Scholars Journal of Agriculture and Veterinary Sciences
OPEN ACCESS
Abbreviated Key Title: Sch J Agric Vet Sci
ISSN 2348–8883 (Print) | ISSN 2348–1854 (Online)
Journal homepage: https://saspublishers.com

Body Weight Improvement in Rats with Type 1 Diabetes Mellitus Treated with Secretome

Dian Bekti Hadi Masithoh[1], Yuda Heru Fibrianto[2], Teguh Budipitojo[3*]

[1]Doctoral Students of Veterinary Science, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
[2]Departement of Physiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia
[3]Department of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

DOI: 10.36347/sjavs.2023.v10i12.002 | **Received:** 14.11.2023 | **Accepted:** 20.12.2023 | **Published:** 23.12.2023

***Corresponding author:** Teguh Budipitojo
Department of Anatomy, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract **Original Research Article**

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by the decreasing of insulin production due to damage to pancreatic β-cells. Secretome is a substance that found in stem cell media have abilityto regeneration cell because contain cytokines and growth factors. The aim of this study was to determine the roleof the secretome in regenerating pancreatic cells to increase body weight in rat with diabetes mellitus type 1. Thisstudy used 60 rats and were divided into healthy control group; diabetes mellitus group; treatment group was induced with streptozotocin and injected with a secretome dose of 0.05; 0,1; 0,2 ml/kg body weight intraperitoneally once a week for four weeks intraperitoneally. The body weight of the rats was measured daily using a digital scale for four weeks. The results of this study showed a significant difference in the body weightsof each group (p = 0.000). The lowest average body weight was observed in the diabetes mellitus group, whereasthe highest average body weight was observed in the healthy control group. In the group that received treatment, the pattern that could be seen was that the more doses given, the more the body weight tended to increase. This study was confirming that there was increasing body weight in diabetes mellitus type 1 that treated with secretome.

Keywords: body weight, diabetes mellitus type 1, pancreas, regeneration, secretome.

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by the decreasing of insulin production due to damage to pancreatic β-cells, which results in an increase in blood glucose levels of more than 200 mg/dL (Swinkels *et al.*, 2018). By 2030, this number is expected to increase to 578 million, representing 10.2% of the world's total adult population and further increase to 700 million in 2045, representing 10.9% of the world's total adult population (Saeedi *et al.*, 2019). T1DM can cause various complications, such as coronary heart disease, heart attacks, high blood pressure, high cholesterol, high triglycerides, stroke and heart failure, hypoglycemia, neuropathy, nephropathy, diabetic ulcers and weight loss (Rawshani *et al.*, 2017; ADA, 2018). Therapy that commonly used in T1DM patients is pancreas transplantation; however, this therapy is often rejected and worsens the patient's condition (Eriksson *et al*, 2009). Therefore, new therapy is needed that can regenerate the pancreas to induce natural insulin formation.

The secretome is a factor found in the stem cell media (McInnes and Gravallese, 2021). The secretome consists of various cytokines, cytokines, chemokines, angiogenic factors, and growth factors (Keshtkar *et al.*, 2018). Unlike stem cells, secretomes are easy to produce, can be packaged in freeze-dried form and are easy to transport. In addition, there is no need for a match between donor and recipient as is the case with stem cells (Brovkina and Dashinimaev, 2020; Warshauer *et al.*, 2020). The secretome plays anti-inflammatory, angiogenic, immunomodulatory, anti-fibrosis, neuroprotective roles, and increases cell proliferation (Vija *et al.*, 2009).

The aim of this study was to determine the role of the secretome in regenerating pancreatic cells to increase body weight in rat with diabetes mellitus type 1. The results of this investigation will add to the knowledge of the influence of the secretome on pancreatic cell regeneration provided in a previous study (Nugroho *et al.*, 2016)

MATERIAL AND METHOD

Ethical Approval

This research was conducted at the Experimental Animal Laboratory, Faculty of Veterinary Medicine, UGM with Ethical Clearance (EC) number No 024/EC-FKH/Int./2018.

Sample

This study used 60 Wistar rats, male and 2 months old. Rats were divided randomly into five groups, namely: Group 1 was a healthy control group that was not given any treatment; Group 2 was the diabetes mellitus group induced with streptozotocin; Group 3 was the treatment group which was induced with streptozotocin and injected with a secretome dose of 0.05 ml/kg body weight; Group 4 was the treatment group that was induced with streptozotocin and injected with a secretome dose of 0.1 ml/kg body weight; Group 5 was the treatment group that was induced with streptozotocin and injected with a secretome dose of 0.2 ml/kg body weight. Diabetes mellitus was induced inRatsusing streptozotocin at a dose of 40 mg/kg body weight intraperitoneally (Guo *et al.*, 2018). Streptozotocin injection was administered for five consecutive days, and diabetes mellitus was achieved if blood glucose was ≥ 200 mg/dl. Secretome injection and data collection. Secretome injection was performed intraperitoneally once a week for four weeks intraperitoneally. The secretome dose used in group 3 was 0.05 ml/kg body weight, group 4 was 0.1 ml/kg body weight, and group 5 was 0.2 ml/kg body weight. The body weight of the rats was measured daily using a digital scale for four weeks.

Analysis

The body weight results were analyzed using the ANOVA test followed by Duncan's post hoc test using the SPSS 16 application.

RESULT

The result of body weight analyzed, there were a significant difference in the body weight of each group (p = 0.000) (Table 1). The lowest average body weight was observed in the diabetes mellitus group, whereas the highest average body weight was observed in the healthy control group. In the group that received treatment, the pattern that could be seen was that the more doses given, the more the body weight tended to increase. There are different notations in each group, except for the 0.2 and 0.1 injection groups which both have the notation d. This shows that the difference in body weight between the healthy control group, the diabetes mellitus group, the 0.05 dose secretome treatment group and the 0.1/0.2 ml/kg body weight dose is quite large and significant. Because they have the same notation, the secretome treatment groups with doses of 0.1 and 0.2 ml/kg body weight can be considered to cause the same effect on body weight, considering that the difference between the two results is not too big.

Table 1: Average comparison of body weight in each group

Group	Mean
Healthy Control Group	185,234[a]
Diabetes Melitus Group	118,643[b]
Secretom 0,05 ml/kg BB	136,750[c]
Secretom 0,1 ml/kg BB	157,262[d]
Secretom 0,2 ml/kg BB	150,548[d]
P value[*)]	0,000

*) Based on Anova test with a confidence level of 95%

Table 2: Average body weight in different weeks

Week	Mean
One week after first injection	142,086[a]
One week after second injection	137,895[a]
One week after third injection	143,381[a]
One week after fourth injection	175,419[b]
P value[*)]	0,000

*) Based on Anova test with a confidence level of 95%

Table 3: Results of weight calculations for all groups

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	531502,324[a]	19	27973,807	383,799	0,000
Intercept	941163,010	1	9411639,010	129127,033	0,000
Group	206274,205	4	51568,551	707,517	0,000
Weeks	94366,590	3	31455,530	431,568	0,000
Group and week	230861,529	12	19238,461	263,950	0,000
Error	29154,667	400	72,887		
Total	9972296,000	420			
Corrected Total	560656,990	419			

a *R Squared* = 0,948 (*Adjusted R Squared* = 0,946).

Table 2 shows that there was a significant difference in body weight from the first to the fourth week after the injection (p = 0.000). The highest average body weight was recorded during the fourth week after injection, whereas the lowest was recorded during the second week after injection. Based on the analysis of the average pattern of body weight each week, body weight decreased in the second week after injection, but increased again in the third week, and increased significantly in the fourth week after injection. In the table, only notations a and b are used for the average body weight studied. No significant differences in body weight were found in the first to third weeks post-injection because all three had notation a. This is different from the results in the fourth week after injection, which are given the notation b, showing that body weight in the fourth week increased significantly compared to the previous weeks after injection. It can be seen that there is an influence of all variables (treatment, post-injection time, and the interaction of the two) on body weight (p = 0.000) (Table 3). The relationship between treatment and post-injection time and body weight was strengthened by an R squared value of 0.948, which means that there was an association between treatment and post-injection time in increasing and decreasing body weight by 94.8%.

DISCUSSION

In Diabetes mellitus type 1 conditions cells loss the ability to transport glucose into cells due to the absence of insulin, resulting in a lack of glucose (Roep *et al*., 2021). Unavailability of glucose results in excessive gluconeogenesis, liver cells increase glucose production from other substrates, which is by breaking down proteins. The amino acids resulting from breakdown are transaminated to produce substrates for the formation of glucose. This event occurs continuously because there is very little or no insulin that limits gluconeogenesis. The glucose produced is then excreted through urine (Bryant *et al*., 2002). As a result, there was a significant reduction in the amount of muscle and adipose tissues. People with diabetes mellitus type 1 lose body weight despite an increase in appetite (polyphagia) and normal or increased calorie intake (Pari and Suman, 2010).

Previous studies have shown that secretome can speed up the healing process for liver disease (such as liver failure, partial hepatectomy) (Driscoll and Patel, 2019), cardiovascular disease, neurological degenerative disease (e.g. Alzheimer's and Parkinson's disease), bone disease (Praveen Kumar *et al*., 2019) or osteoarthritis (Fan *et al.*, 2022). In diabetes mellitus type 1, secretome takes participates in the healing process when different cytokines and growth factors with autocrine and paracrine actions are present. These factors could help in the restoration of pancreatic structure and regeneration of endogenous β cells (Zang *et al.*, 2017). According to Wen *et al.*, (2016), secretome stimulates insulin release by inhibits cell death. β cells pancreas produce insulin. Insulin in bloodstream regulation can occur again, which is indicated by a decrease in blood glucose. The existing insulin causes an increase in glucose uptake into the cells so that the energy supply in the cells is sufficient (Zou *et al.*, 2015). The presence of insulin also limits gluconeogenesis, so that protein breakdown can stop, which has an effect on increasing body weight.

CONCLUSION

Administering secretome injection once a week for four weeks can enhance the regeneration of pancreatic β-cells, as demonstrated by the rats with DMT1 exhibiting increased insulin production accompanied by an increase in body weight.

ACKNOWLEDGEMENTS

The authors are most grateful to Directorate General of Higher Education (DIKTI), Ministry of Research, Technology and Higher Education of Indonesia, with contract number: 3174/UN1.DITLIT/DIT-LIT/PT/2020.

REFERENCES

- American Diabetes Association. (2018). Cardiovascular disease and risk management: Standards of medical care in diabetes. *Diabetes Care*. 41:S86-104.
- Brovkina, O., & Dashinimaev, E. (2020). Advances and complications of regenerative medicine in diabetes therapy. *PeerJ*, *8*, e9746.
- Bryant, N. J., Govers, R., & James, D. E. (2002). Regulated transport of the glucose transporter GLUT4. *Nature reviews Molecular cell biology*, *3*(4), 267-277.
- Driscoll, J., & Patel, T. (2019). The mesenchymal stem cell secretome as an acellular regenerative therapy for liver disease. *Journal of gastroenterology*, *54*, 763-773.
- Eriksson, O., Eich, T., Sundin, A., Tibell, A., Tufveson, G., Andersson, H., ... & Lundgren, T. (2009). Positron emission tomography in clinical islet transplantation. *American Journal of Transplantation*, *9*(12), 2816-2824.
- Fan, Y., Li, Z., & He, Y. (2022). Exosomes in the pathogenesis, progression, and treatment of osteoarthritis. *Bioengineering*, *9*(3), 99.
- Guo, X. X., Wang, Y., Wang, K., Ji, B. P., & Zhou, F. (2018). Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. *Journal of Zhejiang University. Science. B*, *19*(7), 559.
- Keshtkar, S., Azarpira, N., Ghahremani, M.H. (2018). Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. *Stem Cell Res. Ther.* 9(1):63.
- McInnes, I.B., Gravallese, E.M. (2021). Immune-mediated inflammatory disease therapeutics: Past, present and future. *Nat. Rev. Immunol*. 21, 680–686.

- Nugroho, W.S., Kusindarta, D.L., Susetya, H., Fitriana, I., Mulyani, G.T., Fibrianto, Y.H., Haryanto, A., Budipitojo, T. (2016). The structural and functional recovery of pancreatic β-cells in type 1 diabetes mellitus induced mesenchymal stem cell-conditioned medium. *Vet World*. 9(5):535-9.
- Pari, L., Suman, S. (2010). Effcacy Of Naringin on Hepatic Enzymes of Carbohydrate Metabolism in Streptozotocin- Nicotinamide Induced Type 2 Diabetic Rats. *Int. J. Pharm. Biol. Arch*. 1, 280–286.
- Praveen Kumar, L., Kandoi, S., Misra, R., Vijayalakshmi, S., Rajagopal, K., Verma, R.S. (2019). The mesenchymal stem cell secretome: A new paradigm towards cell-free therapeutic mode in regenerative medicine. *Cytokine Growth Factor Rev*. 46, 1–9.
- Rawshani, A., Franzén, S., Eliasson, B., Svensson, A.M., Miftaraj, M. (2017). Mortality and cardiovascular disease in type 1 and type 2 diabetes. *N Engl J Med*. 376:1407-18
- Roep, B.O., Thomaidou, S., van Tienhoven, R., Zaldumbide, A. (2021). Type 1 Diabetes Mellitus as a Disease of the Cell (do Not Blame the Immune System)?. *Nat. Rev. Endocrinol*.17, 150–161.
- Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes atlas, 9th edition. *Diabetes Res Clin Pract*. 157.
- Swinkels, M., Rijkers, M., Voorberg, J., Vidarsson, G., Leebeek, F.W.G., Jansen, A.J.G. (2018). Emerging concepts in immune thrombocytopenia. *Front Immunol*. 9:880.
- Vija, L., Farge, D., Gautier, J.F., Vexiau, P., Dumitrache, C., Bourgarit, A. (2009). Mesenchymal stem cells: Stem cell therapy perspectives for type 1 diabetes. *Diabetes & metabolism*. 35(2):85-93.
- Warshauer, J.T., Bluestone, J.A., Anderson, M.S. (2020). New Frontiers in the Treatment of Type 1 Diabetes. *Cell Metab. 31*, 46–61.
- Wen, D., Peng, Y., Liu, D., Weizmann, Y., Mahato, R.I. (2016). Mesenchymal Stem Cell and Derived Exosome as Small RNA Carrier and Immunomodulator to Improve Islet Transplantation. *J. Control Release.* 238, 166–175.
- Zang, L., Hao, H., Liu, J., Li, Y., Han, W., Mu, Y. (2017). Mesenchymal stem cell therapy in type 2 diabetes mellitus. *Diabetol. metab. syndr*. 9:36.
- Zhou, Y., Hu, Q., Chen, F., Zhang, J., Guo, J., Wang, H. (2015). Human umbilical cord matrix-derived stem cells exert trophic effects on β-cell survival in diabetic rats and isolated islets. *Dis Model Mech*. 8(12):1625- 33.