Oral Presentation (PCS-11)

Mesenchymal Stem Cell-Conditioned Medium Improve the Recovery of Pancreatic α and β Cells in Type 1 Diabetes Mellitus

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Keywords: MSC-CM, pancreatic α-cell, pancreatic β-cell, type 1 diabetes mellitus.

INTRODUCTION

Mesenchymal stem-cell is a pluripotent cells that can be differentiated into many kinds of cells (1) and have abilities to differentiated into mature cell, secreted cytokines, and releasing many kinds of protein functioned in body body regulation (2). However, the therapeutic effect of stem cell arise from their secreted factor such as growth factors, cytokines, chemokines, and metabolits which acts as biologic regulator in the autocrine and paracrine body function (3). Secreted factors of mesenchymal stem cells known as secretome or mesenchimal stem cell conditioned medium (MSC-CM) have therapeutic effect for antiapoptosis, angiogenic, immunomodulatoric, and chemoattraactive activity (4). Mesenchymal stem cell conditioned medium used as the replacement agent for cell therapy because it contains no cells and save for used in different individual (5).

Type 1 diabetes mellitus (DMT1) is a disorder characterized by destruction of the insulin-producing pancreatic β-cell that progressively leads to insulin deficiency and resultant hyperglycemia. Insulin deficiency can lead to progressive metabolic derangement, with worsening hyperglycemia, ketoacidosis, starvation, and death (6). Glucagon is a hormone produced by pancreatic α cells. Glucagon releases by the pancreas to raise the concentration of glucose in the bloodstream when the concentration of glucose in the bloodstream too low (7). Glucagon worked in opposite with insulin, which lowers the extracellular glucose level, while insulin works to increase the extracellular glucose level (8).

The aim of this study was to investigate the role of MSC-CM on the structural and functional regeneration of pancreatic α and β cells in Wistar rat (*Rattus norvegicus*) induced with type 1 diabetes mellitus. The datas in this study will completes the information about the effect of MSC-CM on pancreatic cells regeneration, which has been presented in previous study (9).

MATERIALS AND METHODS

Thirty male Wistar rats (*Rattus* norvegicus) were used in this study. Rats divided into two groups: control and MSC-CM treated group (injection of 0.05 ml/kg b.w. MSC-CM).

Rats induced to DMT1 by single dose 125 mg/kg b.w. alloxan monohydrate intramuscular injection. Rats in MSC-CM treated group received MSC-CM injection at week-1, 2, 3, and 4. Three rats from each groups were euthanized every week respectively. Pancreas was collected for histological examination. Pancreas divided into 3 region: gastric, splenic, and duodenal regions. Histologic preparation was done by paraffin method. Pancreatic tissue then stained with immunohistochemistry method for insulin and glucagon. Results were analyzed descriptively.

RESULT AND DISCUSSION

Histological observation detected the change in profile of pancreatic α and β-cells balance in the islets of treated group after first injection of MSC-CM. The number and size of the islets increased each week after injection in insulin immunohistochemistry stain. The presence of insulin-positive cells increased every weeks for four weeks in all region of pancreas (splenic, gastric,a nd duodenal). The number of glucagon-positive cells were increased on first week of MSC-CM administration and decreased dramatically at second week until the fourth week after the administration of MSC-CM in all region (splenic, gastric, and duodenal) of pancreas.

In the control group, there was no insulin-positive cells detected, while glucagon was detected in small number. In week-1 to 2 after injection, the number of insulin-positive cells and glucagon-positive cells were increasing. In week-3 to 4 after MSC-CM injection, the number of insulin-positive cells keep increasing, however the glucagon-positive cells were decreasing.

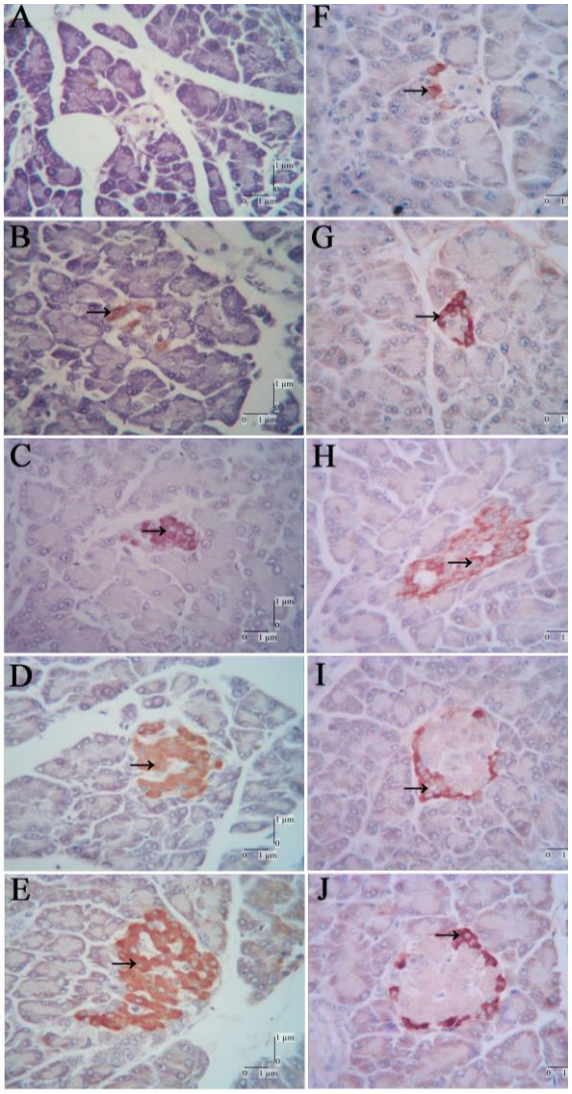

Figure 1. Immunopositive cells of insulin (A-E) and glucagon (F-J) pointed by black arrow in the islet of Langerhans, splenic region of pancreas. There was no insulin-positive cell detected in control (A). Note the size of islet and number of insulin-positive cell was increasing from 1 (B), 2 (C), 3 (D) and reach maksimum size at 4 (E) weeks after MSC-CM injection. The glucagon-positive cells detected in small number in control (F), and the number increasing at 1 (G) and 2 (H) weeks after MSC-CM injection, but decreasing at 3 (I) and 4 (J) weeks after MSC-CM injection. (Insulin and glucagon immunohistochemistry staining, 500x magnification).

We managed to make rats model of type 1 diabetes mellitus with single dose alloxan injection (9), indicated by the absence of insulin-positive cells detected in control group. The presence of small islets and insulin-positive cells started to show in week-1 after MSC-CM injection, and gradualy increased until week-4. This indicated that the administration of MSC-CM successfully increasing the regeneration of β-cells that produce insulin in the pancreas. The increased number of insulin-positive cells then affected the α-cells that produces glucagon. In week-1 to 2 after MSC-CM injection, the glucagon-positive cells detected with high number compared to insulin-positive cells, but they gradually decreasing in week-3 to 4 (Figure 1). The body regulation can maintain the balance of α and β cells in pancreatic islets. The balance of insulin and glucagon production then maintain the glucose concentration in bloodstream. According to the previously study about diabetes mellitus (10), the changes in number of insulin-positive cells in pancreatic islets were matching with glucose concentration in the bloodstream.

Our MSC-CM has been studied before can accelerate the regeneration of burn wound skin (11), skin wound (12), diabetes mellitus type 1 (9), and diabetes mellitus type 2 (10). This study, however, aims to know the effect of MSC-CM injection in DMT1, in relation with regeneration of both α and β-cells in all regions of pancreas (gastric, duodenal, and splenic). Factors contained in MSC-CM have a possibility to help the body to regenerate the damaged in pancreas in relation to DMT1. Injection of MSC-CM in DMT1 rats can increasing the regeneration of the β-cells which is destructed in DMT1 case.

CONCLUSION

Mesenchymal stem cell conditioned medium injection once a week for 4 weeks can improve the regeneration of pancreatic β-cells as indicated by the increasing of insulin-positive cells, followed by the decreasing of pancreatic α-cells as indicated by the small number of glucagon positive cells in rats with DMT1.

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