Original Article

The Study on Possible Gene Therapy on Diabetics Type I Using Insulin Gene under Control of Heat Shock Promoter in Laboratory Animals

Keyvan Ramezani[1,2], Shivasadat Gheflat[1,2], Vahid Jajarmi[1,2], Mojgan Bandehpour[1,2], Bahram Kazemi[1,2*]

[1] Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences
[2] Biotechnology Department, School of Medicine, Shahid Beheshti University of Medical Sciences

Received: 29 July, 2018; Accepted: 16 January, 2019

Abstract

Background: Gene therapy is one of the treatment method for diabetes mellitus. An insulin gene, under control of heat shock inducer promoter was used in the study.

Materials and Methods: Six mice used in this study. Streptozotocin (STZ) induced diabetes mellitus in BALB/C mice and rats. Recombinant plasmid was injected to each animal. Animal's blood sugar (BS) was measured. Warming was done at injection site with a hair dryer to induced gene expression.

Results: Immediately after warming blood sugar was increased in mouse 1 and decreased after one hour. However, blood sugar increased again in mice 2 and 4. Blood sugar increased for two hours after warming in mouse 3. Blood sugar increased to 150 mg/dl after STZ injection and immediately after warming reached to 160 mg/dl, after one hour BS dropped to 116 mg/dl and on the second hour was 126 mg/dl, in mouse 5 BS had an increasing trend, BS in mouse 6 had a similar pattern to mouse 2.

Conclusion: With fixing the defects of the project will be used in gene therapy in future.

Keywords: Diabetes, Gene therapy, Streptozotocin

***Corresponding Author:** Bahram Kazemi, PhD (orcid: 0000-0002-3072-8831), Cellular and Molecular Biology Research Center of Shahid Beheshti University of Medical Sciences, Tehran, Iran, Tel: (+98) 21 22439956; Fax: (+98) 21 89784665; E-mail: Bahram_14@yahoo.com, kazemi@sbmu.ac.ir

Please cite this article as: Ramezani K, Gheflat Sh, Jajarmi V, Bandehpour M, Kazemi B. The Study on Possible Gene Therapy on Diabetics Type I Using Insulin Gene under Control of Heat Shock Promoter in Laboratory Animals. Novel Biomed. 2019;7(2):45-8.

Introduction

Diabetes mellitus is a metabolic disorder with chronic hyperglycemia because of incomplete insulin secretion. Based on WHO reports on April 2016, diabetic people death rate will reach to 1.5 million in 2020[1].

Some patients using insulin regiment should performed self monitoring of blood glucose prior to eating or diving[2]. Other method is Pancras transplantation for treating type 1 diabetes[2], but Van Dellen proposed that probably 22% reject Pancras transplantation[3]. Basta et al. proposed that appropriate donors are scarce and Pancras islands transplantation is laborious. In addition, there is side effects of immunosuppressive drugs used as avoid rejection of transplant[4]. Insulin gene therapy is another treatment for type 1 diabetes[5]. Thule et al used DNA responsive to liver promoter (contained inhibitory insulin response sequence) prepared a liver-targeted insulin transgene and transferred to streptozotocin diabetic mice by recombinant adenovirus vector. In that study, mice in compare to those received empty vector showed normal blood glucose level[6].

Ruihuan Chen et al. proposed a theory that increase liver insulin expression in presence to glucose and reduction of glucose in presence to insulin can achieve to treating type 1 diabetes. Expression of glucose 6 phosphatase in liver stimulate by glucose and inhibit by insulin. The glucose 6 phosphatase promoter in assistance by intronic enhancer of aldolase B gene can be regulated insulin expression in diabetic mouse effectively[7].

Hyun Chul Lee et al. used recombinant adeno-associated virus controlled by hepatocyte-specific L-type pyruvate kinase (LPK) promoter to expression of single-chain insulin analogue (SIA). The recombinant adeno-associated virus could regulate expression of SIA in presence to blood glucose. The expressed SIA can improve autoimmune and STZ-diabetic mouse as long-term period[8].

Methods

Insulin gene: We used insulin gene controlled by heat shock protein 70 (HSP70) promoter constructed previously by our team. It consists of a destination vector named pJTI Fast Dest prepared via gateway system[9]. The construct reduces immune reaction in host cells. The promoter induced by heat and transcription initiate. Although it may not be possible for humans to function for many years, it decided to determine the effect of this structure on the animal's body. It should be noted that in historical sources, the structural issue with non-viral profile and the induction of its promoter with heat was not found. This is the first experiment with this construct on a laboratory animal.

Laboratory animal: Shahid Beheshti University of Medical Sciences ethical committee confirmed the ethical status of animal under experimental test. We used STZ- Balb/c mice and rats for test the construct (Table 1). Mice were diabetic by intra peritoneal injection and rats by subcutaneous injection of STZ. The mail Balb/c mice were received 100mg/kg STZ for 2 weeks. Rats were received 40mg/kg STZ.

Preparations of therapeutic gene construct: Recombinant plasmid (contained insulin gene and heat induced promoter) was transformed into *E. coli* TOP10 strain. Bacterial colony contain recombinant plasmid were cultured and plasmid extraction was done by alkaline method. Recombinant plasmid was confirmed by gel electrophoresis in compare to empty plasmid. Each mouse received 100-microgram plasmid via intra peritoneal injection and each rat was received 100-microgram plasmid through intra muscular injection.

Animal grouping: In this research, three rats and six Balb/c mice were used and two mice as non-diabetic control were considered. Blood sugar was measured by electronic glucometer (ACCU-CHEK) before injection of STZ. Blood glucose were measured through tail vein.

Ethics Committee of Iran National Science Foundation (grant No. 91057380) has approved the protocol for this research project.

Results

After animal injection by therapeutic gene, local injection site was heated by hair dryer for 10 minutes at 42^{o}C, but maybe there was some irregularity in this process (heating). Blood glucose was checked by glucometer and recorded as table 1. Two rats did not recovery and removed from test system.

Table 1 shows blood glucose of first mouse that increased immediately after warming, decreased after one hour, and increased again. Blood glucose of second and fourth mice increased after two hours and not affected by construct (or therapeutic gene not entrance in host cell and removed from system). Only blood glucose of third mouse increased to 150 mg/dl after injection of STZ and immediately increased to 160 mg/dl then decreased so that after 1-hour reach to 116 mg/dl and after 2 hours reach to 126 mg/dl. Blood glucose of fifth mouse after STZ injection increased because did not receive therapeutic gene. Blood glucose of sixth mouse like second mouse increased then decreased again. After heating the blood glucose of third rat increased highly so that glucometer could not record it, but two hours after promoter induction reached to 518 mg/dl, which show the effect of construct.

Discussion

Gene therapy is a process that transfers genetic materials into animal cells to improve a destructed gene function. In this study, we used heat inducing insulin construct. The construct was destination vector

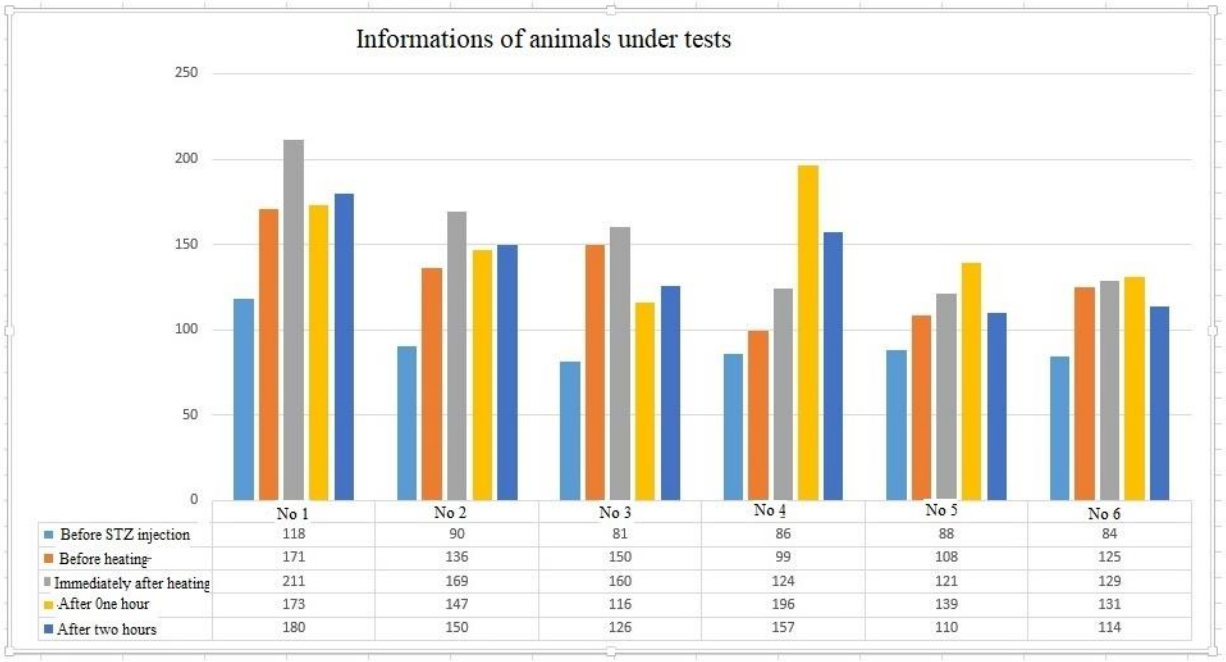

Figure 1. Graph shows results of STZ injection to laboratory animals and heated at injection location by hair dryer.

Table 1: Result of injection of STZ to laboratory animal.

Two hour after heating	One hour after heating	Immediately after heating	Before heating	Before STZ injection	Time / Animal
180	173	211	171	118	Mouse number 1
150	147	169	136	90	Mouse number 2
126	116	160	150	81	Mouse number 3
157	196	124	99	86	Mouse number 4
110	139	121	108	88	Mouse number 5 (control)
114	131	129	125	84	Mouse number 6 (control)
-	-	-	337	-	Rat number 1
-	-	-	378	-	Rat number 2
518	Hi	Hi	374	-	Rat number 3

contained insulin gene and heat shock protein 70 promoter constructed in our previous paper, the construct induced by heat at 42˚C and secreted insulin[9]. It was expected that when injected via IP or IM into animals could enter into the host cells and induced insulin at 42˚C. Resulted were different with different animals, it is possible that therapeutic gene was entered in host cells in some animals but could not be entered into cells of another animal and gene lead to destruct.

Using viruses for gene transfer is a problem because stimulate host immune system and produce antiviral antibody. To reduction of antiviral antibody, He-CX et al. used direct injection of naked DNA, they proposed that it is the immune process but with reduced efficacy for gene therapy in animals[5]. We used plasmid DNA for reducing the risk of antibody secretion. The plasmid was a eukaryotic destination vector named pJTI Fast Dest prepared via Gateway system[9].

It is emphasized that stress and environmental condition might effect on animal and lead to increase blood glucose and promoter induction could not decrease blood glucose. Figure 1 showed that animals' blood glucose was different at different times but immediately after heating (1st, 2nd, 3rd animals) or after one hour (4th animal) blood glucose increased and then decreased. Some trouble of this method including: construct did not enter in the host cells, destruction of construct before cell entrance, inappropriate heating, effect of stress on animal condition, inappropriate destination vector for integration of the therapeutic gene into the host chromosome, or low time for blood glucose regulation.

Conclusion

Results indicated that could be assured for the efficacy of therapeutic gene in future if fix problems. This is the first experiment with this construct is done on a laboratory animal.

Acknowledgment

This paper was extracted from Thesis for General Medical Doctor degree at Shahid Beheshti University of Medical Sciences by Keyvan Ramezani and was done at Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences. This work was supported by Iran National Science Foundation through grant No. 91057380.

References

1. Global report on diabetes. World Health Organization, Geneva, 2016 availabe at http://apps.who.int/iris/bitstream/handle/10665/204871/9789241565257_eng.pdf;jsessionid=8B0019327E5309C002D09E9FEFAF7AE0?sequence=1

2. American Diabetes Association. Glycemic Targets: Standards of Medical Care in Diabetes-2018. Diabetes Care. 2018;41(Suppl 1):55-64.

3. van Dellen D, Summers A, Trevelyan S, Tavakoli A, Augustine T, Pararajasingam R. Incidence and Histologic Features of Transplant Graft Pancreatitis: A Single Center Experience. Exp Clin Transplant. 2015;13(5):449-52.

4. Basta G, Montanucci P, Calafiore R. Islet transplantation versus stem cells for the cell therapy of type 1 diabetes mellitus. Minerva Endocrinol. 2015;40(4):267-82.

5. He CX1, Shi D, Wu WJ, Ding YF, Feng DM, Lu B, et al. Insulin expression in livers of diabetic mice mediated by hydrodynamics-based administration. World J Gastroenterol. 2004;10(4):567-72.

6. Thulé PM, Liu JM. Regulated hepatic insulin gene therapy of STZ-diabetic rats, Gene Ther. 2000;7:1744–52.

7. Chen R, Meseck ML, Woo SL. Auto-regulated Hepatic Insulin Gene Expression in Type 1 Diabetic Rats, Mol Ther. 2001;3(4):584–90.

8. Lee HC, Kim KS, Shin HC. Remission in models of type 1 diabetes by gene therapy using a single-chain insulin analogue, Nature. 2000;408:483-8.

9. Jajarmi V, Bandehpour M, Kazemi B. Regulation of insulin biosynthesis in non-beta cells by a heat shock promoter. J Biosci Bioeng. 2013;116 (2):147e151.