

Treatment of Diabetic Impotence with Umbilical Cord Blood Stem Cell Intracavernosal Transplant: Preliminary Report of 7 Cases

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Abstract

Objectives: Stem cells are characterized by self renewal and multipotent differentiation. We report the effects of intracavernosal transplant of human umbilical cord blood stem cells on diabetic erectile dysfunction.

Materials and Methods: Seven type 2 diabetics who had failed to achieve an erection for at least 6 months despite medications, and who are currently awaiting penile prostheses, participated. All laboratory results were normal, except for impotence and diabetes mellitus. A total of 1.5×10^7 human umbilical cord blood stem cells were infused into the corpus cavernosum. No immunosuppressive measures were taken in any of the patients. International index of erectile function-5, SEP, GAQ, erection diary, blood glucose diary, and medication dosage were followed for 9 months.

Results: The mean age was 69.5 years (range, 57-87 years). Morning erections were regained in 3 participants within 1 month, and for all except 1 by the third month, and maintained for more than 6 months. Rigidity increased as the result of stem cell therapy alone, but was insufficient for penetration. With the addition of PDE5 inhibitor before coitus, 2 achieved penetration and experienced orgasm, and maintained for more than 6 months; however, 1 participant could not achieved penetration at ninth month. All but 1 reported increased desire. During follow-up, 2 returned for prosthesis, 4 returned to a

nonerectile condition at 9 months, and 1 maintained erection sufficient for coitus with medication until the 11th month. Blood glucose levels decreased by 2 weeks, and medication dosages were reduced in all but 1 subject for 4 to 7 months. Glycosylated hemoglobin levels improved after treatment for up to 3 to 4 months.

Conclusions: Human umbilical cord blood stem cell therapy has positive effects on erectile dysfunction and diabetes mellitus. Stem cells and unknown humoral factors of human umbilical cord blood stem cells mediate mechanism may contribute to these positive effects.

Key words: Cell therapy, Erectile dysfunction, Humoral factor, Transdifferentiation.

The current treatments for erectile dysfunction include psychotherapy, oral or injected medication, and penile prosthesis. Oral medication is the most popular of these modalities. Presently, many patients seek non- or minimally invasive, permanent treatments, as opposed to temporary improvement. Gene or cell therapies (1) may hold great promise as new therapeutic modalities for the treatment of impotence, and may in the future represent a satisfactory resolution to the currently unresolved needs of these patients.

In diabetes, the activities of vasoconstrictors such as angiotensin II and endothelin-1 (2) are increased, and the activities of permeability factors, most notably vascular endothelial growth factor (3), are increased, too. The chronic complications of diabetes mellitus affect multiple organs and cause microvascular and macrovascular complications, in addition to nonvascular complications. Erectile dysfunction is the second most-common symptom of diabetes mellitus (4), and affects 32% of men with type 1 diabetes and 46% of men with type 2 diabetes

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(5). From the pathophysiological perspective, erectile dysfunction is caused by the impaired relaxation of corporal smooth muscle cells (6). This condition is thought to be induced by a disorder—or a combination of disorders—in the central or peripheral nervous system, hormone production, vascular integrity, or endothelial smooth muscle, as well as psychological and other factors (7, 8). Additionally, approximately one-third of men with type 2 diabetes evidence lowered levels of free testosterone (9, 10). The current medical therapeutic modalities for type 2 diabetics are oral hypoglycemic agents and insulin.

Stem cells are characterized by a self-renewal, multipotentiality for differentiation, and homing or migration, depending on the stimuli received. Some types of adult stem cells, which exist within matured organs, are now clinically available, and their therapeutic effects have been firmly established (11-15). Adult stem cells may prove invaluable in the development of new therapeutic applications for a variety of incurable or difficult-to-cure diseases. In a previous study, human embryonic stem cell clusters in nestin-selection with minor modifications (16) were reported to evidence a 30-fold increase in insulin production as compared to monolayer cells. Adult stem cell research, by way of contrast, is moving much more rapidly than ESC research, and has reached the clinical trial phase well ahead of ESC research.

Angiogenic and neurogenic potentials of mesenchymal stem cells are important targets of adult stem cell treatments (17, 18). Many angiogenic factors, including vascular endothelial growth factor, basic fibroblast growth factor, platelet derived growth factor AB(PDGF-AB), transforming growth factor- β (TGF- β), and integrin- β 1 have been shown to augment the therapeutic neovascularizing effects of stem cells. In addition to angiogenic factors, some neurotrophic factors, such as ciliary neurotrophic factor (19), brain-derived neurotrophic factor, and neurotrophin-4/5 (NT4/5) (20), are secreted by stem cells and may promote neural regeneration. Additionally, stem cells have been induced by isobutylmethylxanthine to differentiate into neuronlike cells (21); this process is mediated by the insulinlike growth factor 1 signaling pathway (22).

Reports on improvements of vascular insufficiencies (23, 24) and neuropathies (25, 26) are accumulating rapidly, and stem cell therapies are

already being regarded as a new potential therapeutic modality for the treatment of vascular and neurologic insults. The available evidence points to the possible role of stem cells as replacement cells for the routine maintenance of normal tissues, such as kidney tissues (27), and for the repair of damaged tissues, such as in an infarcted heart (28). Adipose tissue-derived stem cells may be induced by isobutylmethylxanthine to differentiate into neuronlike cells (21); this process appears to be mediated by the IGF-I signaling pathway (22).

Among the variety of types of adult stem cells, which would reside in virtually all postnatal organs and tissues (29), bone marrow stem cells, adipose tissue derived stem cells, and umbilical cord blood stem cells can be used for practical, clinical, cell-based therapeutic purposes. Among these 3 types, umbilical cord blood stem cells are the most-attractive adult stem cell type, because they can be readily and noninvasively collected from donors, and their collection and use raise no problematic ethical implications.

The principal routes for stem cell transplant are intravascular infusion (30), via lumbar puncture (31), and during surgery (32) when used to treat cardiovascular or vascular lesions, internal organ lesions, and nervous lesions, including lesions in the brain or spinal cord. Surgical transplant is, of course, the most-accurate route. Vascular and neural lesions are an important cause of diabetic erectile dysfunction, and the many dramatic reports currently emerging on stem cell therapies led us to conduct a prospective study on the effects of allogeneic human umbilical cord blood stem cell therapy on erectile dysfunction. Thus, we report the results of stem cell therapy via intracavernous infusion of human umbilical cord blood stem cells in patients with diabetic erectile dysfunction.

Materials and Methods

Characteristics of participants

Ten, diabetic impotent patients, all of whom had type 2 diabetes for a mean of 29.4 years (range, 12-52 years), participated in this study. All patients had proven unresponsive to previous medical therapies (oral phosphodiesterase 5 inhibitor, PDE5 inhibitor, or prostaglandin E 1, PGE1 injection) for more than 6 months, and all were awaiting penile prostheses. The 10 participants were grouped according to the order

in which they were entered into the study—for every 3 patients, the first 2 were assigned to the experimental group and the third to the control group. Thus, 7 patients ultimately were included in the experimental group, and 3 were in the control group.

This study was conducted as a single-blind study and all participants were informed of the materials (stem cells) and the nature of treatments to be used; additionally, informed consent was obtained from all participants. This study, which conforms with the ethical guidelines of the 1975 Helsinki Declaration, was also approved by the relevant institutional review board. Written informed consent was obtained from all of the subjects. Information related to sexual status, international index of erectile function-5 and sexual encounter profile questionnaires, and standard erectile dysfunction evaluations, including medical history, thorough physical examination, testosterone and prolactin levels, and penile duplex Doppler ultrasonography were performed for all patients. Among many candidates, only those individuals with a body mass index between 20 and 25 were included in this study.

To exclude patients that might have diseases (including cancer) other than diabetes mellitus and erectile dysfunction, all participants were subjected to ancillary tests, including blood and serologic studies (CBC, serologic series 12 including lipids, tumor markers < AFP, CEA, CA19-9, PSA>, VDRL, AIDS, hepatitis Ag/Ab, *H. pylori*, electrolytes, C-reactive protein, ASO titer, ESR, RA factor) tests, urine studies (urine analysis, microscopic examination), imaging studies (ultrasound examinations for thyroid, heart, liver, kidney, and prostate; and computed tomography scans for brain, chest, and abdomen), electrocardiogram, funduscopy, and direct inspections of the gastrointestinal systems (gastroscopy and colonoscopy). All participants had only diabetes mellitus and erectile dysfunction but were within normal range for testosterone and prolactin (testosterone levels were examined every visit during follow-up). Anybody with an abnormal finding on these ancillary studies was excluded from this study. No immunosuppressive measures were applied. International index of erectile function-5 and blood glucose were monitored for 5 to 11 months. The patients' hormonal levels were checked every visit.

Characteristics of human umbilical cord blood stem cells

The human umbilical cord blood stem cells were supplied by Histostem Co. Ltd. (Seoul, Korea) and were deemed acceptable for clinical use by the Korean government. The cells were ABO, HLA-ABC, DR, and sex-matched for each participant, and had been obtained from donors without any specific family medical history, including diabetes mellitus. The human umbilical cord blood stem cells were as follows: CD13(+), CD14(-), CD29(+), CD31(-), CD34(-), CD44(+), CD45(-), CD49e(+), CD54(+), CD90(+), CD106(-), AMSA(+), SH2(+), SH3(+), HLA-ABC(+) and HLA-DR(-).

Umbilical cord blood stem cell treatment procedures

Human umbilical cord blood stem cells, in a total cell number of around 1.5×10^7 , were injected into both corpus cavernosa of each patient. The penile root was clamped with a band before injection and released 30 minutes after the human umbilical cord blood stem cells were injected prolong the transplanted cell residence time, and hopefully increase the graft. After stem cell treatment, penile duplex Doppler ultrasonography was not conducted, owing to ignorance about the possible effects of vasoactive drugs on the transplanted stem cells. Normal saline was injected into the cavernosa of the 3 diabetic impotent controls.

Follow-up

All participants submitted an international index of erectile function questionnaire at every visit, before a follow-up interview. Dosages of a hypoglycemic agent or insulin declined during periods in which the blood glucose level dropped below 5.55 mmol/L (100 mg/mL). All patients were instructed to use the PDE5 inhibitor when they wished to engage in sexual intercourse after 3 months from the time of the stem cell therapy. All patients monitored their fasting blood sugar at home every day and monthly at the hospital, and their glycosylated hemoglobin levels also were checked monthly at the hospital. Participants were encouraged to reduce their medication dosage when the fasting blood sugar fell below 5.55 mmol/L (100 mg/mL) to prevent hypoglycemia. For each patient, an erection diary, blood glucose diary, and medication dosage were recorded daily, and the patients were followed for 11 months.

Results

The 10 participants' ages ranged from 57 to 87 years (average, 69.5 years). Penile duplex Doppler ultrasonography was conducted for all 10 participants before stem cell therapy, and these records demonstrated that all had reduced peak systolic velocities in both cavernosal arteries—less than 25 cm/sec. All participants exhibited some inconsistent changes in testosterone levels on their follow-ups, but these variations were within normal limits (data not shown). Additionally, some changes in international index of erectile function-5 scores were noted (Table 1).

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. pylori test, Electrolyte, Testosterone, C-reactive protein, ASO titer, EKG, ESR, prolactin, RA factor, visual acuity, funduscopy

Abbreviations: AFP, alpha-fetoprotein; AIDS, acquired immunodeficiency syndrome; ASO, antistreptolysin O; CBC, complete blood count; CEA, carotid endarterectomy; EKG, electrocardiogram; ESR, erythrocyte sedimentation rate; PSA, prostate-specific antigen; UA, urine analysis; VDRL, venereal disease research laboratory.

Among the 7 experimental group participants who, before treatment, had no erections in the morning or during sexual activity, 3 experienced morning erections by 1 month after treatment, and all but 1 regained morning erections by the second month (question 1); this was maintained for at least 3 months. The 3 control patients experienced no changes (no morning erections). Six of the experimental participants experienced increases in penile hardness with time from stem cell therapy only; however, the degree of increased hardness was still insufficient for effective penetration. With the addition of a PDE5 inhibitor (sildenafil citrate 100 mg), 2 (cases 2 and 3) could achieve penetration, maintenance, and orgasm, and retained that ability (questions 3 and 4) at the fifth month after treatment (Table 2). At 9 months' follow-up, case 3 reported not being able to maintain penetration, even when using the PDE5 inhibitor. The control experienced no changes in penile rigidity. All expressed desire before and after cell therapy, and all but 1 participant

Table 2. International Index of Erectile Function scores. International Index of Erectile Function Questions.

Patient	Before treatment	Post-Tx 1M	Post-Tx 3M	Post-Tx 6M	Post-Tx 9M	Post-Tx 11M
1	1	1	2	2	1	1
2	1	2	3	3	3	3
3	1	2	3	3	2	1
4	1	1	2	2	PP	PP
5	1	2	2	2	1	1
6	1	1	2	2		
7	1	1	1	PP		
Control 1	1	1	1	1	1	
Control 2	1	1	1	1	1	
Control 3	1	1	1	1	1	

Patient	Before treatment	Post-Tx 1M	Post-Tx 3M	Post-Tx 6M	Post-Tx 9M	Post-Tx 11M
1	1	1	1	1/1	1/1	1/1
2	1	1	1	2/3	2/3	2/3
3	1	1	1	1/3	1/2	1/1
4	1	1	1	1/1	PP	PP
5	1	1	1	1/2	1/1	1/1
6	1	1	1	1/2		
7	1	1	1	PP		
Control 1	1	1	1	1/1		
Control 2	1	1	1	1/1		
Control 3	1	1	1	1/1		

Patient	Before treatment	Post-Tx 1M	Post-Tx 3M	Post-Tx 6M	Post-Tx 9M	Post-Tx 11M
1	1	1	1	1/1	1/1	1/1
2	1	1	1	1/3	1/3	1/3
3	1	1	1	1/2	1/1	1/1
4	1	1	1	1/1	PP	PP
5	1	1	1	1/1	1/1	1/1
6	1	1	1	1/1		
7	1	1	1	PP		
Control 1	1	1	1	1/1		
Control 2	1	1	1	1/1		
Control 3	1	1	1	1/1		

Patient	Before treatment	Post-Tx 1M	Post-Tx 3M	Post-Tx 6M	Post-Tx 9M	Post-Tx 11M
1	2	3	3	3	2	2
2	2	3	3	3	3	3
3	2	2	3	3	3	2
4	2	2	3	3	3/PP	3/PP
5	2	3	3	3	3	2
6	2	3	3	3		
7	3	3	3	3/PP		
Control 1	2	1	2	2		
Control 2	2	2	2	2		
Control 3	1	2	1	2		

Patient	Before treatment	Post-Tx 1M	Post-Tx 3M	Post-Tx 6M	Post-Tx 9M	Post-Tx 11M
1	1	1	1	1/2	1/1	1/1
2	1	1	2	2/3	2/3	2/3
3	1	1	1	1/3	1/2	1/2
4	1	1	1	1/1	PP	PP
5	1	1	1	1/1	1/1	1/1
6	1	1	1	1/1		
7	1	1	1	PP		
Control 1	1	1	1	1/1		
Control 2	1	1	1	1/1		
Control 3	1	1	1	1/1		

Patient	Before treatment	Post-Tx 1M	Post-Tx 3M	Post-Tx 6M	Post-Tx 9M	Post-Tx 11M
1	1	1	1	1/1	1/1	1/1
2	1	1	2	2/3	2/3	2/3
3	1	1	2	2/3	1/2	1/2
4	1	1	1	1/2	PP	PP
5	1	1	1	1/2	1/2	1/1
6	1	1	1	1/1		
7	1	1	1	PP		
Control 1	1	1	1	1/1		
Control 2	1	1	1	1/1		
Control 3	1	1	1	1/1		

Abbreviations: PP, penile prosthesis; X / Y X, without sildenafil citrate; Y, sildenafil citrate was combined after the third month;

Patient 6: Followed for 7 months

Patient 7: Followed for 5 months, and penile prosthesis was done at fifth month

reported an increased desire in both frequency and intensity after stem cell treatment (question 11).

The control showed some fluctuation in desire, but this may be attributable to changes in their hormone levels, which varied within normal range. Hormonal levels exhibited individual and intrapersonal variations at each test, but again, all remained within normal range (data not shown). During 11 months' follow-up, 2 participants turned for prosthesis, 1 returned to nonerectile status, 3 could achieve only poor erections at 5 to 9 months, and 1 patient maintained erection sufficient for coitus with the help of medication. Two of the patients who could achieve penetration and maintenance also experienced orgasm. With regard to the effects of stem cells on erectile dysfunction, 3 (3/7) of the subjects agreed that stem cell therapy applied alone had some effect on erectile dysfunction, although this effect was insufficient (question 13), and 5 (5/7) of the subjects regarded stem cell therapy as effective for erectile dysfunction when combined with a PDE 5 inhibitor (Table 3).

Table 3. Sexual Encounter Profile (SEP) question at fifth month.

Patient	SEP 2 (Insert penis into vagina)	SEP 3 (Maintenance)
1	0/5	0/5
2	5/8	3/8
3	3/6	2/6
4	0/4	0/4
5	1/5	0/5
6	1/5	0/5
7	0/6	0/6
Control 1	0/3	0/3
Control 2	0/2	0/2
Control 3	0/2	0/2

Subjects' blood glucose levels began to drop from 2 weeks after treatment, and 6 of 7 evidenced some reduction in fasting blood sugar by 1 month. The insulin dosage had to be dropped at 1 month in 2 cases (cases 4 and 5) owing to lower fasting blood

sugar (lower than 5.55 mmol/L (100 mg/mL); and at 3 months, a hypoglycemic agent had to be reduced (Table 5) to maintain blood glucose levels at above 5.5 mmol/L (100 mg/mL). The most elderly of the subjects (No. 7) evidenced no changes in blood glucose levels after stem cell therapy. Glycosylated hemoglobins improved beginning in the third month after treatment in all patients. The lowered blood glucose levels in each patient, relative to the pretreatment levels, were maintained for 4 to 7 months. As compared to the experimental group, the controls exhibited no consistent improvements in their diabetic conditions. There were no adverse effects after stem cell therapy, even without immune suppression.

Overall, only 1 patient reported confidence in the effects of stem cell therapy on erectile dysfunction without a PDE5 inhibitor, and 1 other patient expressed confidence in a combination treatment consisting of stem cell therapy and a PDE5 inhibitor (Table 4).

Table 4. Global Assessment Questions (GAQ) for efficacy of stem cell therapy.

Patient	Did stem cell improve your erection?	
	Without sildenafil citrate	With sildenafil citrate
1	No	Yes
2	Yes	Yes
3	Yes	Yes
4	No	No
5	No	Yes
6	Yes	Yes
7	No	No
Control 1	No	No
Control 2	No	No
Control 3	No	No

Discussion

Umbilical cord blood stem cells are the most ontogenetically immature form of adult stem cells, are relatively less-exposed to immunologic challenges, are available in abundance, and can be harvested without risk to the donor. Their use raises no ethical problems, and they evidence regenerative capabilities similar to other adult stem cells. Adult stem cell treatments have proven effective for cardiac ischemia (14), peripheral vascular impairments (33, 34), neurologic disorders (32, 34, 35), congenital disorders (13), and a host of other conditions. However, most of these therapies are in initial or trial stages. The participants in this study were all men with type II diabetes and had complications of erectile failure and impotence. Before we designed

this study, we personally communicated with endocrinologists on the topic of improved erections among diabetics undergoing stem cell therapy for type II diabetes mellitus. Although we were not cognizant of the exact mechanisms, we believed that the improvement of erectile function-related scores should be closely related to stem cell therapy and improved erectile functions in diabetics as compared with controls. When we designed this study, we attempted to remove any variables that would affect interpretation of the study data, because diabetes is a condition that affects multiple organs. Ultimately, among many candidates, we recruited type 2 diabetics who scored normally on a variety of ancillary tests.

When stem cells were injected into both cavernosa, we did not anticipate that all of the stem cells would be successfully implanted into the cavernosal tissues. Thus, in an effort to prolong the residence time and improve the graft, we clamped the penile roots for 30 minutes. We expected the systemic spread of some infused stem cells via the blood stream, in accordance with Min's hypothesis (36), and we also anticipated homing to the disordered organ (in this case the pancreas), and that the homed stem cells would exert favorable effects on blood glucose levels. Although this was not the target of our study, the data did reflect some blood glucose-lowering effects, regardless of the

use of a hypoglycemic agent or insulin for diabetes mellitus control (Table 5).

We injected stem cells into both cavernosa, which is not how injections of vasoactive agents such as PGE1 are conducted. The reason for this is that stem cells are qualitatively different from any type of vasodilation agent (37-39).

Stem cell therapy, administered as a single modality, improved many erectile dysfunction-related indices; this indicates that stem cells may play some important roles in the correction or amelioration of diabetic erectile dysfunction. All of the patients in this study reported increased libido—of which multiple factors could be the cause. In an attempt to focus in on this issue, we reviewed changes in the patients' hormone levels before and after the implants. However, owing to the lack of consistency in those levels, and the substantial interpersonal and intrapersonal differences in those levels (although all remained consistently within normal range), we are hesitant to draw any conclusions regarding improvements in libido reported by our study subjects. It may be attributed to something as simple as not having strictly scheduled examinations.

Enhanced penile erection with the addition of a PDE5 inhibitor is the consequence of a complex set

Table 5. Changes of fasting blood glucose and glycosylated hemoglobin.

Fasting blood glucose (mmol/L)														Types of treatment	Age
Patient	Before treatment	Post-Tx 1M	Post-Tx 2M	Post-Tx 3M	Post-Tx 4M	Post-Tx 5M	Post-Tx 6M	Post-Tx 7M	Post-Tx 8M	Post-Tx 9M	Post-Tx 10M	Post-Tx 11M			
1	12.21	9.99	7.77	6.11	5.66	5.27*	6.05	6.55	6.22	7.22	8.66	7.94	HA	78	
2	8.60	7.66	6.16	4.99*	5.99	5.66	5.88	6.49	6.33	6.88	7.88	7.16	HA	57	
3	9.27	7.22	5.88	5.22*	5.88	6.05	5.66	5.94	6.44	6.55	8.16	7.49	HA	66	
4	5.94	5.44*	6.27	5.83	4.99*	6.11	5.38*	5.99	6.11	6.33	6.11	6.22	Insulin	71	
5	5.66	5.22*	5.83	5.16*	5.44*	5.66	5.55	5.94	6.22	5.72	5.99	5.94	Insulin	68	
6	9.27	8.10	7.05	5.88	5.27*	6.27	5.66	6.33	6.72	5.99	6.27		HA	60	
7	6.66	6.72	6.27	6.60	6.22	6.83	7.05	6.60	6.99	6.72			Insulin	87	
Control 1	8.49	8.27	9.38	8.38	7.33	9.10	8.71	8.60	7.55	8.38	9.10	8.38	HA	67	
Control 2	6.55	6.66	6.49	6.72	6.60	6.94	6.66	6.38	6.77	6.66	7.49	6.88	HA	73	
Control 3	8.91	8.77	6.94	8.66	8.20	7.49	8.55	7.71	7.33	8.33	7.94	8.49	Insulin	61	

Glycosylated hemoglobin (% of total hemoglobin)														Types of treatment	Age
Patient	Before treatment	Post-Tx 1M	Post-Tx 2M	Post-Tx 3M	Post-Tx 4M	Post-Tx 5M	Post-Tx 6M	Post-Tx 7M	Post-Tx 8M	Post-Tx 9M	Post-Tx 10M	Post-Tx 11M			
1	8.7	8.4	8.6	8.6	7.9	7.4	7.3	7.3	7.1	7.1	7.4	7.5	HA	78	
2	8.2	8.3	8.1	8.2	7.8	7.5	7.4	7.5	7.3	7.3	7.4	7.4	HA	57	
3	8.3	8.1	8.2	7.9	7.5	7.6	7.4	7.4	7.3	7.3	7.3	7.4	HA	66	
4	6.8	6.8	6.7	6.7	6.6	6.4	6.5	6.4	6.4	6.5	6.4	6.5	Insulin	71	
5	6.9	6.9	6.8	6.6	6.6	6.3	6.2	6.4	6.3	6.4	6.5	6.5	Insulin	68	
6	8.4	8.5	8.4	8.1	7.9	7.6	7.4	7.4	7.5	7.5	7.4		HA	60	
7	7.4	7.3	7.5	7.4	7.4	7.3	7.5	7.5	7.6	7.5			Insulin	87	
Control 1	8.4	8.5	8.4	8.4	8.4	8.3	8.5	8.2	8.4	8.1	8.3	8.4	HA	67	
Control 2	7.3	7.2	7.3	7.3	7.3	7.2	7.3	7.3	7.3	7.3	7.5	7.4	HA	73	
Control 3	7.9	8.3	8.4	8.8	8.4	8.2	8.4	8.1	7.9	8.2	7.9	8.0	Insulin	61	

Abbreviations: HA, oral hypoglycemic agents; Insulin, injectable insulin.
*Blood glucose below 5.55 mmol/L and recommended lowering dose of medication.

of mechanisms, but it is difficult to neglect the indirect role of nitric-oxide-related events in this regard. Although we did not biopsy the patients' cavernosal tissues, the actions of a PDE5 inhibitor would definitely require a responding counterpart. Thus, we suppose that there could have been some positive changes in the cavernosal tissues as the result of stem cell therapy (40) among those who did not respond to a PDE5 inhibitor before therapy.

In this study, we used human umbilical cord blood stem cells without modification. However, mesenchymal stem cells modified with endothelial nitric oxide synthase (eNOS) have been shown to correct erectile dysfunction even more effectively when injected into the corpora cavernosa of old rats (41). After the report (42) demonstrating that stem cells transduced with eNOS could improve the erectile function of aged rats, embryonic stem cells transfected with brain-derived neurotrophic factor were previously reported to restore the erectile function of rats whose cavernous nerves had been experimentally damaged (43) and Bivalacqua and associates (40) reported the same findings as Deng (42). Song and associates (44) reported previously that the v-myc-transfected immortalized human bone marrow stem cells that were transplanted into rat corpus cavernosum proved capable of differentiating into endothelial and smooth muscle cells.

Diabetes mellitus is a representative disorder that has a familial history. It would be difficult to exclude the underlying genetic disorder (45) from a patient's each cell in any organ.

Because all participants in this study were type II diabetic, we used allogeneic human umbilical cord blood stem cells rather than autologous bone marrow or adipose tissue-derived stem cells. The stem cell donors were selected among those with no family histories of diabetes mellitus. The total volume of blood collected from 1 umbilical cord was approximately 100 mL, and the stem cell numbers that can be collected from 1 unit of umbilical cord blood are far smaller than can be drawn from adipose tissue or bone marrow. We infused approximately 15 million stem cells per patient, corresponding to about 15 mg of lean tissue. Thus, the possibility remains that the insufficient improvement for normal sexual life achieved using our protocol might be a function of the low total number of stem cells used for treatment.

The causes of erectile dysfunction can be neurogenic, vasculogenic, or endothelium-related, hormonal, psychogenic, or combinations of any of these. The participants in this study were selected from a group of people whose testosterone levels were within normal limits. All patients had organic (diabetic) erectile dysfunction and did not respond to medical therapy. During the 11-month follow-up, the reacquisition of morning erections began in the first month after treatment—all patients had regained morning erections by the second month. We did not anticipate that these patients would regain their morning erections as early as 1 month after treatment. Although we do not know the mechanism underlying the early reacquisition of morning erections, albeit to a level of rigidity insufficient for penetration or maintenance, we suppose that some unknown humoral (paracrine, cytokine) factors might be involved, in addition to the stem cells. Aside from erections, we also noted down-regulations of blood glucose levels. Koblas and associates (46) previously proposed some possible mechanisms for the generation of umbilical cord blood stem cells-derived β cells, and "the direct transplant of un-manipulated stem cells and their homing to the pancreas with their subsequent differentiation" seems consistent with our findings.

In this study, we used no immunosuppressive therapy, either before or after the infusion of allogeneic stem cells, into the diabetes mellitus patients. Immunologic considerations, however, are a very important part of any stem cell-based treatment. Mesenchymal stem cells evidence immunomodulatory, reparative, and anti-inflammatory properties, and these are generally attributed to preferential homing to damaged tissues and the secretion of humoral (or paracrine or cytokine) factors (47).

Additionally, these properties are believed to be potentially applicable to clinical disorders, including the treatment of therapy-resistant acute graft-versus-host disease, and prevention and treatment of rejection following the transplant of either hematopoietic stem cells or solid organs, as well as in autoimmune diseases. Mesenchymal stem cells are characterized by immune suppression and immune avoidance (48, 49) mediated by the inhibition of T-cell responses to a variety of stimuli (50-52), and mesenchymal stem cells suppress lymphocyte proliferation *in vitro* (47). The observed

immunomodulatory effect is likely attributable to humoral factors secreted by the mesenchymal stem cells (53), such as TGF-1 (transforming growth factor -1), HGF (hepatocyte growth factor), IL-6 (interleukin-6), PGE-1 (prostaglandin E2), indoleamine 2,3-dioxygenase-mediated tryptophan depletion, or NO (nitric oxide) (54). Mesenchymal stem cells down-regulate CD25 (the interleukin-2 receptor) and CD38 on phytohemagglutinin-activated lymphocytes (55). In addition to bone marrow-derived mesenchymal stem cells, mesenchymal stem cells derived from dental pulp (56), adipose tissue (57, 58), and amniotic tissue (59) were immunosuppressive, and the immunosuppressive properties of mesenchymal stem cells have previously been exploited for the successful treatment of severe human graft-versus-host disease (60). Mesenchymal stem cells evidence immune avoidance ability, and thus reduced immunogenicity. This reduced immunogenicity causes a lack of acute rejection response by the host in xenografts performed on immune-competent animals (61, 62). Immune escape from the host's immune system may modulate host dendritic and T-cell functions owing to the induction of divisional anergy in T cells (63) and the promotion of regulatory T-cell induction, as well as limited alloantigen expression (64).

Weiss and associates (65) previously demonstrated that umbilical cord mesenchymal stromal cells (UCMSC, Wharton's jelly-derived stem cells) were immunosuppressive, evidence-reduced immunogenicity, express HLA-G, do not express costimulatory molecules, and express cytokines that modulate immune function. All this appears to suggest that human umbilical cord blood mesenchymal stem cells may be tolerated in allogeneic transplants. Porcine umbilical cord blood mesenchymal stem cells transplanted into rat brains resulted in no obvious untoward behavioral effects or host immune responses (66, 67). Human umbilical cord blood-derived stem cells suppress lymphocyte proliferation, and the xenografting of human umbilical cord blood stem cells into rats resulted in no rejection, but a weak immunogenicity (68). Siepe and associates (69) demonstrated that neonatal thymus-derived mesenchymal stromal cells had immunomodulatory properties, also, and did not stimulate an allogeneic reaction. Further, they did not express MHC-I, and do not appear to be able to do so. Rasmusson (70) previously reported on the

immunomodulatory properties of mesenchymal stem cells occurring as the result of the inhibition of T-cell proliferation. Human umbilical cord blood stem cells cocultured with peripheral lymphocytes in the presence of phytohemagglutinin (PHA) or IL-2 inhibited lymphocyte proliferation. Human umbilical cord blood stem cells also suppressed the proliferation of regulatory T cells (71), which was assumed to be an immune-related event associated with tolerance. We supposed that immune tolerance might be an applicable-related effect on immune responses when using stem cells. Before we designed this study, others, studying human umbilical cord blood stem cells for clinical trials, informed their unpublished data that showing no problems when using ABO, HLA-AB, DR, and sex-matched cell therapies without immune suppression (unpublished data). These reports compelled us to design this study, wherein we treated the participants without using immune suppression. Indeed, we used no immune suppressants with any of the participants.

In stem cell therapy, we expect the implanted cells to differentiate into the target cells, and thus effect a recovery of the function of those cells. This study is, to the best of our knowledge, the first clinical report describing the use of unmodified stem cell therapy for the treatment of the diabetes mellitus complication—diabetic erectile dysfunction. The results of this study showed that erectile functions were improved by the therapy, but some questions remain regarding the relevant mechanisms and features of umbilical cord blood stem cell therapies. The total cell number we used was quite small relative to the unit body weight as compared with other studies, and we do not know the rates of implantation and differentiation of stem cells in the corpus cavernosa during the follow-up. The earliest indicator of improved penile erection—the recovery of morning erections—is another salient issue. We suppose that the early improvement of erectile dysfunction could be mediated by unknown humoral factors (growth factors/cytokines/paracrine factors) rather than by the differentiated graft stem cells, as the interval from implantation to differentiation was quite short.

However, in the study of Ning and associates (72), the injected Adipose-tissue-derived stem cells were reported to have localized to the sinusoid endothelium and formed tubelike structures that were mediated by fibroblast growth factor 2 (FGF2). Lin and associates (73) reported that the insulinlike

growth factor/insulinlike growth factor receptor (IGF/IGFR) pathway participates in neuronal differentiation, whereas the fibroblast growth factor 2 (FGF2) pathway is involved in endothelial differentiation; thus, the activation of endogenous stem cells (74) in cavernosal tissue by humoral factors or factors secreted by the stem cells themselves might be a mechanism underlying the improvements of diabetic erectile dysfunction observed herein.

Additionally, we surmise that the late onset of symptom improvement, as compared to routine hormonal action onset, rules out hormonal effects as a causal factor. Many agree that stem cells secrete multiple humoral factors, and that some of these are vasculogenic (75). We suppose that the possibility of transdifferentiation of grafted stem cells into new cavernosal cells cannot be denied in at least 1 patient who has maintained satisfactory erections with PDE5 inhibitor, until at least 11 months after treatment. Erectile dysfunction is predominantly a disease of vascular origin, featuring dramatic changes in the endothelium. The role of stem cells, then, may be related to some changes in the corporeal endothelium. In this study, a trend was noted specifically that, with passing time, the effects of stem cells gradually increased, followed by a later decline. These gradually changing patterns of effects should be interpreted later with other factors, such as cell numbers and other possibly relevant issues.

In conclusion, herein we report simultaneous improvements in the libido, erectile dysfunction, and blood glucose levels in patients with diabetic erectile dysfunction after intracavernous transplant of human umbilical cord blood stem cells, without immune suppression. Despite the observed positive effects on diabetic erectile dysfunction, we cannot currently elucidate the exact mechanisms underlying these effects. We surmise that these effects may be attributable to as-yet-unidentified humoral factors generated by the stem cells themselves, the mechanisms relevant to our findings warrant further inquiries into this topic, although we could not rule out the transdifferentiation in mechanism.

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