Case Report

Stem Cell & Regenerative Medicine

Activation of PPARγ by SB Cells® Treatment for Type 2 Diabetes Patients: A Case Study

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ABSTRACT

Management of blood glucose levels is vital for the health and well-being of those with type 2 diabetes mellitus. Current research has identified novel targets for regulating glucose and fat metabolism, with peroxisome proliferator-activated receptors (PPAR) emerging as a candidate in treating type 2 diabetes. Diabetes mellitus medications may target the PPAR pathway, and the class of thiazolidinediones is a popular treatment that functions as PPARγ agonists. While this treatment is an established method for lowering blood glucose through increasing insulin sensitivity, patients may experience serious adverse effects such as hepatotoxicity and heart failure. Therefore, there is a need for safe and effective type 2 diabetes therapies. Stem cell research regarding diabetes mellitus has provided promising therapies for improving insulin sensitivity and normalizing blood glucose. The objective of this study is to examine the effects of the StemBios stem cell therapy on maintaining healthy glucose levels through the PPARγ pathway in type 2 diabetes patients. In this study, we tracked PPARγ levels before and after intravenous SB cells® (StemBios cell) infusion. The treatment led to an increase in PPARγ levels and stabilized blood glucose levels after the treatment, suggesting that the SB cells® treatment offers a therapeutic benefit for those with type 2 diabetes.

Keywords
PPARγ, Type 2 diabetes, Small stem cells.

Abbreviations
SB cells®: StemBios cells, T2DM: Type 2 diabetes mellitus, PPARγ: Peroxisome proliferator-activated receptor-gamma, TZD: Thiazolidinedione, GLUT4: Glucose transporter type 4, IRS1: Insulin receptor substrate 1, IRS2: Insulin receptor substrate 2, VSEL: Very-small embryonic-like stem cell, HSC: Hematopoietic stem cell, BLSC: Blastomere-like stem cell, Lgr5: Leucine-rich repeat-containing G-protein coupled receptor 5, EDTA: Ethylenediaminetetraacetic acid, cDNA: Complementary DNA, RT-PCR: Real-time polymerase chain reaction, ΔΔCt: Delta-delta cycle threshold.

Introduction
Type 2 diabetes mellitus (T2DMM) is a metabolic disorder that inhibits the uptake of glucose into cells, and is the result of insulin resistance or scarcity [1]. The prevalence of T2DM has steadily increased around the world, due to technological and agricultural advancements that changed diets and increased lifespans. The long-term health risks from high blood glucose levels include retinopathy, neuropathy, cardiovascular disease, and a life expectancy shortened by ten years, among other complications [2]. Afflicted individuals currently focus on lifestyle interventions, with the aid of anti-diabetic medications, to maintain normal blood glucose levels [3]. The population of type 2 diabetics worldwide, however, is sharply increasing, driving the imminent need for safe therapies that can prolong life expectancy as well as stabilize blood glucose levels.

Current research into the molecular pathways that control glucose metabolism has found viable targets for blood glucose management. In particular, the peroxisome proliferator-activated

receptors (PPARs) are a family of nuclear transcriptions factors that mediate lipid storage and glucose metabolism through transcriptional activation and repression [4]. The most abundant factor of the PPAR family, PPARγ, has been implicated in the pathology of obesity and diabetes mellitus. It has been determined that increased PPARγ expression enhances muscle, adipose, and liver sensitivity to insulin by stimulating the differentiation of fibroblasts into adipocytes and increasing Glucose transporter type 4 (GLUT4) and insulin receptor substrates 1 and 2 (IRS1, IRS2) expression in skeletal muscle and adipose [5,6]. Investigations into the medical relevance of PPARγ have shown that agonists have been useful in treating hyperlipidemia and hyperglycemia [7].

The class of medications known as thiazolidinediones (TZD) or glitazones, are PPARγ agonists that lower blood glucose levels without increasing pancreatic insulin secretion [8]. Thiazolidinediones have been used as a treatment for T2DM in the United States since 1997 and the group includes troglitazone, rosiglitazone, and pioglitazone [9]. While these drugs have been approved for use in T2DM patients, there are drawbacks that reduce the appeal and effectiveness of the drugs.

The blood glucose lowering effects of TZDs is slow compared to other medications, between 4-12 weeks, due to transcriptional level activation and repression [10]. The safety of the drugs has also been questioned, with the first pharmaceutical agent, troglitazone, pulled off the market in 2000 for hepatotoxicity [11]. The remaining two drugs are still commonly used for treatment, but studies have suggested that they fail to lengthen life expectancy and may increase cardiovascular disease risk. Rosiglitazone, in particular, has been evaluated for adverse cardiovascular risk. It has been determined in 2007, by the Endocrinologic and Metabolic Drugs Assessor Committee, that rosiglitazone use is indeed associated with ischemic events in the heart, when compared to other diabetes medication such as metformin or sulfonyl urea [12]. The delayed effectiveness of thiazolidinediones in lowering blood glucose levels, coupled with increasing myocardial risk, suggests a need for safer T2DM treatments.

Novel approaches to treating chronic diseases have emerged with the advancement of stem cell research. In particular, StemBios stem cells have shown promise in treating multiple diseases triggered by chronic inflammation. The SB cells® (StemBios cells) are adult multipotent stem cells that have the ability to differentiate into different cell lineages. These cells are derived from human bone marrow, and a patient's own cells are used, reducing the possibility of immuno-rejection [13]. It has been previously determined through flow cytometry that the purified and isolated SB cells®, obtained after incubation at 4˚C for 48 hours, are distinct from erythrocyte and leukocyte populations and contain few inactivated platelets. The cell population is within the 2-6 µm range and the cells have been shown to be CD133-, CD34-, and CD66e-, suggesting that the population is free of very-small embryonic-like stem cells (VSELs), hematopoietic stem cells (HSCs), and blastomere-like stem cells (BLSCs). The SB cells® also expressed distinct cell markers: Lgr5 and CD349. This analysis suggests that the collection technique enables the procurement of a cell population that contains primarily SB cells®, and is suitable for the therapy.

These cells have displayed therapeutic benefits for different degenerative diseases, and the application of SB cells® in T2DM shows a promising avenue for the management of PPARγ. In this study, we examined the effects of the SB cells® treatment on two T2DM patients.

Methods

Isolation of the SB cells® mixture

Patients were given fucoidan pills, an algae-based supplement, (Patent Publication Number: 20140178886; manufactured by Kansou Mozuku, Okinawa, Japan) two hours before blood collection. This was done to facilitate with stem cell mobilization from bone marrow into circulating blood. The SB cells® were then collected from patients using the purification protocol previously outlined by StemBios Technologies, Inc [12]. The cells were injected intravenously per the guidelines set forth in the protocol for IRB SB-IN-4112.

Total RNA collection

Patient peripheral blood was drawn into EDTA-coated collection tubes and inverted thoroughly to prevent coagulation. Patient blood was collected and analyzed at three time points during each round of treatment: before, 24 hours after, and one week after the SB cells® injection. The blood samples were processed with 6% HetaStarch solution (StemCell Technologies; catalog number: 07906) and incubated at 37°C for 1 hour. The resultant top layer was isolated and total RNA was extracted using the RNeasy Mini Kit (Qiagen; catalog number: 74104). Total RNA concentration was measured with a Synergy H1 Hybrid Reader (Biotek).

PPARγ expression detection

Total RNA was reverse transcribed into cDNA using the PTC-100 Programmable Thermal Controller from MJ Research Inc. Each reaction utilized 100ng of total RNA and qScript cDNA SuperMix (Quanta Bioscience; catalog number: 95048-500), and was run according to the manufacturer's instructions. The cDNA samples were subsequently diluted with nuclease-free water to 0.5ng/µL and stored at -20°C.

Real-time PCR was performed with iQ™ SYBR® Green Supermix (Bio-Rad; catalog number: 1708882), patient cDNA samples, anchored oligo-dT primers, and nuclease-free water in 96-well, thin wall hard-shell PCR plates (Bio-Rad; catalog number: HSP9601). The reaction was performed with the CFX96 Touch System and the C1000 Touch Thermal Cycler (Bio-Rad). The samples were tested for PPARγ expression levels, with cyclophilin serving as the housekeeping, reference gene. The reference gene were tested in all runs and used as an inter-run calibrator. The fold-change in expression was calculated by computing delta-delta (ΔΔ) Cycle Threshold (Ct), with cyclophilin as the baseline reference for normalization to the test primer PPARγ. The PCR products were analyzed on a 1.8% agarose gel to confirm the gene of interest.

Primer and run information can be found below:

Cyclophilin: Forward primer: 5`-AGG GTG GTG ACT TTA CAC GCC ATA-3`, Reverse primer: 5`-CAA AGA CCA CAT GCT TGC CAT CCA-3`
PPARγ: Forward primer: 5`-GCT GTG CAG GAG ATC ACA GA-3`, Reverse primer: 5`-GGG CTC CAT AAA GTC ACC AA-3`

RT-PCR running program:
95°C, 2:00
95°C, 0:05
60°C, 0:30
Plate read
Go to 2, 39X
95°C, 0:05
65°C, 0:31
65°C, 0:05 +0.5°C/cycle
Plate read
Go to 8, 60X

Results

Patient Medical History

Prior to the SB cells® treatment, both patients were advised to maintain their normal lifestyles, including amount of exercise and type and dosage of medications and supplements, immediately before and for 90 days after the treatment.

Patient 73 is a 68 year old, Asian female with the comorbidity of T2DM and hypertension. She currently takes Glipizide to control her blood glucose levels, and Diovan to control her blood pressure. She consumes supplements for vitamin B12, vitamin D3, vitamin B complex, and Omega-3. She regularly consumes Move Free®, Zantac®, and CholestOff®, to improve joint mobility, stomach acid reflux, and cholesterol levels. Prior to the treatment, the patient regularly had blood glucose levels in the 160-180 mg/dl range. For two weeks after the SB cells® treatment, the patient's blood glucose level was consistently read at the 125-130 mg/dl level. The patient continued to experience lower blood glucose readings, within the 120-130 mg/dl range for 90 days after the treatment, when tested at home in the morning and during a check-up with a physician. Three months after the treatment, the patient was advised by her physician to half her dosage of anti-diabetic medication. In addition to lower blood glucose levels, patient 73 experienced a 2.6-fold increase in PPARγ gene expression 24 hours after the SB cells® treatment (Figure 1).

Patient 118 is a 61 year old, non-white Hispanic male with T2DM. He is currently not taking anti-diabetic medication. The patient uses Xanax for pain management, particularly for headaches and joint pain. Prior to the treatment, the patient regularly had blood glucose levels in the 170-180 mg/dl range. The patient experienced blood glucose levels in the 120-130 mg/dl range after the SB cells® treatment, and the lower readings were maintained for the 90 days. The readings were performed in the patient's home and obtained in the morning, before food consumption, and at a physician's office. Patient 118 also experienced a 17.0-fold increase in PPARγ gene expression 24 hours after the SB cells® treatment (Figure 1).

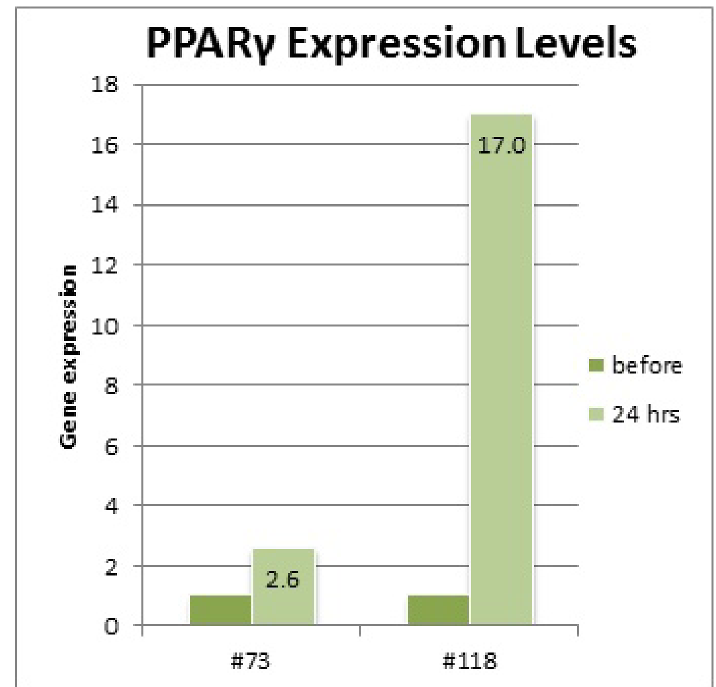

Figure 1: PPARγ levels were tracked for Patient 73 and Patient 118, using RT-PCR for total RNA collected before and 24 hours after the treatment.

Discussion

Diabetes mellitus is currently managed through lifestyle choices such as food and exercise, and medication. It has been shown that lifestyle modifications, such as weight loss and physical activity, increase insulin sensitivity for those with T2DM. While these changes are a component of diabetes management, older and elderly individuals may be physically incapable of strenuous lifestyle changes. Targeting the PPARγ pathway remains a valuable approach toward improving insulin sensitivity, as increased PPARγ gene expression causes increased GLUT4 and insulin receptor substrates 1 and 2 protein production. This, in turn, increases insulin sensitivity and glucose uptake into muscle, adipose, and liver cells. The classes of PPARγ agonists, the thiazolidinediones, are commonly used medications and have been effective at reducing blood glucose levels. The drugs, however, act slowly, have been shown to cause adverse effects in the liver and heart, and fail to increase life expectancy.

Therefore, it is vital to develop safe therapies that are accessible for all populations, regardless of age and lifestyle. For the SB cells® treatment, both patients were instructed to continue with their regular lifestyles, by maintaining prior physical activity levels and medication regimes. This was suggested to thoroughly examine the effects of the treatment on blood glucose levels. After the first treatment, there was an increase in the expression of PPARγ for both patients, coupled with a long-term reduction in blood glucose levels, over a 90 day period. The ability of the therapy to increase PPARγ gene expression, without the

introduction of pharmacological agents, provides a novel approach toward modulating insulin sensitivity via the PPARγ pathway. These findings suggest that the treatment aids in the maintenance of healthy blood glucose levels and provides beneficial outcomes for T2DM patients.

In order to further explore the effects of the SB cells® treatment for those with T2DM, we propose a larger study involving multiple treatment plans. There would be four arms of this trial, with 25 patients per arm. The first arm involves patients receiving a single round of the intravenous SB cells® treatment. The second arm involves two consecutive rounds of intravenous SB cells® treatment, performed weekly. The third arm involves the patients receiving three consecutive rounds of our intravenous SB cells® treatment, performed weekly. The control group of patients will receive an intravenous saline solution. Patient PPARγ levels will be measured at 0, 7, 14, 30, and 90 days after each SB cells® treatment. Blood glucose values will be recorded upon waking in the morning, every day after each SB cells® treatment, for 90 days.

Conclusion

The StemBios cell therapy has been shown to immediately enhance PPARγ gene expression, conferring long-term stabilization of blood glucose levels. This maintenance of healthy blood glucose levels persisted for 90 days, without the need for lifestyle modifications or additional medication. In addition to reducing blood glucose, SB cells® have been shown to modulate the PPARγ pathway without the need for additional pharmacological substances. These findings suggest that the SB cells® treatment is a valuable and effective method for treating type 2 diabetes.

Competing Interests

The authors have received funding a commercial source. One or more of the authors are employed by a commercial company, StemBios Technologies, Inc. Both of these affiliations do not alter the authors' adherence to all the JSCR policies on sharing data and materials.

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