal, diabetic and treated Rats

Volume of urine (mL)	Consumed food (g)	Consumed water (mL)	Blood C-peptide (ng mL^{-1})	Blood insulin (m IU mL^{-1})	Blood glucose (mg dL^{-1})	STZ injection (mg kg^{-1})	Age of Rats (days)	Body weight (gr)	Number of rats	State of rats
10±1	10±2	30±5	0.056±0.001	2±0.2	135±5	0	75-90	250-300	6 head	Normal
130±5	45±5	145±5	0.046±0.002	1.5±0.2	500±20	60	75-90	250-300	6 head	Diabetic
35±5	30±5	40±5	0.053±0.009	2±0.2	145±10	60	75-90	250-300	19	Treated

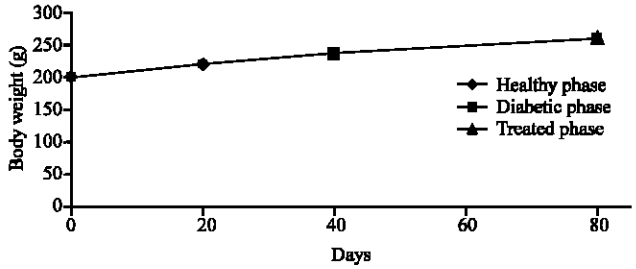

Fig. 5: The continuous changes in average of body weight in 8 non-adult rats in three healthy, diabetic and treated phases

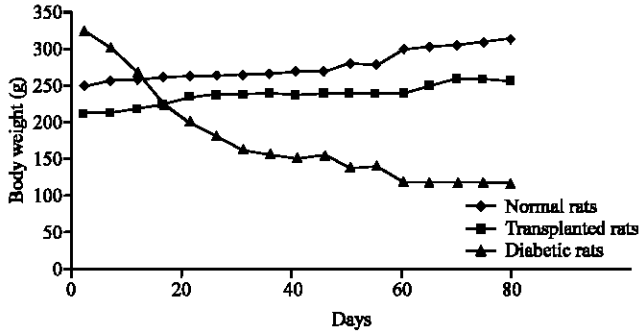

Fig. 6: A comparison of the curves relating to the average changes in 19 body weight in the three groups of healthy, diabetic and transplanted rats. These curves reveal loss of weight and thinness due to streptozotocin used for diabetes induction in adult rats and elimination of these effects after transplantation of pancreatic Langerhans islets cells during 80 days

volume in healthy adult rats was 10±1 mL. In diabetic rats the levels of glucose, insulin and C-peptide were measured as 500±20 mg dL^{-1}, 1.5±0.2 m lU L^{-1} and 0.042±0.002 ng mL^{-1}, respectively. Daily consumption of water and food in them were measured as 145±5 mL and 45±4 g. Daily urine volume in diabetic rats was measured as 130±5 mL. Levels of glucose, insulin and C-peptide in treated adult rats were measured as 145±10 mg mL^{-1}, 2±0.2 m IU L^{-1} and 0.053±0.009 ng mL^{-1}, respectively. Daily consumption of water and food in treated adult rats were measured as 40±5 mL and 30±5 g, respectively. Daily urine volume in treated adult rats was measured as 35±5 mL (Table 1). Changes of weight in adult and non-adult diabetic rats vary. Since the non-adult rats are in the growing age, diabetic loss of weight is not seen in them and they even show a slight weight gain. Figure 5 shows continuous changes in average of body weight in 8 non-adult rats in three healthy, diabetic and treated phases and a slight increase in the weight of non-adult rats in the three healthy, diabetic and treated phases. But in adult rats, however, diabetes is accompanied by loss of weight. Two to four weeks after diabetes induction and observing its effects, transplantation of purified Langerhans islets cells was carried out by healthy rats' pancreas with modified collagenase digestion method with 91% beta cells in the cellular suspension for treatment of diabetes. Figure 6 shows the average changes in the body

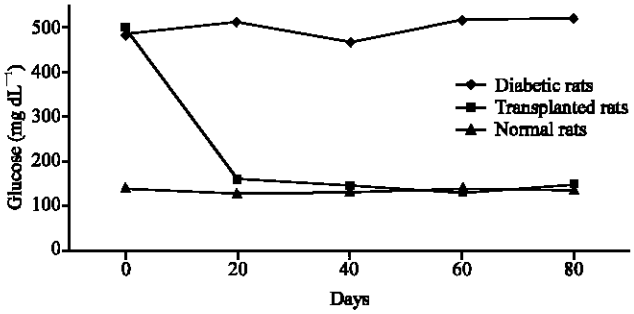

Fig. 7: The changes of average level of glucose in serum of 19 diabetic rats treated by transplantation of Langerhans islets cells and normal ones during 80 days

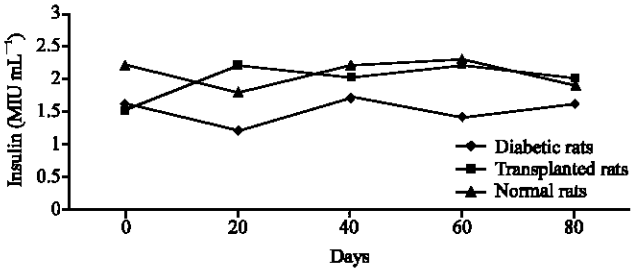

Fig. 8: The average in changes in the level of insulin in serum of 19 diabetic rats treated by transplantation of Langerhans islets cells and normal ones during 80 days

weight of 19 diabetic rats treated by transplantation of Langerhans islets cells and normal ones during 80 days. This diagram reveals the loss of weight and thinness due to streptozotocin used for diabetes induction in adult rats and elimination of these effects after transplantation of pancreatic Langerhans islets cells. By analyzing of variance (ANOVA) with SPSS. 12, the Standard Error Mean (SEM) is found to be 8.19, $F = 40.87$, $df = 2, 57$, $p<0.001$, which well indicates the weight loss in diabetic adult rats. By carrying out this operation, signs of recovery were gradually observed in the rats, so that the levels of glucose, insulin and C-peptide in transplanted rats were 145±11.2 mg dL^{-1}, 2±0.2 m IU L^{-1} and 0.053±0.009 ng mL^{-1}, respectively. Figure 7 shows the average of the changes in the level of glucose in blood serum of 19 diabetic rats treated by transplantation of Langerhans islets cells and normal ones during 80 days, that by using ANOVA on transplanted and diabetic rats, we found the standard error mean (SEM) to be 48.1, $F = 903.18$, $df = 2, 11$, $p<0.001$, which well indicates the glucose loss in diabetic adult rats. By carrying out this operation, signs of recovery were gradually observed in the rats. Figure 8 shows the average changes of insulin in blood serum of 19 diabetic rats treated by transplantation of Langerhans islets cells and normal ones during 80 days, in which the ANOVA on transplanted and diabetic rats shows that the standard error mean (SEM) is equal to 0.088, $F = 8.53$, $df = 2, 12$, $p<0.005$, which well indicates the insulin loss in diabetic adult rats. By carrying out this operation, signs of recovery were gradually observed in the rats. Figure 9 shows the average of the changes in the level of C-peptide in blood serum of 19 diabetic rats treated by transplantation of Langerhans islets cells and normal ones during 80 days, in which the ANOVA on transplanted and diabetic rats shows that the standard error mean (SEM) is equal to 0.002, $F = 4.85$, $df = 2, 12$, $p<0.029$, which well indicates the C-peptide loss in diabetic adult rats. By carrying out this operation, signs of recovery were gradually observed in the rats consequently, data analysis of glucose, insulin and C-peptide show the high significant difference between transplanted and diabetic rats serum and

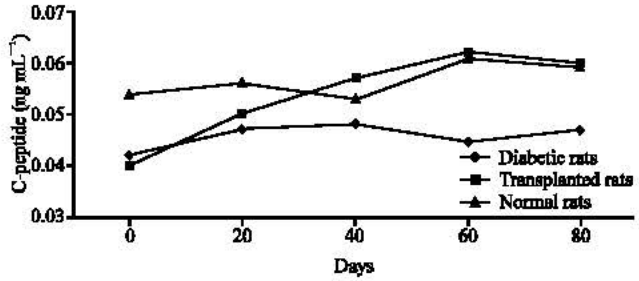

Fig. 9: The changes of average level of C-peptid in serum of 19 diabetic rats treated by transplantation of Langerhans islets cells and normal ones during 80 days

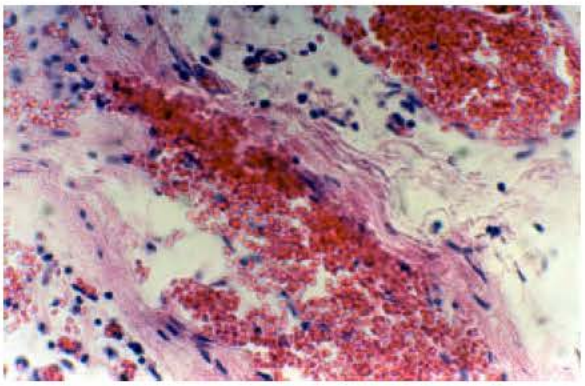

Fig. 10: The existing transplanted Langerhans islet cells under the testis subcutaneous of the diabetic rat which has been treated via transplantation of Langerhans islet cells. Observed after taking a tissue from the inside the testis subcutaneous and stabilizing in 10% formaldehyde solution

confirm the success of the transplantation project. Moreover, the daily consumption of water and food reached to the relatively normal limit of 40±5 cc and 30±5 g, respectively and daily urine in treated rats was measured as 35±5 cc. Pancreatic biopsy of normal and diabetic rats confirmed that the islet cells were destroyed due to the effect of streptozotocin in diabetic rats. The comparison of pancreatic biopsy of normal and diabetic rats shows that the tissue of Pancreatic Langerhans and the beta cells of diabetic rats have been degenerated irreversibly, while no change is observed in the tissue under the testis subcutaneous of the normal and diabetic rats.

The diabetic rats did not have a natural living or natural length of life while the healthy and treated rats had a natural life. The diabetic rats gradually lost their eyesight while the healthy and treated rats had natural vision till the end of their life. No discrepancy was seen between the results of our study and those of other researchers. The only newly found, inexpensive and available method now is transplantation of Langerhans islets cells for the treatment of insulin-dependent diabetes mellitus since it is inexpensive, easy to do, available and effective. One of the most important phases of transplantation is standardization of the conditions of dissociation and purification of the Langerhans islets cells. This is easy to do and practical and the researchers, after achieving and stabilizing such a method, will be able to save the diabetic patients by transplanting the Langerhans islets and solve the problem of more than 1.5 M diabetic patients in Iran and 200 M diabetic patients throughout the world. However, the method of transplantation of the Langerhans islets inside the testis subcutaneous of diabetic rats (Fig. 10) successfully shows the transplanted Langerhans islets, i.e., in an immunity-quarantined space, it prevents access of the immunity cells to the external transplanted

cells and prevents rejection of the transplantation. The rat is cured 100% as a result of secretion of the transplanted Langerhans islets cells under the testis subcutaneous. In this method, approximately 5,000 islets are needed for each kg of rat body weight. This method is important because of its simplicity and accessibility.

Discussion

Streptozotocin prevents DNA synthesis in mammalian and bacterial cells. Streptozotocin, which is used intravenously by rapid injection or constant short diffusion, stimulates the tissues. Metabolically, a slight deviation of the glucose-bearing pain from the normal limit has been seen in patients treated with a certain dose of streptozotocin, which is generally reversible. However, the insulin shock, which is one of its other effects, is irreversible (Weiss, 1982; Ikebukuro *et al*., 2002). In this study, the clinical manifestations and also the amount of glucose, insulin and C-peptide after using a 60 mg kg^{-1} dose of streptozotocin, ensured induction of diabetes in rats. Hyperglycemia, hypoinsulinemia, polyphagia, polyuria and polydipsia accompanied by weight loss were seen in adult rats within three days of streptozotocin treatment and within one week to ten days, the amounts of the relevant factors were almost stable, which indicates irreversible destruction of Langerhans islets cells moreover, Researchers around the world have used streptozotocin to create experimental diabetes because it is a simple, inexpensive and available method (Gray and Morris, 1987; Pipeleers *et al*., 1991a). Our results are similar with those of Ikebukuro (2002a) and Elias (1994). With the transplantation of the cellular suspension obtained, it was expected that, due to secretion of insulin by transplanted beta cells, the level of blood serum glucose would fall to the normal, healthy limit and the amount of insulin and C-peptide of the plasma would increase and the clinical manifestations of the disease, such as polyuria, polyphagia and polydipsia would be eliminated. All these were clearly seen immediately and completely in the day after transplantation. The considerable point in this research is that inbred rats were not used as receptor or donor of the transplantation. In spite of this, however, no sign of rejection of the transplantation was observed (Wiukel *et al*., 1982). To explain this, one must say that the phenomenon of immuno-isolation due to the effect of transplantation of Langerhans islets cells, in an immunity-quarantined space, prevented access of the immunity cells to the external transplanted cells and rejection of the transplantation (Rabinovitch *et al*., 1982). Transplantation of Langerhans islets cells is generally used for treating a type of diabetes that results from the autoimmune destruction of beta cells of the islets. Therefore, as expected, this autoimmune process also continues with respect to the transplanted beta cells. In this research, by carrying out transplantation of Langerhans islets cells in parts of the body with a special immuno-isolated position, the risk of destruction of the transplanted beta cells by the autoimmune process in the transplantation receptor was completely eliminated (Yasumizu *et al*., 1987). The technique of transplantation of the Langerhans islets cells inside the testis, subcutaneously, in the absence of immunological inhibitors to support the transplanted tissue against the host immunity system is a new way of success in this path (Thomas *et al*., 1999; Rastellini and Shapiro, 1997). In this process, the islets can be encircled in a semi-permeable membrane that allows food and oxygen to reach to the Langerhans islets and the insulin to be released into the blood flow while, at the same time, it creates a mechanical barrier separating the potentially destructive immunity cells and the antibodies from the islets cells and thus preventing the rejection of the transplantation (Titus *et al*., 2000). Statistical data relating to F, df, values of p, food, water, urine, body weight and SEM in the entire test group, compared to the findings (Lanza and Ecker, 1999; Pipeleers *et al*., 1991; Gray and Morris, 1987) show the greater success of the transplantation of the Langerhans islets in rats as achieved in their work.

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