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Preoperative exercise facilitates abundant bone marrow collection in patients with type 2 diabetes for mononuclear cell therapy

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Abstract

Background aims. Traditional bone marrow (BM) collection is inadequate for separation of abundant mononuclear cells (MNCs). We aimed to investigate the effects of preoperative exercise on BM collection in patients with type 2 diabetes mellitus (T2DM). *Methods.* Sixty patients with T2DM were randomly assigned to either a control group or an exercise group ($n = 30$ each). The patients in the exercise group exercised before the collection. All patients underwent routine surgical care. The collected BM volume, operation duration, collecting speed, puncture times and pain scores were recorded. BM samples were tested before and after MNCs separation for CD34+ flow cytometry and whole blood cell count. *Results.* The collected BM volumes were significantly larger and collection speed was faster in the exercise group (379.77 ± 4.93 mL and 1.40 ± 0.14 mL/s) than those in the control group (356.67 ± 15.36 mL and 0.89 ± 0.16 mL/s, $P = 0.00$ for both). Puncture times were significantly less and pain scores were lower in the exercise group (2.07 ± 0.25 and 2.67 ± 1.56) than those in the control group (2.50 ± 0.63 and 3.43 ± 1.76, $P = 0.00$ and 0.02, respectively). CD34+ cells and whole blood cell count variables were comparable in the 2 groups. *Conclusions.* Preoperative exercise facilitates BM collection by increasing collected volume, improving collecting speed, relieving patients' pains and ensuring MNC quality.

Key Words: *bone marrow, exercise, mononuclear cells, type 2 diabetes*

Introduction

Stem cells have recently become a topic of interest because of their multipotency for rejuvenation and regeneration. Stem cell therapy has been applied in the treatment of diabetes mellitus in both animal models and clinical trials [1–3]. The cellular regimens include homogenous stromal cells and heterogenous bone marrow (BM) mononuclear cells (MNCs) [4]. Abundant BM MNCs are used in clinical practice to achieve maximal therapeutic effects. MNCs are separated from the autologous BM of the patients. The traditional BM collection procedure is unfavorable because it yields a small quantity of BM. Preliminary studies have shown that abundant BM collection was arduous in patients with type 2 diabetes mellitus (T2DM), which might be attributable to the patient's high blood viscosity and BM's hypercoagulable characteristic [5]. Operational modification is needed to facilitate abundant production. Studies have shown that physical exercise can increase bone blood flow, which might facilitate BM collection [6,7]. The present study aimed to investigate the effects of a single exercise session on the collection of abundant BM and the quality of MNCs from patients with T2DM.

Methods

Subjects and eligibility

Subjects who were diagnosed with T2DM according to 2008 American Diabetes Association diagnosis criteria were recruited [8]. Inclusion criteria were aged 40–65 years, body mass index <35 kg/m^2, age of onset ≥ 40 years, diabetes history ≥ 2 years and ≤ 15 years, glycosylated hemoglobin of 7.5–12%, basic C-peptide of 0.3–2.0 ng/mL, and daily total insulin dose of <100 units. Patients with tumors, pancreatitis, cirrhosis, hemorrhagic diseases, abdominal aortic

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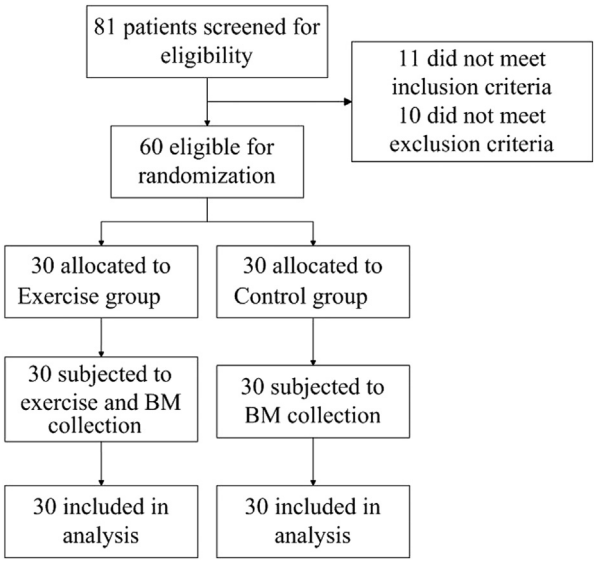

Figure 1. Screening, randomization, and completion of the study. Eleven patients did not meet inclusion criteria. Ten patients met inclusion criteria but did not meet exclusion criteria.

aneurysm, chronic infection, myocardial ischemia, untreated proliferative diabetic retinopathy, or severe physical dysfunction were excluded. This study is registered at ClinicalTrials.gov (NCT00767260).

Flow design

Included patients were randomly assigned to either a control or an exercise group. The control group underwent BM collection. Under local anesthesia with 2% lidocaine, BM was aspired from both posterior superior iliac spine to obtain a minimum of 300 mL and a maximum (target) of 400 mL [4]. Patients were generally punctured twice. Some were punctured 3 times when target BM volumes were not achieved with 2 punctures. At maximum, 4 punctures were permitted. Puncture times for each individual were recorded. To prevent BM coagulation, we pretreated the 20-mL syringes with 2 mL of heparin sodium (25 U/mL). Procedure duration was recorded using a second counter. The BM collecting speed was calculated by dividing the total volume (milliliters) by the total time (seconds).

Table I. The demographic and baseline data of the patients.

Group	Exercise	Control	*P* value
Age (y)	57.9 ± 5.2	55.5 ± 5.2	0.08
Men	17/30	17/30	0.60
Weight (kg)	67.0 ± 8.8	68.8 ± 10.4	0.49
Body mass index (kg/m^2)	24.3 ± 1.97	24.9 ± 2.18	0.22
Waist circumstance (cm)	86.5 ± 10.9	84.9 ± 12.3	0.61
Diabetic duration (y)	9.6 ± 3.2	11.0 ± 3.0	0.07
Systolic pressure (mm Hg)	128.3 ± 8.3	122.6 ± 10.1	0.45
Diastolic pressure (mm Hg)	75.7 ± 6.2	69.8 ± 5.9	0.55

The exercise group was subjected to exercise before the procedure. The patients jogged on a treadmill (SH5176D) with submaximal exercise heart rate maintained for 10 min [5] (85% of maximum heart rate; maximum heart rate for males = 205 − age/2 in years; maximum heart rate for females = 220 − age in years). They were allowed to rest for 15 min, and then BM collection was initiated. All patients' operations were performed by 1 surgeon who was blind to the patient randomization. This trial was conducted from January 2010 to March 2012.

Pain score recording during the operation

A visual analog scale was used to evaluate the pain during the operation. The pain intensity was interpreted by a number from 0 to 10 (0 = *no pain*; 10 = *severest pain*). The patients indicated pain level by selecting a number.

BM mononuclear cell production

BM was mixed with 20,000 U heparin and preserved in the primary bag of a Quadruple Collection Bag (Terumo Medical Products Co Ltd). BM samples were tested for CD34+ flow cytometry (FC500, Beckman Coulter) and whole blood cell count (peroxidase method, ADVIA2120, Siemens). The primary bag was placed upside down and centrifuged (Beckman, J-26) at 2000 *g* for 15 min. The bottom layer red cells were gravitated into the second bag and discarded; the median layer buffy coat was collected in the third bag, and the upper layer plasma and fat were discarded. The buffy coat was washed and resuspended in isotonic normal saline in the third bag, which was ∼500 mL in volume. The bag was centrifuged again at 2000 *g* for 5 min to remove residual plasma and fat from the buffy coat. It was then sampled for flow cytometry and complete blood cell count again.

Statistical analysis

A computer-generated block randomization was used to assign each subject to 1 of the 2 groups. Statistical analysis was performed using SPSS for Windows (version 17.0; SPSS). Data were presented as means ± SD. Intergroup comparisons were performed using independent sample *t*-test. Tests resulting in a *P* value <0.05 were considered to be statistically significant. Power and sample size considerations assume a 10% increase of collected BM volume after exercise from average 350 mL or 10% increase of collecting speed from average 0.9 mL/s of patients with T2DM. Student's *t*-test of independence considered 2