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BRIEF REPORT

Stem Cell Treatment for Complicated Diabetes

Jong Yoon Bahk[1], Hoon Han[2], Youn Soo Lee[3]

[1]*Department of Urology, Gyeongsang National University Medical Graduate School, Jinju,* [2]*Histostem Co. Ltd. and* [3]*Cho & Lee's Urology Clinic, Seoul, Korea*

Background and Objectives: Self renewal, homing or migration and multipotent differentiation are characteristics of stem cell. We studied the effect of stem cell treatments on diabetes complicated with impotencies using human umbilical cord blood stem cells (hUCBSCs). **Methods and Results:** The patients who had no erection more than 6 months, were not responded to any medication more than 6 months and were waiting penile prosthesis due to type 2 diabetics were participated and number was 5. All had normal laboratory findings except diabetes mellitus related one. Prepared hUCBSCs were ABO, HLA-AB, DR and sex identical to each patient. Total 1.5×10^7 hUCBSCs were infused into both corpus cavernosa. Immune suppression was not done. The blood glucose, medication dose and erection diary were recorded and followed for 9 months. Mean age of participants were 61 (57～66). The blood glucose dropped from second week, and insulin or hypoglycemic agent doses were reduced in all patients for 6～7 months. The level of glycosylated hemoglobin was improved from post-treatment for 3～4 months. The libido was improved and morning erection was regained from 3 weeks. During the follow-up, one patient turned out for prosthesis, two patients were returned to non-erection state at 8 and 9 months and two patients maintained erections with medication. **Conclusions:** The hUCBSCs has positive effect on blood glucose and erectile dysfunction, although it is not sufficient. We suppose that the stem cell effects might be caused by combination of unknown humoral factors from hUCBSCs and hUCBSCs themselves.

Keywords: Umbilical cord blood stem cell, Diabetic impotence, Cell therapy

Introduction

Although much is yet to be understood about the nature of the putative increased permeability with respect to diabetes, intracellular hyperglycemia causes abnormalities in blood flow and increased vascular permeability. Hyperglycemia decreased activities of vasoconstrictors such as angiotensin II and endothelin-1, and the increased activities of permeability factors such as vascular endothelial growth factor (VEGF) (1). The chronic complications of diabetes mellitus (DM) affect many organs and causes vascular complications, such as microvascular and macrovascular, and nonvascular complications. Erectile dysfunction (ED) is the most common symptom of the diabetes (DM). Diabetic ED is believed to be caused by one or combination of some disorders in central or peripheral nervous system, hormone production, psychological factors, vascular integrity, endothelial smooth muscle, and so on (2). Diabetic neuropathy is a serious common earliest complication of diabetes involving genitourinary system (3). About one-third of type 2 diabetic men have lower levels of free testosterone (4). Current therapeutic modalities for type 2 diabetics involve insulin and oral hypoglycemic agents, and human embryonal stem cells clusters in nestin-selection protocol with minor modifications (5, 6) showed 30-fold increased production of insulin compared to those increased by monolayer cells.

The most prominent characteristics of stem cells are self renewal and multi potential differentiation, and homing and migration with ability to replace or treat the damaged tissues. Adult stem cells (ASCs), discovered from various

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Correspondence to **Jong Yoon Bahk**
Department of Urology, Gyeongsang National University Medical Graduate School, 92, Chilam-dong, Jinju 660-751, Korea
Tel: +82-55-751-8816, Fax: +82-55-751-8807
E-mail: jybahk@hanmail.net

mature organs, are used clinically and showed many therapeutic results (7-9). ASCs offer hopes for the development of new therapeutic applications for a large numbers of diseases which are incurable or difficult to cure. Angiogenic potential of mesenchymal stem cell (MSC) is one of the representative roles of stem cell. Many angiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet derived growth factor AB (PDGF-AB), transforming growth factor-β (TGF-β), and integrin-β1 can augment therapeutic neovascularization effects of stem cells. Immaturity and easy accessibility of hUCBSC without ethical problem emerged as an attractive tool for cell based therapy. Stem cells transplanted during surgery (10), lumbar puncture (11) and intravenous route (12) for brain and spinal cord lesions showed favorable results for neuroregeneration. Data of improvements in vascular insufficiencies and peripheral neuropathies accumulated rapidly, and stem cell therapy was believed to be a new potential approach for vascular and neurological insults.

In diabetic impotence, vascular and neural lesions are very important causes and many dramatic reports attract us to study the effects of allogenic human umbilical cord blood stem cell (hUCBSC) therapy on erectile dysfunction, prospectively.

Materials and Methods

Five diabetic impotent patient waiting penile prosthesis, unresponsive to medical therapies (PDE5 inhibitor or PGE1 injection) more than 6 months, were participated. All materials were approved by IRB and Ethical Committee, and informed consents were received. IIEF-5 questionnaire and standard ED evaluations including medical history, physical examination, testosterone and prolactin level, penile duplex Doppler ultrasonography and some other studies including cancer screening (Table 1) were done. Immune suppression was not done. The blood glucose (BG) and symptoms were monitored for 9 months. The hUCBSCs were supplied from Histostem Co. Ltd. (Seoul, Korea). They were ABO, HLA-ABC, DR and sex identical to each patient. hUCBSCs were CD13(+), CD14(−), CD29(+), CD31(−), CD34(−), CD44(+), CD45(−), CD49e(+), CD54(+), CD90(+), CD106(−), AMSA(+), SH2(+), SH3(+), HLA-ABC(+) and HLA-DR(−). Total cell numbers were around 1.5×10^7 and they were supplied in undifferentiated state.

Table 1. Pretreatment studies

Imaging	Ultrasound examination on thyroid, heart, liver, Kidney and Prostate CT on brain, chest and abdomen
Blood & serology	CBC, Serologic series 12
Endoscopy	Gastroscopy, colonoscopy
Urine	UA, Microscopic examination
Cancer markers	AFP, CEA, CA19-9, PSA
Others	VDRL, AIDS, Hepatitis Ag/Ab test, H. pyroli test, Electrolyte, Testosterone, C-reactive protein, ASO titer, EKG, ESR, Prolactine, RA factor

Before stem cell treatments, above items are examined thoroughly to rule out other diseases, including cancer, in all participants. When there were some abnormal findings aside from diabetes related one, the patients were not included in study.

hUCBSCs were injected into both corpus cavernosa. Penile root was clamped with rubber band before injection, and released at 30 minutes after injection to prolong the residence time hoping to increase the graft. Three diabetic impotent controls were injected with saline. The doses of hypoglycemic agent or insulin were modified according to BG level. Patients recorded daily fasting glucose, penile erection and medication doses every day. After treatment, penile duplex Doppler ultrasonography was not done. Patients took the PDE-5 inhibitor to assist the erection.

Results

The BG were begun to lower at second weeks and needed to reduce the medication doses from one month in insulin and three months in hypoglycemic agent (Table 2), and gylcosylated hemoglobins were improved in all. There was little change in IIEF-5 score except improved libido (Table 3) and erection (Table 4). There was no significant change in testosterone and prolactine, within normal values. All regained the morning erections within 3 weeks, and three had the full penile erections with PDE-5 inhibitor addition. Although, sometimes, it was not satisfactory, all gained the full erection within 2 months. There was no side reaction. During the follow up, three patients were returned to non-erection at 8 and 9 months and other two maintained insufficient erections for coitus but maintained full erections with oral medication.

Discussion

Umbilical Cord Blood Stem Cell (UCBSC) is the most immature form of ASCs, free from ethical problem and has similar regenerative capability (13) with other form. Adult stem cell (ASC) treatments are effective in cardiac

Table 2. The changes of the fasting blood glucose and glycosylated hemoglobin

Patient	Fasting blood glucose										Types of treatment
	Before treatment	Post-Tx. 1 M	Post-Tx. 2 M	Post-Tx. 3 M	Post-Tx. 4 M	Post-Tx. 5 M	Post-Tx. 6 M	Post-Tx. 7 M	Post-Tx. 8 M	Post-Tx. 9 M	
1	127	120	106	94	106	109	102	107	116	105	HA
2	115	108	101	90	108	102	106	117	109	104	HA
3	125	120	108	93	102	105	109	118	112	118	HA
4	107	98	113	105	90	110	99	118	110	114	Insulin
5	102	94	105	93	98	102	100	107	112	103	Insulin
Control 1	125	129	119	121	132	124	127	125	126	121	HA
Control 2	118	120	117	120	119	125	120	115	122	120	HA
Control 3	132	128	125	126	129	135	124	129	132	130	Insulin

Patient	Glycosylated hemoglobin										Types of treatment
	Before treatment	Post-Tx. 1 M	Post-Tx. 2 M	Post-Tx. 3 M	Post-Tx. 4 M	Post-Tx. 5 M	Post-Tx. 6 M	Post-Tx. 7 M	Post-Tx. 8 M	Post-Tx 9 M	
1	7.4	7.3	7.4	7	6.8	6.8	6.8	6.7	6.5	6.7	HA
2	6.9	6.9	6.8	6.7	6.7	6.6	6.5	6.4	6.2	6.4	HA
3	7.6	7.5	7.6	7.2	6.9	6.6	6.8	6.8	6.6	6.8	HA
4	6.8	6.8	6.7	6.7	6.6	6.4	6.2	6.3	6.3	6.2	Insulin
5	6.9	6.9	6.8	6.6	6.6	6.3	6.2	6.1	6.3	6.4	Insulin
Control 1	7.4	7.5	7.4	7.4	7.4	7.3	7.3	7.3	7.4	7.5	HA
Control 2	7.3	7.2	7.3	7.3	7.3	7.2	7.3	7.3	7.3	7.3	HA
Control 3	7.4	7.5	7.5	7.6	7.6	7.4	7.5	7.4	7.5	7.4	Insulin

All participants checked the fasting blood sugar (FBS) at home every day and monthly at hospital, and glycosylated hemoglobin at hospital. Table showed the gradual decrease in FBS and the participants were recommended to reduce the hypoglycemic agent or insulin dose when FBS below 100 mg/mL to prevent hypoglycemia. All 5 participants controlled the blood sugar relatively well before treatments. From one month after stem cell treatment, two using insulin for blood sugar control showed FBS levels lower than 100 mg/mL and they reduced the insulin doses. Participants who control the blood sugar with hypoglycemic agents showed FBS levels below 100 mg/mL from third months after stem cell therapy. The glycosylate hemoglobin
level was reduced from third month after treatment in all participants. The control showed little change.
HA: Hypoglycemic Agent, Post-Tx.: Post treatment.

ischemia (14, 15), peripheral vascular impairment (16), neurological disorders (10, 17) and congenital disorders (8, 9).

Patients were type II diabetes and they controlled BG in good compliances. When we designed this experiment, we did expected homing of stem cells in some proportion via blood stream into pancreas like Min's report, (18) and anticipated favoring effects on blood glucose. As we expected, data (Table 2) showed lowering effects of BG regardless of the hypoglycemic agent or insulin. And the results show that, although we do not know the mechanism of these results, there are improvement in libidos (Table 3) and erection (Table 4). We suppose that results related with desire and erection would be caused by unknown humoral factors of stem cell. Koblas et al. (6) proposed three possible generation of β cells derived from hUCBSC, and "the direct transplantation of un-manipulated stem cells and their homing to the pancreas with their subsequent differentiation" would be applicable to our results.

All experienced the improved penile erection and libido, different from others (19, 20), because all were free from cancer or radio-chemotherapy and much less psychological burdens. MSC modified with endothelial nitric oxide synthase injected into corpora cavernosa for correction of the erectile dysfunction in old rat improved erectile function (6) although we used hUCBSC without modification.

We did not use the immune suppressant. Other's hUCBSC treatment results, no problem in ABO, HLA-AB, DR and sex identical cells without immune suppression (unpublished data), encouraged us to treat our patients without immune suppression. Rasmusson et al. (21) reported the immuno-modulatory properties in MSC, inhibition of T-cell proliferation. And hUCBSCs with peripheral lymphocyte in the presence of phytohaemagglutinin (PHA) or IL-2 inhibit the lymphocyte proliferation. hUCBSCs also suppressed the proliferation of regu-

Table 3. IIEF questions

Patient No.	Question 11 (Felt desire)					Question 12 (Level of desire)				
	Before Tx	Post-Tx 1 M	Post-Tx 3 M	Post-Tx 6 M	Post-Tx 9 M	Before Tx	Post-Tx 1 M	Post-Tx 3 M	Post-Tx 6 M	Post-Tx 9 M
1	2	3	3	3	2	2	3	3	3	3
2	2	3	3	3	3	2	3	3	3	3
3	2	2	3	3	3	2	3	3	3	3
4	2	2	3	3	PP	2	2	2	3	PP
5	2	3	3	3	3	2	3	3	3	3
Cont. 1	2	1	2	2	2	2	2	2	2	2
Cont. 2	1	2	2	2	2	2	2	2	2	2
Cont. 3	1	1	1	2	1	1	2	1	1	1

Level of desire. Participants were asked every month on the changes in desire. IIEF (International Index of Erectile Function) Question 11 is "Over the past 4 weeks, how often have you felt sexual desire?" and scales are divided into 5 (Almost always or always), 4 (Most times, much more than half the time), 3 (Sometimes, about half the time), 2 (A few times, much less than half the time) and 1 (Almost never or never). IIEF Question 12 is "Over the past 4 weeks, how would you rate your level of sexual desire?" and scales are divided into 5 (Very high), 4 (High), 3 (Moderate), 2 (Low) and 1 (Very low or none at all). Table showed that all participants increased desire although they were not high. But control had the a little change.

Table 4. IIEF question 1

Patient No.	Before Tx.	Post-Tx 1 M	Post-Tx 3 M	Post-Tx 6 M	Post-Tx 9 M
1	1	1	2	2	1
2	1	2	3	3	3
3	1	2	3	3	3
4	1	1	2	2	PP
5	1	2	2	2	2
Cont. 1	1	1	1	1	1
Cont. 2	1	1	1	1	1
Cont. 3	1	1	1	1	1

Level of frequency in erection at coitus. Participants were asked every month on the condition of penile erection when they attempted sexual intercourse. IIEF Question 1 is "Over the past 4 weeks, how often were you able to get an erection during sexual activity?" and the scales are 5 (Almost always or always), 4 (Most times, much more than half the time), 3 (Sometimes, about half the time), 2 (A few times, much less than half the time), 1 (Almost never or never), 0 (No sexual activity). All participants replied that they never or almost never erected before treatment but regained the penile erection at posttreatment one month. But control never regained penile erection.

latory T cells (22) and regarded as the tolerance (23). We assume that this would be due to immune tolerance induction.

Although there are many complications in diabetes, this study may be the first report on the unmodified stem cell therapy for DM complication, diabetic impotence. In stem cell therapy, the functional differentiation of implanted cells would be expected, and our patients showed improvements, we have some questions on the mechanisms of the hUCBSC actions because the total cell number was small. And, we don't know the implantation and differentiation rates occurred during follow-up. Early improvement of penile erection would be due to unknown humoral factors because the time interval was very short from treatments. And hormonal effect could be ruled out due to late onset of symptom improvement to other hormone action.

In conclusion, we report the simultaneous improvements of the BG and ED in diabetes with intracavernous infusion of hUCBSC, the remote sites from pancreas, without immune suppression but we do not know the exact mechanisms. We suppose that these effects would be combined effects of unknown humoral factor produced from stem cell and stem cells, and the mechanism of action should studied further more.

Potential Conflict of Interest

The authors have no conflicting financial interest.

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