Histomorphology of Pancreatic Islet in Physiological Aging Female Rats Post Intravenous Human Wharton's Jelly Mesenchymal Stem Cell Injection

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ABSTRACT

The increasing population of aged people will have the important role in the life, but the function of their bodies will decrease because of aging. Aging will increase the risk of degenerative disease, one of example is diabetes. The disease is related to the aging in the pancreatic organ which progressively declines by age. The aimed of the experiment was to determine the effect of human wharton's jelly mesenchymal stem cells by injecting intravenously in aging female rats. This study used 3 young female rats (3 months) and 6 aging female rats (24 months). The experiment consisted of three groups. The young control group (A), the aging control group (B) that received NaCl (0.9%) 0,4 mL, the aging treatment group (C) received 1 x 10^6 cells/kg of human wharton's jelly mesenchymal stem cells 0,4 mL. The aging control and the aging treatment group were injected 4 times with the interval in 3 months. The end of the experiment (12 months), the rats were anesthetized and sacrificed. The pancreatic tissues were collected to examine the pancreatic islets by histology studies. Changes of the pancreatic islet in control and treated groups were examined using hematoxylin and eosin staining. These findings conclude that injecting human wharton's jelly mesenchymal stem cell increase the diameter and total pancreatic islet in the treatment group. In other side, the cell population of pancreatic islet also have significant differences ($P<0.05$) in treated physiological aging female rat groups than control aging female rat group.

Keywords: aging, pancreatic, rat, stem cells

INTRODUCTION

The proportion of elderly people (≥ 65 years old) has been increasing in the world. It is about 450 million people and will increase through the time (Kane *et al.,* 2009). It also will play an important role in life, but the body's function will reduce and increase the risk of various degenerative diseases (Hadley *et al.*, 2005). One of example is diabetes type II which is affected by pancreatic aging (Monickaraj *et al.,* 2012). The glucose metabolism, insulin sensitivity and insulin secretion are factors related to the function of pancreatic organs (Cuesta *et al.*, 2013).

Many kinds of treatment have been done to repair the pancreatic function, one of example is transplantation. It is to be the best way to overcome the diabetes (Hering *et al.,* 1993). Over the time, there are some of transplantation

type such as transplantation of organ and transplantation of cell. But, transplantation of organ have a big risk to the patient related with infection, bleeding until immune rejection post operation. Meanwhile, transplantation of cell is better than transplantation of organ. It can repair the right damaged cell or organ and minimizes the implantation of unnecessary tissue or organs (Halim *et al.,* 2010).

Stem cell is the gold standard therapy to increase the pancreatic function in type 1 diabetes (Guo and Hebrok, 2009) and type 2 diabetes (Guan *et al*., 2015). Transplantation of mesenchymal stem cell can improve the pancreatic function in type 2 diabetes patient (Guan *et al.,* 2015). However, in other study says that transplantation of wharton's jelly mesenchymal stem cell (hWJ-MSCs) intravenously can repair the β cell function and decrease the damaged pancreatic islet (El-Hossary *et al.,* 2016). The purpose of this study was to know histomorphology of pancreatic islet in physiological aging female rat post intravenous xenotransplantation human wharton's jelly mesenchymal stem cell.

METHODS

Experiment Animal

This study used 3 young female rats (3 months) and 6 aging female rats (24 months) of Sprague-Dawley (SD) which purchased from Veterinary Medicine Laboratory Animal Bogor Agricultural University (UPHL). This study had approved by Animal Care and Use Committee (ACUC) Veterinary Hospital of Bogor Agricultural University, Indonesia (license: 21-2016 ACUC RSHP FKH-IPB). Rats were given feed and standard drinking water *ad libitum*. The condition of study was in 12h light and in 12h dark.

Isolation and Experimental Design of Human Wharton's Jelly Mesenchymal Stem Cell

For the isolation and identification of human wharton's jelly mesenchymal stem cell (hWJ-MSCs), we used the same procedures as previously described (Widowati *et al.,* 2014). The time of this study was 12 months. All rats were sacrificed in the end of this study. They were divided into 3 groups:

Group A : Young female rats (3 months, n : 3) with no treatment.

Group B : Aging female rats (24 months, n : 3) received NaCl (0.9%) 0,4 mL was injected 4 times with the interval in 3 months (control) through the tail vein injection.

Group C : Aging female rats (24 months, n : 3) received 1 x 10^6 cells/kg of human wharton's jelly mesenchymal stem cells was injected 4 times with the interval in 3 months (treatment) through the tail vein injection.

Histology

The preparation of the sample and the histology method was done according to Haematoxylin-Eosin (HE) staining protocols (Kiernan, 1990). Microscopic studies of the islet pancreatic was observed from each treatment of histology slide using Olympus CX31 light microscope (Olympus, Tokyo, Japan. The number of

pancreatic islet and the population cell of pancreatic islet were counted. Pancreatic islet diameter was also measured with micrometer representation and was compared with control for statistical analysis.

Statistical Analysis

The data was examined using SPSS statistical package (SPSS version 23). Data were analyzed by completely randomized design for diameter and number of pancreatic islet also the population cell of pancreatic islet at P<0.05 (95%). The observation of histological analysis were determined descriptively.

RESULT

The average of islet diameter in young control rats was 137,43 ± 37,43 µm. It was different with the aging control rats which had the greater diameter than young rats. It was 189,68 ± 22,32 µm (**Figure 1**). But, the average of number of islet was lower to 9,66 ± 0,57 in aging control rat model than the young rats 13,66 ± 1,15 (**Figure 2**) and the population cell of pancreatic islet in control aging rat was significantly reduced to 54,20 ± 7,79 than the young control group 139.13 ± 31.15 (P<0.05) **(Figure 3).** The data showed that there were morphological changes and decreasing population cell significanly (P<0.05) related with age in aging female rat. After injecting hWJ-MSCs dose 1 x 10^6 cells/kg, the diameter of treatment aging groups increased to 262.15 ± 80,00 µm, the number of pancreatic islet increased to 13, 66 ± 1,52 and the population cell of pancreatic islet increased significanly to 190 ± 65.39. These result indicate that hWJ-MSCs intravenous transplantation to physiological aging female rat could repair morphological changes and increase population cell significanly (P<0.05).

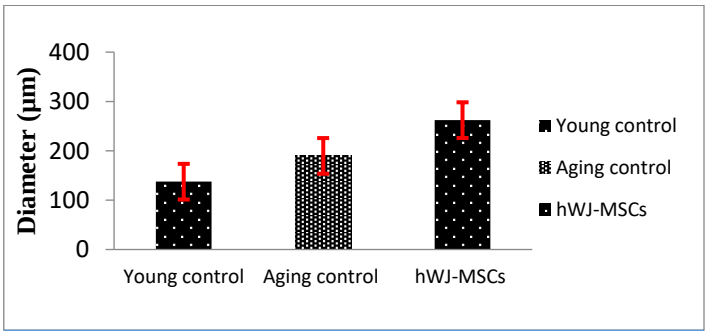

Figure 1. Diameter average graph, group control and group hWJ-MSCs (1 x 10^6 cells/kg).

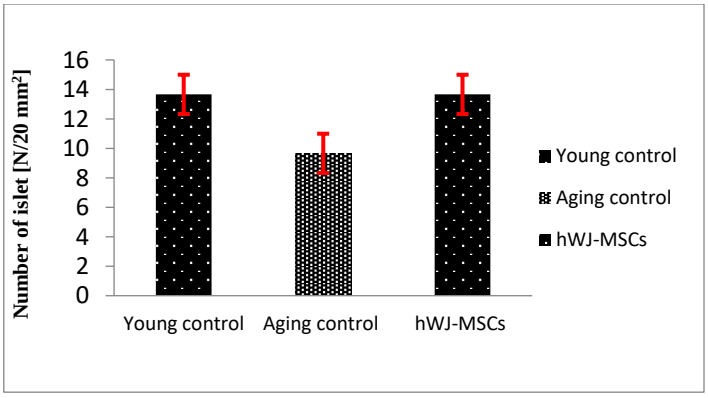

Figure 2. Number of islet average graph, group control and group hWJ-MSCs (1 x 10^6 cells/kg).

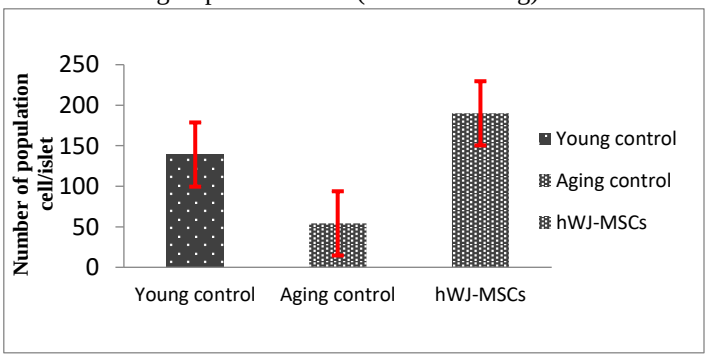

Figure 3. Number of population cell in pancreatic islet, group control and group hWJ-MSCs (1 x 10^6 cells/kg) Note:* (P<0.05).

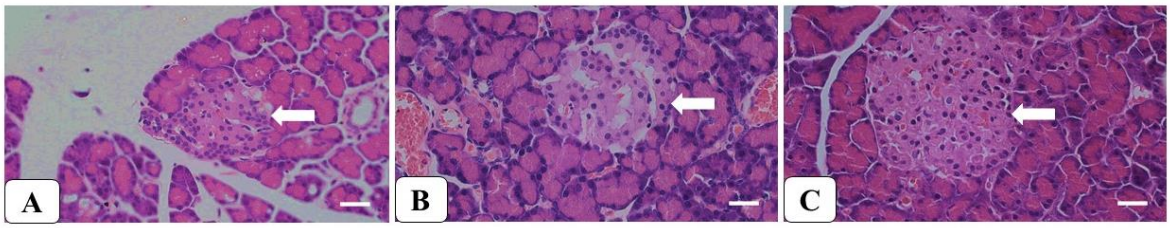

Figure 4. (A) Histology of pancreatic in young control group, (B) Histology of pancreatic in aging control group (C) Histology of pancreatic in the treatment group, by using hematoxylin and eosin staining. Magnification: 40x; Scale bar: 50 πm.

DISCUSSION

The histomorphometric study showed that the aging control rat had greater diameter of pancreatic islet than the young, but the total of pancreatic islet in aging rat was lower than the young. In other hand, mean of total of population cell decreased significanly. It proved that the morphology of aging pancreatic changed and accelerated with age. Pancreatic cell function are affected by aging (Adelman, 1989).Aging is one of factor which is related with β cell change and the reduction of population cell in pancreatic tissue. These more occur in aging rat (1-2 years old) than in young (5 weeks and 2,5-7,5 months) (Aguayo *et al.*, 2017). Aging factor, obesity and hyperglicemia change the morphology of pancreatic islet and the total of producing insulin cell ((Winarto *et al.*, 2001). The other factor which make pancreatic function alteration is the sensitivity of islet to ER (endoplasmic reticulum) stress. Aged mice is more sensitive than young and it makes apoptosis in islet. CHOP (CCAAT-enhancer-binding protein homologous protein) has main function to make apoptosis, ER stress also cell destruction in pancreatic islet (Puthalakath *et al.*, 2007).

Injecting wharton's jelly mesenchymal stem cell intravenously at a dose 1 x 10^6 cells/kg to aging rat had effect to increase diameter, total and population cell of pancreatic islet in aging rat. The data showed that injecting wharton's jelly mesenchymal stem cell intravenously at a dose 1 x 10^6 cells/kg to aging rat had the best way to repair the pancreatic islet morphology. In other study proves that injecting human wharton's jelly mesenchymal stem cell intravenously in diabetic rat model can repair the damage of pancreatic islet. It increase total, area and diameter of pancreatic islet (El-Hossary *et al.*, 2016). in hypoxia condition, pancreatic islet which are cultured with MSC increase function, repair the form and decrease the apoptosis (Lu *et al.*, 2010).The morphological amelioration of pancreatic islet by injecting mesenchymal stem cell is influenced by chemokines (El-Hossary *et al.*, 2016). The damaged pancreatic islet express CXCL12, CX3CL1, CXCL16, CCL19 and CCL3 [17] which struggle to link with the chemokines expression released from mesenchymal stem cell (CXCR4, CX3CR1, CXCR6, CCR1 dan CCR7) (Sordi *et al.*, 2009). This mechanism explain how mesenchymal stem cell make home to injured site (Sordi, 2009). When homing to damaged part, mesenchymal stem cell can produce bioactive features to form a microenvironment that encourages cell regeneration and prevents T-cell reaction (Bell *et al.*, 2012).

CONCLUSION

The conclusion of this study show that pancreatic islet morphology in physiological aging female rat change related with the age. Injecting wharton's jelly mesenchymal stem cell intravenously at a dose 1 x 10^6 cells/kg in physiological aging female rat can repair pancreatic islet morphology by increasing diameter, total and population cell significanly.

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