Research Article

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SB Cells® Treatment Reduces IL-6 in Type 1 Diabetes Mellitus Patients

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Abstract

Type 1 diabetes mellitus is an auto-immune disease that results in the destruction of insulin-producing β-islet cells of the pancreas. Current research has shown that IL-6, an inflammatory cytokine, is elevated in those with type 1 diabetes, and may be involved in disease progression. Despite advancements in type 1 diabetes research, the primary therapy for mediating glucose uptake in patients with type 1 diabetes remains to be daily injections of exogenous insulin or insulin-analogues. While these treatments are established methods for lowering blood glucose, daily injections can be burdensome for patients. In order to aid patients and ease the lifelong dependence on injections, alternative approaches to type 1 diabetes disease pathology must be investigated. In particular, stem cell research has shown promising results in reducing inflammation. This study aims to investigate the effects of StemBios stem cell therapy on reducing inflammatory markers and stabilizing blood glucose levels. In order to quantify the effects of the SBcells® (StemBios cells) treatment, glycated hemoglobin (HbA1c) and interleukin-6 (IL-6) levels were recorded before and after the treatment. The study patient's HbA1c and IL-6 levels both decreased during the treatment and these findings suggest that the SB cells® treatment can ameliorate the inflammatory process and provide beneficial outcomes for type 1 diabetics.

Keywords: IL-6, HbA1c, Type 1 Diabetes, Stem Cells

Abbreviations

SBCell®: StemBios cells
T1DM: Type 1 Diabetes Mellitus
IL-6: Interleukin-6
HbA1c: Glycated Hemoglobin
β-islet: Beta-islet
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
ELISA: Enzyme-linked Immunosorbent Assay
IV: Intravenous

Introduction

Type 1 diabetes, also known as insulin-dependent diabetes or juvenile diabetes, involves an auto-immune attack on the insulin-producing β-islet cells of the pancreas [1]. The auto-immune response involves an expansion of auto-reactive CD4+ T-helper cells and CD8+ T cells, auto antibody-producing B cells, and activation of the innate immune system. This attack halts the production of insulin, which prevents glucose from entering the body's cells [2]. The buildup of glucose in the blood increases one's risk for cardiovascular disease, kidney, neuropathy, as well as vision issues. Type 1 diabetes is a prevalent genetic disorder that affects approximately one to three million people in the United States, and the exact course of disease development is still unclear [3]. Despite the development of insulin-analogues and efficient insulin-delivery systems, the current and most common treatment is lifelong dependence on daily insulin injections [4]. The daily injections, alongside a carefully planned diet, are a burdensome feat for those with type 1 diabetes.

Those with type 1 diabetes must also remain diligent about hyperglycemia, as excessive blood glucose levels can lead to serious physical manifestations, such as blurry vision and pain. Physicians utilize glucose levels to evaluate and track the health of type 1 diabetics and a standard marker that is commonly used for diabetes progression is glycated hemoglobin A1c (HbA1c) [5]. When a persistently high concentration of glucose circulates through the body's blood vessels, hemoglobin within red blood cells can chemically react with glucose to form glycated hemoglobin. It has been clinically verified that there is a positively correlated relationship between amount of circulating glucose and fraction of glycated hemoglobin. Therefore, glycated hemoglobin can serve as an accurate proxy for average plasma glucose concentration, over a three month period [6].

In addition to HbA1c, the interleukin (IL) family of cytokines has been identified as marker in the disease progression of diabetes mellitus [7]. Interleukins have been implicated in affecting glucose

homeostasis and metabolism and IL-6 is a prominent marker that is active as a pro-inflammatory cytokine and an anti-inflammatory myokine. It is a chemical factor that is secreted by T-cells and macrophages to stimulate an immune response during infection or after trauma [8]. It has been shown to stimulate the inflammatory and auto-immune processes in many diseases, including diabetes, atherosclerosis, and depression [9]. Islet cell inflammatory pathways, mediated by IL-6, have been found to contribute to diabetes risk, with high levels of IL-6 correlated to diabetes onset [10]. It has also been demonstrated that improvements to hyperglycemia in those with T1DM are associated with a decrease in IL-6 [11]. Therefore, monitoring both HbA1c and IL-6 levels, provides an assessment of blood glucose levels and one's diabetic state.

Advancements in the field of stem cell research have introduced stem cells as possible therapeutics for treating chronic disease, such as type 1diabetes. In particular, SBcells® (StemBios cells) have shown promise due to their regenerative abilities. These cells are adult multipotent stem cells that have the ability to differentiate into different cell lineages, are obtained from human bone marrow, and used for autologous intravenous applications, reducing the likelihood of immuno-rejection. These purified and isolated SBwcells® have been verified through flow cytometry analysis to be distinct from erythrocyte and leukocyte populations. This population contains platelets that have been deactivated during the incubation process. The cells range from 2 to 6 μm and have been shown to be CD133-, CD34-, CD66e-, and express distinct markers, Lgr5 and CD349 [12]. These combination of markers suggests that the population is free of very-small embryonic-like stem cells (VSELs), hematopoietic stem cells (HSCs), and blastomere-like stem cells (BLSCs). The cell procurement technique isolates principally SB cells® offering a pure population that is suitable for cell therapy.

These cells have displayed therapeutic benefits for different degenerative diseases, and it has been hypothesized that SB cells® may aid those with type 1 diabetes by replenishing damaged β-islet cells or reducing the autoimmune and inflammatory response. In this study, we examine the effects of the SB cells® treatment on a type 1 diabetes patient through quantification of blood serum HbA1c and IL-6 levels.

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 outlined by StemBios Technologies, Inc [12]. The cells were injected intravenously following the guidelines in the protocol for IRB SB-IN-4112.

Serum collection

Patient peripheral blood was drawn into EDTA-coated collection tubes and inverted thoroughly to prevent coagulation. The blood was collected and analyzed before, 24 hours after, and one week after the SB cells® injection. The blood was prepared for analysis through incubation at 4°C for 48 hours. The resultant top layer was isolated and centrifuged at 3000rpm for 15 minutes to pellet cells and platelets. The serum was removed from the pellet and immediately stored at -80°C for subsequent analysis.

IL-6 concentration detection

A human IL-6 Quantikine ELISA kit (R&D Systems, catalog number: D6050) was used to measure IL-6 concentrations in serum. Patient serum was collected at three time points during each round of treatment. Serum was acquired before, 24 hours after, and one week after the SBcells® injection. The samples and standards were then loaded into a 96-well plate according to the manufacturer's instructions and the absorbance of the samples and standards were measured at 450 nm. Levels of IL-6 was also analyzed via Luminex Assay at the Clinical Immunobiology Correlative Studies Laboratory at City of Hope, Duarte, California.

HbA1c percentage detection

A direct enzymatic Glycated Hemoglobin A1c (HbA1c) Kit (Diazyme; Catalog Number: DZ168A-K) was used to measure HbA1c percentages in blood. Patient peripheral blood was drawn into EDTA-coated collection tubes and inverted thoroughly to prevent coagulation. Whole blood was acquired before, 24 hours after, and one week after the SB cells® injection. The samples and standards were then loaded into a 96-well plate according to the manufacturer's instructions and the absorbance of the samples and standards were measured at 700 nm.

Results

Patient Medical History

Prior to receiving the SB cells® treatment, the study participant was advised to maintain her regular routine involving exercise, medication course, and diet immediately before and for 90 days after the treatment. The subject received the treatment twice, once a week, for two weeks. Patient 74 is a 34 year old, Asian female with type 1 diabetes. She is currently taking Humalog and Lantus to manage her blood sugar levels. She does not take additional vitamins or supplements, and maintains an active lifestyle by performing vigorous exercise 2-3 times per week. Blood glucose readings were performed in the patient's home and obtained in the morning, before food consumption, and at a physician's office.

SB cells® treatment affects type 1 diabetes markers

HbA1c levels for patient 74 changed from 7.248% before the first injection to 7.692% 24 hours after the SB cells® treatment, and decreased to 7.008% one week after the treatment. Patient 74 also experienced a drop in HbA1c levels after the second injection, with HbA1c levels dropping from 6.627% to 6.357% 24 hours after the second injection. The IL-6 concentration of Patient 74 also decreased from 6.54 pg/mL before the first injection to 4.12 pg/mL 24 hours after the SB Cells® treatment, to 3.53 pg/mL one week after the treatment. Patient 74 also experienced a reduction in IL-6 levels after the second injection, with the concentration decreasing from 4.25 pg/mL before the second injection to 2.02 pg/mL 24 hours after the second injection.

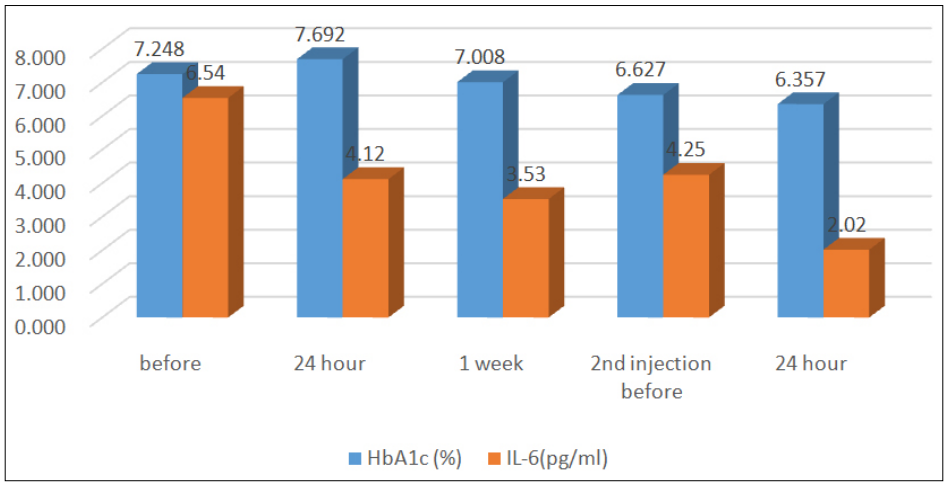

Figure 1: HbA1c and IL-6 levels tracked for Patient 74 over the course of two treatments. The blue bars signify the percentage of HbA1c in circulating blood. The orange bars signify IL-6 levels in pg/mL in serum.

Discussion

Those with type 1 diabetes control blood glucose levels through active dieting and insulin supplementation. Blood glucose readings, such as HbA1c, provide patients with valuable health status information and allow them to modify their lifestyles to avoid hypo- and hyperglycemic episodes. While this broad approach of diet and monitoring are important for T1DM management, it fails to target the molecular pathways that may contribute to disease pathology. In order to dampen the changes associated with β-islet cell destruction, T1DM therapies should shift focus toward inflammatory targets and auto-immune processes. Research into IL-6 in β-islet cell inflammatory pathways have found an increased diabetes risk with increased IL-6 expression. Therefore, modulating IL-6 levels and reducing chronic inflammation can be a powerful method toward ameliorating diabetes progression.

Novel investigations into the efficacy of stem cells in reducing inflammation has shown promising outcomes for those with T1DM. The SB cells® treatment has been shown to modulate IL-6 and HbA1c levels immediately after the treatment. A decrease in the percentage of HbA1c to non-diabetic levels, below 6.5%, was observed after the second round of treatment. After each round of treatment, HbA1c levels continued to decrease, with a lower second "before" HbA1c percentage compared to the first "before" percentage. This pattern of consistent decrease was also observed with IL-6 levels after the two rounds of treatment. A reduction in IL-6 levels to normal levels, less than 4.7 pg/mL, was observed after the second round of treatment. The long-term changes of HbA1c and IL-6 levels to normal, healthy ranges, after two rounds of SB cells® treatment suggest high efficacy in reducing inflammation and maintaining normal levels of blood glucose.

The SB cells® treatment and its effects for those with T1DM have shown encouraging results. Therefore, we propose further investigation into the relationship between SB cells® treatment and blood glucose management via involvement of the IL-6 pathway. In this expanded study, there would be four arms with 25 patients per arm. In the first arm, patients would receive a single round intravenous (IV) SB cells® treatment. In the second arm, patients receive two consecutive rounds of IV SB cells® treatment, performed weekly. In the third arm, patients would receive three consecutive rounds of IV SB cells® treatment, performed weekly. The control group of patients will receive an intravenous saline solution. Patient IL-6 and HbA1C levels will be measured at 0, 7, 14, 30, and 90 days after each SB cells® treatment. Fasting blood glucose values will be recorded upon waking in the morning, every day after each SB cells® treatment, for 90 days.

The association between SB cells® treatment and reduction in IL-6 levels suggests that the treatment may be able to ameliorate other IL-6 associated diseases, such as atherosclerosis and depression.

Conclusion

The study has presented support for the utilization of SB cells® therapy in reducing IL-6 levels and inflammation in a type 1 diabetic patient. This decline in cytokine expression was sustained, and correlated with a long-term decrease in blood glucose concentration. Levels of HbA1c and IL-6 fell within normal ranges after the second injection, or two weeks after the initial injection. These study findings suggest that the SB cells® treatment offers immediate and sustained improvement through modulation of the inflammatory pathway, and can offer therapeutic benefit for those with type 1 diabetes mellitus.

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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