Research Article

Stem Cell & Regenerative Medicine

# Increasing HMOX-1 Expression with SB cells® Treatment Ameliorates Chronic Hypertension

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## ABSTRACT

*Hypertension has been known to be one of the most common chronic inflammations of the body affecting lives of many worldwide. Constant elevation of blood pressure limits the organ from performing at its optimal rate, and its effects are more widespread throughout the body. Many prescription drugs have been created and used by affected individuals at a cost-severe financial loss and/or inescapable side effects. The race to discover other alternative treatment leads to newfound attention to stem cell therapy where patients are treated with their own stem cells in the hope of minimizing cost and side effects. The intention of our study is to use SB cells® cells to approach hypertension syndromes by testing the gene Heme oxygenase decycling-1 (HMOX-1). HMOX-1 is a critical enzyme that breaks down heme to produce carbon monoxide (CO). CO is an important metabolite that participates in vasodilation and many other anti-inflammation processes. We observed a significant increase in HMOX-1 expression in the blood 24-hours and/or 1-week post-SB cells® treatment. Additionally, blood pressure level of tested patients significantly decreased post treatment.*

*We envision that this application could one day be used to treat other HMOX1 related diseases such as inflammation as well as cancer.*

## Keywords

Hypertension, Small stem cells, HMOX-1.

## Abbreviations

SB cells®: Stembios cells; CO: Carbon monoxide; HMOX-1: Heme oxygenase decycling-1; VSELs: Very-small embryonic-like stem cells; HSC: Hematopoietic stem cells; BLSC: Blastomere-like stem cells; HTN: Hypertension; HT: Hypertension; HBP: High blood pressure; cGMP: Cyclic guanosine monophosphate; MAPK: Mitogen-activated protein kinase; TNFα: Tumor necrosis factor alpha; IL-1β: Interleukin-1 beta; IV: Intravenous ; RBC: Red blood cells; WBC: White blood cells; ΔΔ Ct: Delta-delta Cycle Threshold; ACE: Angiotensin converting enzyme; NSAIDS: Nonsteroidal anti-inflammatory drugs; CIRM: California Institute of Regenerative Medicine; hES: Human embryonic stem cell; AE: Amniotic epithelial; HGF: Human growth factor.

## Introduction

SB cells® are adult pluripotent stem cells isolated from adult blood and bone marrow. SB cells® are capable of differentiating into different cells type of the endoderm, mesoderm, and ectoderm such as osteocyte, neurons, muscle cells, and cardiomyocyte. With self-renewing and differentiation capabilities SB cells® have distinctive characteristics closer to embryonic stem cells than other types of adult stem cells, thereby eliminating the concerns over teratoma formation and immune rejection. Previous publication of flow cytometry analysis shows the presence of SB cells® in the small cell like region (3-7μm) devoid of white blood cells and red blood cells. SB cells® are less than 6μm in diameter, expresses Lgr5+ and CD349+ but not CD133-, CD45-, and CD66e-; thereby, concluding properties dissimilar to very-small embryonic-like stem cells (VSELs), hematopoietic stem cells (HSCs), and blastomere-like stem cells (BLSCs) [1,2].

Hypertension (HTN or HT), also known as high blood pressure (HBP), is a long-term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure itself usually does not cause symptoms. However, long-term high blood pressure would be a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss, and chronic kidney disease [3,4,5]. A typical treatment of hypertension would consist of the administration of several classes of antihypertensive medications, which includes thiazide-diuretics, calcium channel blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blocks. These medications could be used alone or in combination, with the latter option serving to minimize counter-regulatory mechanisms that act to revert blood pressure values to pre-treatment levels. Most people would require more than one medication to control their hypertension more efficiently [6].

Heme oxygenase decycling-1 (HMOX1) is a human gene that encodes the enzyme heme oxygenase I (EC1.14.99.3). Heme oxygenase mediates the first rate-limiting step of heme catabolism, cleaving heme to form biliverdin, which converts to bilirubin by biliverdin reductase, ferrous iron, and carbon monoxide (CO). The ability of the oxygenase I enzyme to catabolize free heme produces metabolites that have anti-oxidant, anti-inflammatory, and vasodilatory properties. CO generated by HMOX1 activates the enzyme guanylate cyclase to produce cyclic guanosine monophosphate (cGMP) that helps with vasodilation, anti-platelet aggregation, and anti-proliferation [7]. CO could also act on hemoprotein targets to modulate hemoprotein functions, and participate in the anti-inflammation pathway with its effector activating p38 mitogen-activated protein kinase (MAPK) that inhibits pro-inflammatory cytokine production such as tumor necrosis factor alpha (TNFα), Interleukin-1 beta (IL-1β) [8,9].

HMOX-1 gene is highly expressed in all types of cells when exposed to various oxidative stressing stimuli such as heavy metals, ultraviolet light, cytokines, and hypoxic conditions. This is due to the fact that there is a plethora of stress activating recognition site on the hmox1 promoter, making this enzyme a highly sensitive and reliable indicator for various oxidative stress related experiment [10].

The up regulation of HMOX-1 serves as a homeostatic mechanism that quickly protects cells against oxidative damage; it has the potential of being a therapeutic target of cardiovascular related diseases. The extent to which HMOX-1 is feasible in treating such diseases is highly dependent on the level of expression, cell-type or organ specificity expression.

The identification of the amelioration of hypertension syndrome is based on (1) the purification process of SB cells®, (2) the delivery of autologous SB cells® through intravenous (IV) treatment, and (3) the detection of blood HMOX1 expression through real time PCR. Based on our findings, we propose that the application of SB cells® may play a role in the treatment of hypertension syndrome.

## Materials and methods

### Isolation of SB cells®

Procedure begins by having participating patients take Fucoidan (patent publication number: 20140178886); a natural supplement, an hour prior to having their blood collected.

SB cells® were collected from patients using SB cells® purification protocol as mentioned in our previous publication. SB cells® were then injected back to patients following the guidelines described in our procedural IRB (IRB protocol number SB-IN-4112) [1].

### RT-PCR

Total RNA from patient's blood plasma was extracted with Qiagen RNA extraction kit (catalog number: 74104). Concentration of each RNA sample was measured using Synergy H1 hybrid reader (Biotek).

Reverse transcription of RNA to cDNA synthesis was carried out using PTC-100 Programmable Thermal Controller from MJ Research Inc. In brief, 100ng of each total RNA sample was reverse transcribed using qscript cDNA supermix (catalog number: 015770) from Quanta Bioscience according to the manufacturer's instructions and subsequently diluted with nuclease-free water to 0.5ng/μL cDNA and stored at -20°C.

Real-time PCR was performed by adding SYBR green supermix from BioRad (catalog number: 170-8882) into patients' cDNA samples and analyzed with BioRad CFX96 Real-time system, C1000 Touch Thermal Cycler. Patients' cDNA samples were added to BioRad Hard-Shell PCR plates 96-well, thin wall (catalog number: HSP9601) and tested for gene HMOX-1 with housekeeping gene cyclophilin. The reference gene were tested in all runs and used as inter-run calibrators. The PCR products were analyzed on a 1.8% agarose gel to confirm the bands on the gel correspond to the gene of interest. Primer 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`)

**HMOX-1:** (Forward primer: 5`-CTC TGG AAA GGA GGA AGG AG-3`, Reverse primer: 5`-TTG AGA CAG CTG CCA CAT TA-3`)

**RT-PCR running program:**

95°C, 2:00
95°C, 0:05
60°C, 0:30
Plate read
Goto 2, 39X
95°C, 0:05
65°C, 0:31
65°C, 0:05 +0.5°C/cycle
Plate read
Goto 8, 60X

## Results

### A: Patient's medical history

Patient number 83 is an Asian female of age 68, a housewife. She had been diagnosed with hypertension with underlying type 2 diabetes for over 18 years. She is currently taking metformin to improve her blood sugar control, glucosamine and liquid calcium for joint pain from arthritis. She is not taking any anti-hypertensive medication. According to the American Heart Association's blood pressure chart, patient number 83's blood pressure prior to SB cells® treatment is categorized under high blood pressure hypotensive stage I with a value of systolic 145 and diastolic 90. After treatment with SB cells®, patient's blood pressure dropped to prehypertension blood pressure with systolic 120 and diastolic 80 (Figure 1).

Patient number 118 is a Hispanic male of age 61. He works long hours and his job is labor-intensive. He had also been diagnosed with hypertension with underlying type 2 diabetes for over 18 years, he claims that he had headaches and joint pains frequently and is currently taking Xanax for pain management. According to the American Heart Association's blood pressure chart, patient number 83's blood pressure prior to treatment is categorized under high blood pressure hypotensive stage I with a value of systolic 145 and diastolic 90. After treatment with SB cells®, patient's blood pressure dropped to prehypertension blood pressure with systolic 120 and diastolic 80 (Figure 1).

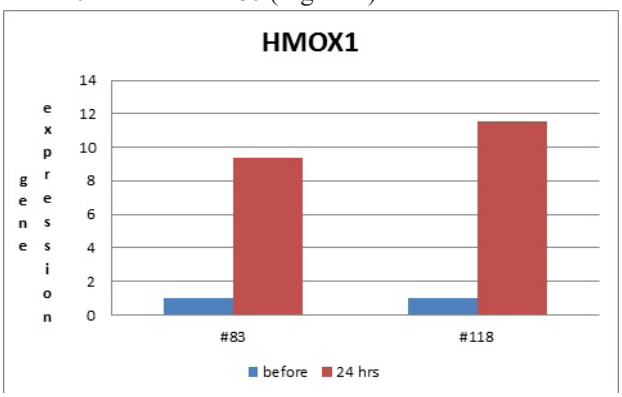


**Figure 1:** HMOX-1 gene expression. Expression of HMOX-1 gene on patient 83 and 118 shown as fold differences from before and 24Hr post treatment. X-axis as patient number and Y-axis as the 2 times exponent of normalized HMOX-1 Ct values against Cyclophilin, a housekeeping gene as baseline control, Ct values (not shown).

### B: qRT-PCR analysis

24 hours after administration of SB cells® treatment, gene expression of HMOX-1 have shown to increase 9-folds for patient #83, and 11-folds for patient #118 (Figure 2).

The amount of fold increment was calculated by computing delta-delta (ΔΔ) Cycle Threshold (Ct). A Ct value is defined as the number of cycles it takes for the fluorescent signal to cross threshold level.

**Cyclophilin:** a housekeeping gene was used in this study as a baseline reference for normalization to the test primer HMOX-1. The differences in Ct values (ΔCt) of Cyclophilin, before and 24hr after treatment were adjusted against the Ct value of HMOX-1 at the 24hr time point. A 2-fold exponent of the second difference of Ct values (ΔΔ Ct) between HMOX-1 before and adjusted 24hr post treatment were computed to obtain the final value of HMOX-1 gene expression.

| Hypertension | #83 | #118 |
|---|---|---|
| Before treatment | 145/90 | 145/90 |
| After treatment | 120/80 | 120/80 |

**Figure 2:** Blood pressure monitoring. Blood pressure measurements from patients prior to and following SB cells® treatment taken within the exact hour of the day to minimize irregularities.

## Discussion

Patients who received SB cells® treatment followed by a blood draw 2 days after the treatment have a significant increase in HMOX-1 gene expression level. This allow us to hypothesize that intravenous (IV) administration of SB cells® gives the body a boost of underlying HMOX-1 level increasing its level by 9-11 folds that leads to a decrease in patients' blood pressure. However, how SB cells® causes the increase in HMOX-1 level and decreases blood pressure remains unclear and requires further investigation.

High blood pressure have been the most common chronic medical problems paralyzing many Americans well-being for many years, prompting visits to primary health care providers frequently. This disease affects between 16%-37% of the population worldwide, and in 2010, hypertension was believed to have indirectly been a factor of 18% (9.4 million) deaths. In the United States, 80% of people with hypertension are aware of their condition, 71% take some form of anti-hypertensive medication, but only 48% of the people are aware that their hypertension has been adequately controlled. Health care providers were taunted with many obstacles, such as drug reaction when combining multiple medications to achieve control of their patients' blood pressure [11].

There are many drugs in the market that helps to lower blood pressure; however, most of them come with detrimental side effects. Thiazide diuretics can increase the incidence of new onset diabetes especially when combined with beta-blockers. Beta-blockers may trigger asthma attacks for patients who are asthmatic. Angiotensin converting enzyme (ACE) inhibitors drug efficacy is decreased when combine with nonsteroidal anti-inflammatory drugs (NSAIDS) [12]. The treatment of hypertension can be very costly, with direct costs, such as prescription drugs, physician

visits, hospital care, nursing home care, and home health care, up to $54.2 billion. And indirect costs, such as loss of productivity due to mortality and morbidity, amounting to $19.2billion [13]. Being a relatively known disease for many years, the types of drugs used and frequencies of treatment are better known and readily available than a new method, such as stem cell therapy. It is also more users friendly and less invasive as compared to stem cell therapy, ingestion versus injection.

Many scientists and physicians have turned their attention to stem cell therapy in the hope to minimize the side effect and the cost of treating hypertension. In 2008, physicians announced successful results from a clinical study of pulmonary hypertension in which a patient was treated with autologous adult stem cells from his own peripheral blood. The California Institute of Regenerative Medicine (CIRM) has used human embryonic stem cell (hES)-derived neural stem cells with human amniotic epithelial (AE) cells to develop an "alternative" stem cell therapy to treat hypertension [14].

SB cells® could have the potential to alleviate hypertension symptoms with the cell's innate ability to increase HMOX1 level. Since SB cells® have properties closer to embryonic stem cells, it will not cause tumor formation and autologous immune-mediated rejection. SB cells® would be a cheaper alternative to prescription drugs with no side effects. However, there are questions going forward with SB cells® treatment, (1) Would allergies to mobilizing agent ingredient affect the chance of a patient success in such treatment, (2) Would efficiency decrease when patient miss a treatment, (3) Would underlying disease, such as cancer, complicate a patient success in the treatment?

In the future, we hope to expand the number of patients and the number of times each patient received SB cells® treatment weekly. Our future directions are as proposed; first arm would include one round of IV treatment with cells for 25 patients, second arm would include two consecutive rounds of IV treatment with SB cells®, performed weekly for 25 patients, third arm would include three consecutive rounds of IV treatment with SB cells®, performed weekly for 25 patients, and a control round of saline for 25 patients. We would want to test for human growth factor (HGF) and HMOX-1 level changes at time points of 0, 7,14,30,90 days after each SB cells® treatment. We would also keep a record of patient's blood pressure measured after morning prior to SB cells® treatments and 3 months after.

## Conclusion

In summary, SB cells® have effectively shown to maintain patients' blood pressure to the optimal level as well as increases the gene expression of HMOX1 similar to those function of many traditional drugs currently prescribed. However, improvements on existing treatment have to be made in order to confirm the findings. SB cells® could be a therapeutic alternative to patients seeking hypertensive treatment in the future.


## Funding and Competing Interests

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