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Full Length Research Paper

Autologus MSC bone marrow stem cell and allogenic pancreatic stem cell for repair of beta pancreatic cell in experimental diabetes mellitus

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Alternative therapies in diabetes mellitus (DM) management include the use of stem cells. Stem cells derived from the bone marrow and pancreatic cells from allogenic donors were used in this experimental animal model to restore glucose control. DM was induced in Wistar rats with 50 mg/kg alloxan. DM rats were divided into 4 treatment groups, Group 1 was transplanted with autologous bone marrow derived mesenchymal stem cells (MSC) by intraperitoneally injection. Group 2 was given allogenic pancreatic cells intraperitoneally. Group 3 was given insulin subcutaneously, and Group 4 served as control (no treated). The dosage was 200,000 cells/rat. Post therapy results in group 1 revealed significant decrease of blood sugar levels, an increase in insulin levels, and increased C peptide levels. In group 2, there were more pronounced changes, improvements in glucose control compared to group 1. Those receiving only insulin the levels of blood sugar decreased but less so compared to those receiving MSC or pancreatic cells (p = 0.002). In a DM Wistar rat model the intraperitoneal administration of pancreatic cells resulted in better restoration of glucose control than intraperitoneally of bone marrow derived MSC, which in turn was better than only insulin.

Key words: Diabetes mellitus (DM), stem cell, allogenic, autologous.

INTRODUCTION

Diabetes mellitus (DM) is found worldwide, but it more often (especially type 2) occurs in developing countries. The increase in prevalence was greatest in Asia and Africa, as a result of the trend of urbanization and life style changes, including the adoption of a "Western-style" diet (Wild et al., 2004). A wide range of medicinal interventions and life style changes have been attempted so far for the prevention and treatment of diabetes, but its prevalence continues to rise. Therefore (Diabetes Control, 1993; ADA, 2008), we attempted to provide an alternative in the management of diabetes by using stem cells. Alternative therapy used here was based on mesenchymal stem cells, derived from the bone marrow, which were compared to transplanted pancreatic cells harvested from an allogeneic donor. Autologous adult stem cells constitute a source of stem cells to replace pancreatic cells, rejection does not occur after being transplanted. So far, bone marrow has been considered as the preferred source of stem cells in the adult.Mesenchymal stem cells (MSCs) derived from bone marrow have multipotent properties. MSCs will grow and differentiate according to their milieu or environment. *In vivo*, when MSC is inserted into the pancreas, it can be expected that MSCs will differentiate into pancreatic cells, that have both exocrine and endocrine functions. Thus, MSCs transplantation from bone marrow stem cells may repair the pancreas in its role as paracrine organ.

In this comparative experimental *in vivo* study, cells were transplanted to improve the function of the pancreas through two ways, with autologous bone marrow derived MSCs or with allogenic pancreatic cells *in vivo*.

METHODS

Samples

Wistar rats were used, which were divided into 4 groups.

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Tabel 1. Level of blood glucose, C-peptide and insulin before and after aloxan injection.

	Glucose level (mg/dl)				Insulin level (pg/L)	p	C Peptide level (NG/l)	p
	BSN	p	2 PP	p				
Rat before aloxan	85.7 ± 5.33	0.000	98.45 ± 4.67	0.000	0.298 ± 0.242	0.000	0.262 ± 0.177	0.002
Rat after aloxan	135.05 ± 21.25		206.65 ± 58.27		0.057 ± 0.041		0.168 ± 0.132	

Group 1 was treated with MSC transplantation, Group 2 was treated with pancreatic cell transplantation, Group 3 was treated with insulin and Group 4 served as controls. All animals were first rendered DM with alloxan injection in a dose of 50 mg/kg intraperitoneally, and then fasted for 72 h, and levels of C peptide and insulin were measured by ELISA (Mercodia). The results were compared between pre-injection and post-injection. In addition, we measured blood sugar levels Accu-chek active stick (Roche, Indonesia) both before and after aloxan injection.

The diagnosis of diabetes was established by measuring blood glucose, C-peptide and insulin levels (Purnamasari, 2009). Blood sugar level was determined by measuring venous blood taken from the tail vein and measured using Accu-chek active stick. Glucose levels were measured at fasting and 2 h after meals. Normal sugar level in mice was 60 to 120 mg /dl. Determinationof C-peptide and insulin levels was doneusing ELISA method. Table 1 shows that alloxan treatment was effective in reducing insulin and C-peptide levels and in raising the blood glucose levels in these animals.

Mesenchymal stem cells isolation and culture from bone marrow

Mesenchymal stem cells were derived from bone marrow using aspiration and separation on Histopaque-1.077 (Sigma). Harvested cells were cultured in Dulbecco's Modified Eagles Medium (DMEM) containing 1.0 g / L glucose. MSC characterization was performed by analyzing the expression of CD44 + and CD 105 + by using DAB immunostaining and FACS (BD).

Pancreatic isolation and culture

Pancreatic cells were isolated from pancreatic organs taken from Wistar rats (Demeterco et al., 2000). To obtain pancreatic cells, minced pancreatic organs were digested using trypsin (Sigma) for dissociation of tissue for 40 min at 37°C. Subsequently, 1.5 ml foetal bovine serum (Gibco) was added to halt further digestion and the suspension was centrifuged at 1600 rpm for 10 min, and the supernatant was discarded. The pellet was cultured in RPMI medium and Insulin transferin selenium medium (ITS).

After 21 day cultured, cell was characterized by examining the levels of insulin and C-peptide secreted and nestin expression in pancreatic cells (Shapiro et al., 2000). C-peptide and insulin were measured using ELISA method, whereas nestin immunofluorescence was examined using indirect methods (Rantam et al., 2009). When pancreatic cell was confluence, the cells were harvested and transplanted. Transplantation: DM rats were divided into four groups, each consisting of 6 animals.

Group 1 was given with 200,000 MSC cells,Group 2 was treated with 200,000 pancreatic cells, Group 3 was treated with subcutaneous insulin 1 unit/kg BW, 3 times 15 min before meal, and untreated Group 4 served as control. Cells were given intraperitoneally. After cells transplantation we measured the levels of blood glucose, C-peptide, andinsulin on indicated intervals and immunohistochemistry to examine expression of HE and PDX1.

RESULTS

Three months after aloxan injection, we measured the level of blood glucose, C-peptide and insulin. We also examine immunohistochemistry. Revealed of blood glucose, C-peptide and insulin level as seen on Table 1.

Level of blood glucose (fasting and 2 h post prandial) was significantly increasing after aloxan injection. Level of C-peptide and insulin were significantly decreasing after aloxan injection.

Mesenchymal stem cell (MSC) and pancreatic cell characterization

Stem cells were characterized before being administered. In this study MSCs phenotypes characterization used DAB immunostaining to observe CD 44+ and CD105+ expression. The positive result was shown in brown color as seen in Figure 1.

After going through the stages of isolation and culture of pancreatic cell, the results obtained are shown in Figure 2.

Characterization of pancreatic cells in this study used ELISA method for the measurement of insulin and C peptide levels, and indirect immunofluoresence methods to observe nestin expression (Figure 4). Measurement of insulin level and C peptide revealed insulin was secreted by pancreatic cell beginning passage , increasing on every passage and have a peak level on passage 4. C peptide was secreted also on passage 1, increasing by passage and have a peak level on passage 4 (Figure 3).

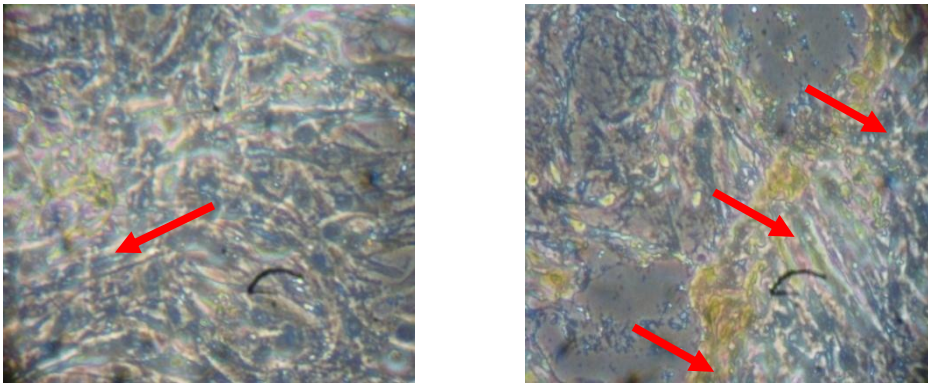

Figure 1. Characterization of MSCs by DAB immunostaining. Left, CD44 expression. Right, CD 105 expression. 40× magnification.

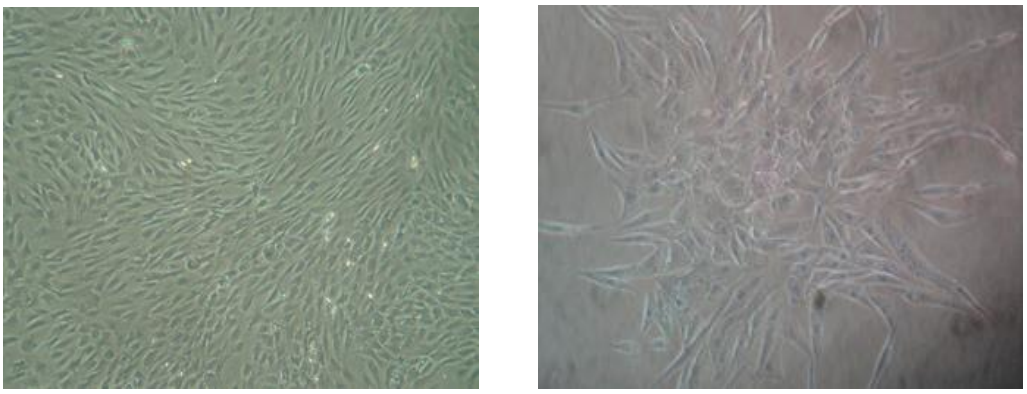

Figure 2. Pancreatic cells in culture. Left, the growth of pancreatic cells on day 21 showing cells in distinct clusters. Right, pancreatic cell growth at day 5 showing confluent layer of cells. Inverted microscope, 40× magnification.

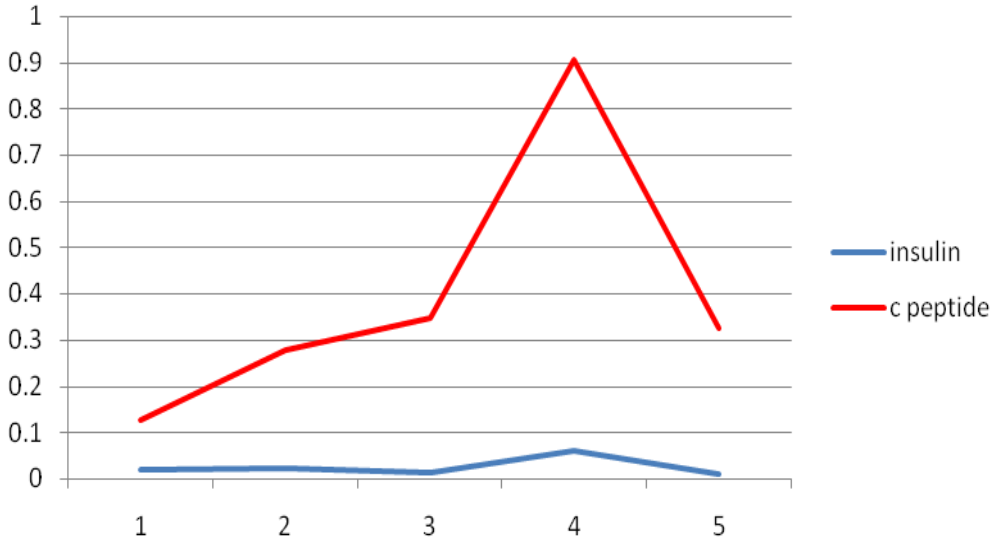

Figure 3. C peptide and insulin levels measured by ELISA in the cultured pancreatic cell supernatant, increasing by passage cell and have peak level on passage 4.

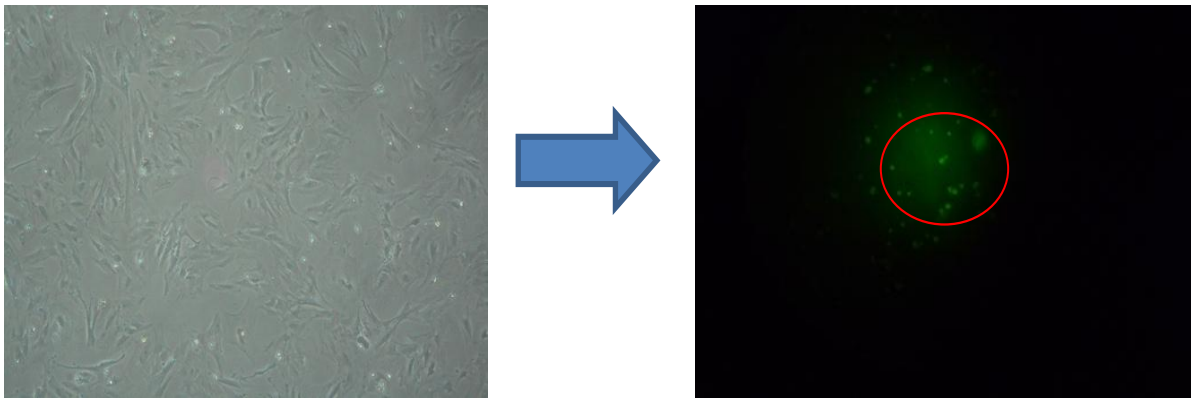

Figure 4. Nestin expression of *invitro* explanted pancreatic cells. Left, mature pancreatic cells Right, cells showing nestin-specific green fluorescence. 40× magnification

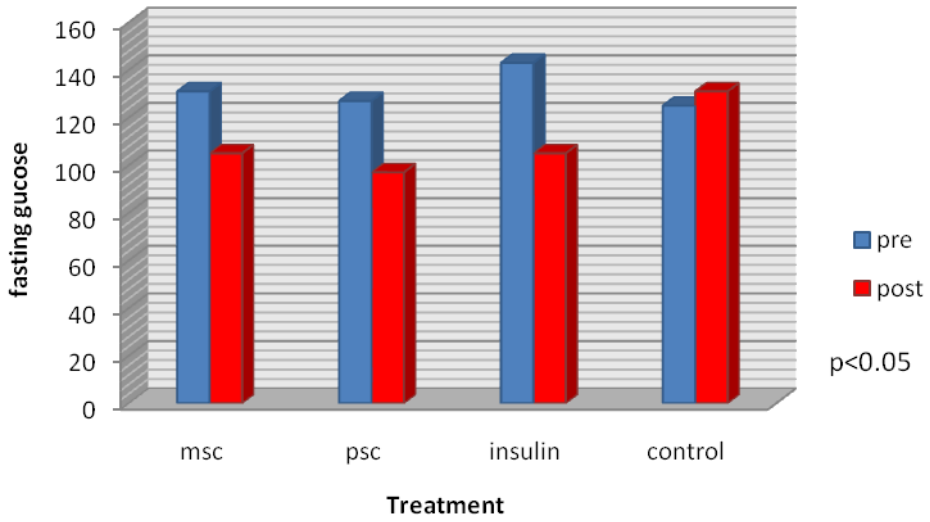

Figure 5. Fasting plasma glucose level before and after transplantation of autologus MSCs, allogenic PSCs compare with insulin dan control.

Mesenchymal stem cells (MSCs) and pancreatic cell application

Result of fasting plasma glucose dan 2 h post prandial post-transplant using autologus MSCs 2 × 10^6/kg BW, allogenic PSCs 2 × 10^6/kg BW compare with insulin sub cutan and control as seen Figures 5 and 6.

Fasting and 2 h post prandial plasma glucose level was significantly decreasing after transplantation of MSCs and PSCs, also after insulin treatment but not in control group

We measure level of insulin and C peptide after autologus MSCs and allogenic PSCs transplantation compare with insulin treatment and control. The result is shown in Figures 7 and 8.

C peptide and insulin level was significantly increasing after transplantation of MSCs and PSCs, also after insulin treatment but not in control group.

We examine immunohistochemistry after transplantation of autologus MSCs and allogenic PSCs compare with insulin treatment. Marker for immunohistochemistry used PDX1 as insulin promotoer and HE as recovery of B cell pancreas. The result of immunohistochemistry is shown in Figure 9.

Normal pancreatic rat expression PDX1 more than 75%, after induce by aloxan 50 mg/kg the PDX1 expression on rat DM was decreasing below 25%. After transplantation with autologus MSCs and allogenic PSCs, the PDX1 expression become increasing. The PDX1 after allogenic PSCs transplantation increased with over 75%. That was more higher than PDX1 expression after autologus MSCs transplantation was range 25 to 50%. The result PDX1 expression after insulin treatment did not increasing below 25%.

Similarity with PDX1 expression, HE expression on

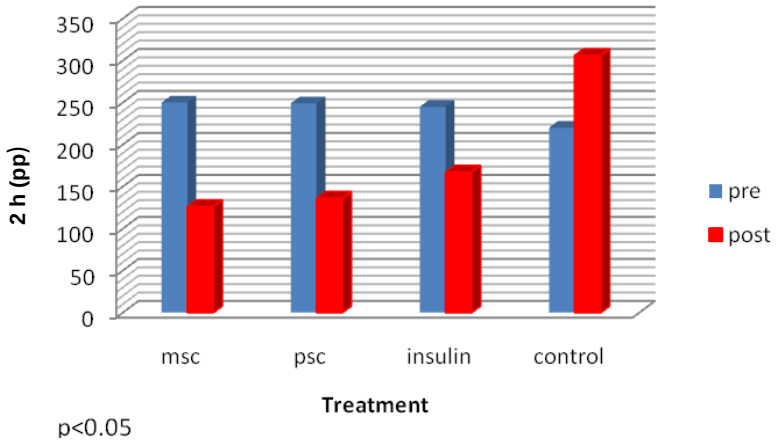

Figure 6. Two hours post prandial plasma glucose level before and after transplantation of autologus MSCs, allogenic PSCs compare with insulin and control.

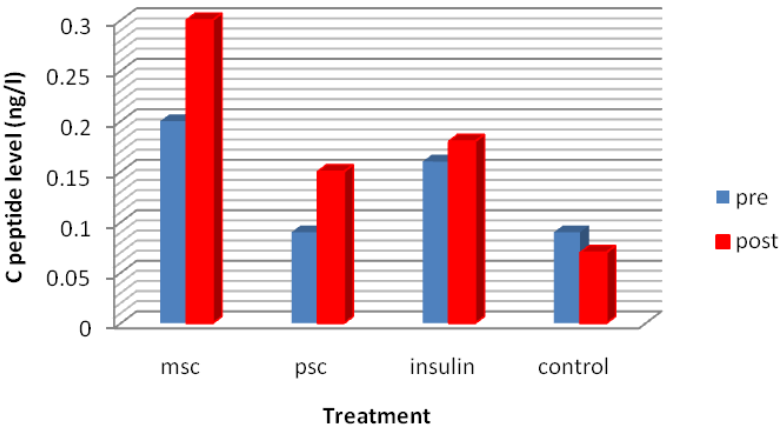

Figure 7. C peptide level before and after transplantation of autologus MSCs, allogenic PSCs compare with insulin and control.

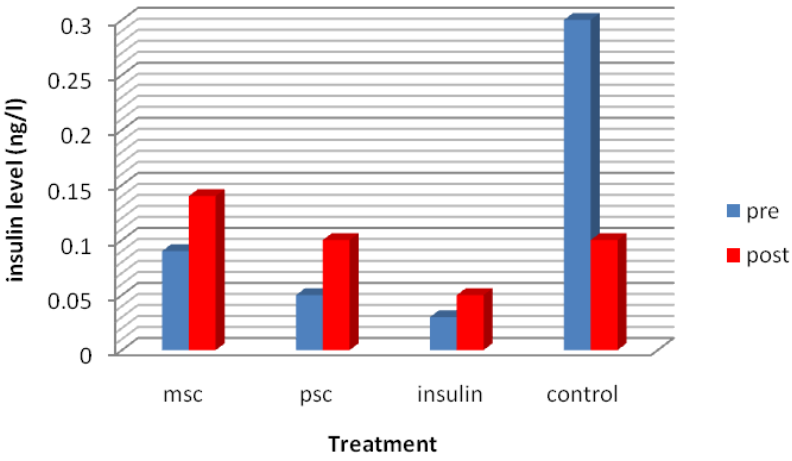

Figure 8. Insulin level before and after transplantation of autologus MSCs, allogenic PSCs compare with insulin and control.

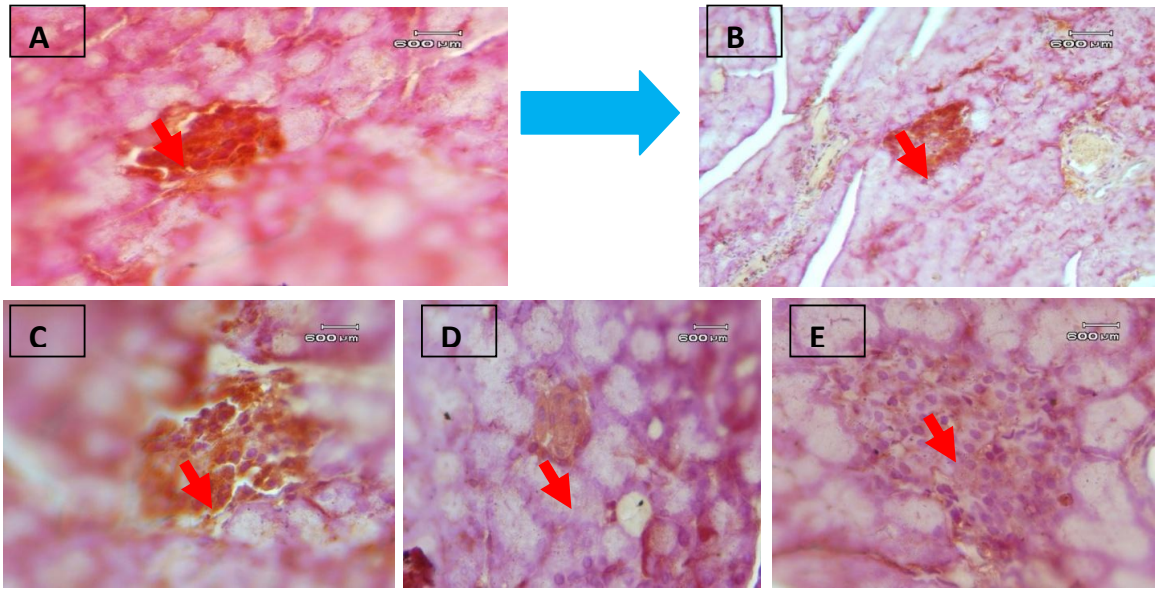

Figure 9. PDX1 expression by immunohistocemistry.A. Normal pancreatic rat. B. Diabetic pancreatic rat. C. Diabetic pancreatic rat after 1 month insulin treatment. D. Diabetic pancreatic rat 1 month after allogenic PSC transplantation. E. Diabetic pancreatic rat 1 month after autologus MSC transplantation.

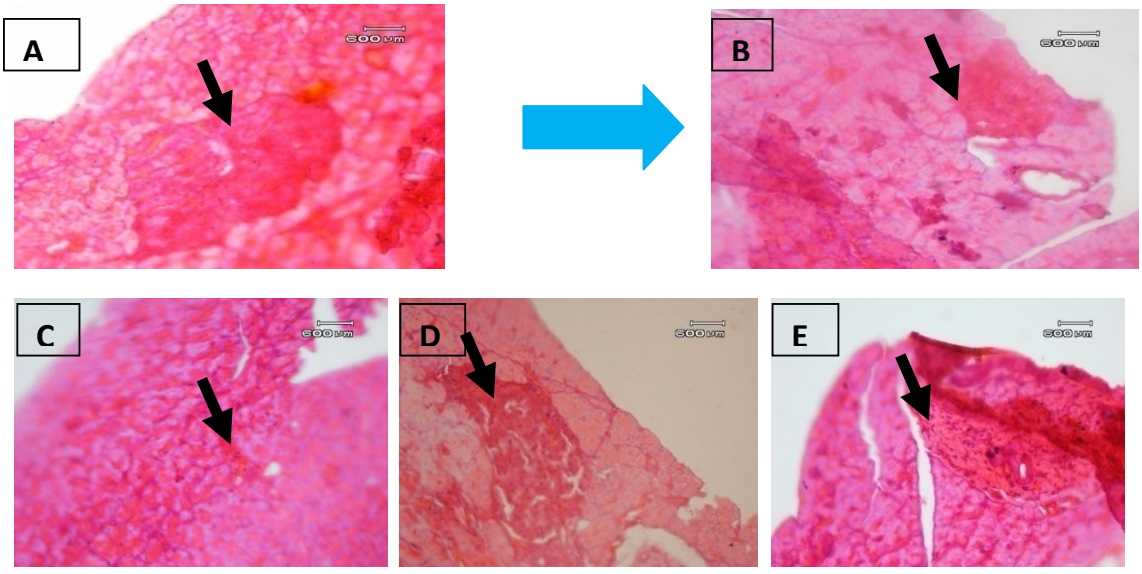

Figure 10. HE expression by immunohistocemistry.A. Normal pancreatic rat. B. Diabetic pancreatic rat. C. Diabetic pancreatic rat after 1 month insulin treatment. D. Diabetic pancreatic rat 1 month after allogenic PSC transplantation. E. Diabetic pancreatic rat 1 month after autologus MSC transplantation.

normal pancreatic rat was over than 75% and become decreasing after injection by aloxan. Increasing of HE expression was shown after autologus MSCs transplantation (more than 50 to 75%) and allogenic PSCs transplantation (more than 75%), but HE did not express too much on diabetic rat with insulin treatment, only below 25% (Figure 10).

DISCUSSION

Diabetes mellitus is caused by absolute reduction of insulin production, thus requiring chronic supplementation with exogenous insulin (DeFronzo et al., 2004). It would be biologically preferable to regenerate the capacity of the pancreas to produce insulin itself. In this study we

showed that injection of bone marrow derived autologous stem cells and allogeneic pancreatic cells can at least in part, reconstitute the insulin production in an established animal model of DM. Prior to use, MSCs suspensions were first validated for its purity level, so that they could be directly. In this study, the level of purity was measured using CD105 and CD44 expression. This indicated that the cells were indeed mature MSCs (Dominici et al., 2006)

Mesenchymal stem cells are precursors of non-hematopoietic tissues (for example, muscle, bone, tendons, ligaments, adipose tissue, and fibroblasts) that are relatively easily obtained from autologous bone marrow. These cells remain multipotent after *in vitro* expansion, suggesting a relatively low immunogenicity, and easy to store at subzero degrees. MSCs are hypoimmunogenic and can escape from host immune elimination. MSCs will grow and differentiate according to their environment. *In vivo*, when injected into the pancreas, it is expected that MSCs will differentiate into pancreatic cells that have both exocrine and endocrine functions. Thus, transplantation of MSCs from bone marrow stem cells can repair the pancreas in its role to provide paracrine effects and other cell differentiation effects. There was a report that bone marrow stem cells can differentiate into cells that can secrete insulin in vitro (Baksh and Tuan, 2007; Sotiropoulou et al., 2006) but there are some researchers who were not able to confirm this finding (Bartholomew et al., 2002), so the claim remains in doubt.

The pancreasis an organ that has limited ability to proliferate (Soria et al., 2001). However, these cells can proliferate and differentiate *in vitro* (Demeterco et al., 2000). As they are cultured *in vitro*, pancreatic cells will proliferate and differentiate into islet cells (Bouwens, 1998). To obtain the desired result of pancreatic cells were cultured in serum free media insulin-transferrin selenium (ITS), consisting of nicotinamide and keratinocyte growth factor. In a few days the cells grow confluent with tridimensional formation of that produces insulin and glucagon. Before being applied, the pancreatic cells were first validated for purity levels using insulin, C peptide, and nestin. Insulin and c peptide examination using elisa found levels of insulin and C peptide in cultured cells supernatant, suggesting that those cells secrete insulin and c peptide. Characterization of pancreatic cells also used nestin expression through immunonofluorescence examination, revealing positive nestin expression. Positive nestin expression showed a specific character of pancreatic stem cells, both in its exocrine and endocrine functions.

In post-therapy using MSCs, pancreatic stem cell and insulin, it was found that the regaining proper glucoase control, insulin and C peptide secretion and repair B cell pancreas also was best attained by pancreatic cells intraperitoneally. Possibly, this was because pancreatic cells populations used contain progenitor cells that function directly in endocrine and exocrine manners, whereas MSCs are multipotent stem cells, which take time to differentiate into pancreatic cells.

In conclusion intraperitoneal pancreatic cell injections provide better responses than intraperitoneal MSC and subcutaneous insulin in controlling and repair B cell on diabetes indiabetic Wistar rats.

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