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ORIGINAL ARTICLE

Randomized Controlled Trial

Autologous bone marrow derived stem cell therapy in patients with type 2 diabetes mellitus - defining adequate administration methods

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Abstract

AIM

To carry out randomized trial for evaluating effects of autologous bone marrow derived stem cell therapy (ABMSCT) through different routes.

METHODS

Bone marrow aspirate was taken from the iliac crest of patients. Bone marrow mononuclear cells were separated

and purified using centrifugation. These cells were then infused in a total of 21 patients comprising three groups of 7 patients each. Cells were infused into the superior pancreaticoduodenal artery (Group Ⅰ), splenic artery (Group Ⅱ) and through the peripheral intravenous route (Group Ⅲ). Another group of 7 patients acted as controls and a sham procedure was carried out on them (Group Ⅳ). The cells were labelled with the PET tracer F18-FDG to see their homing and *in vivo* distribution. Data for clinical outcome was expressed as mean ± SE. All other data was expressed as mean ± SD. Baseline and post treatment data was compared at the end of six months, using paired *t*-test. Cases and controls data were analyzed using independent *t*-test. A probability (*P*) value of < 0.05 was regarded as statistically significant. Measures of clinical outcome were taken as the change or improvement in the following parameters: (1) C-peptide assay; (2) HOMA-IR and HOMA-B; (3) reduction in Insulin dose; subjects who showed reduction of insulin requirement of more than 50% from baseline requirement were regarded as responders; and (4) reduction in HbA1c.

RESULTS

All the patients, after being advised for healthy lifestyle changes, were evaluated at periodical intervals and at the end of 6 mo. The changes in body weight, body mass index, waist circumference and percentage of body fat in all groups were not significantly different at the end of this period. The results of intra-group comparison before and after ABMSCT at the end of six months duration was as follows: (1) the area under C-peptide response curve was increased at the end of 6 mo however the difference remained statistically non-significant (*P* values for fasting C-peptide were 0.973, 0.103, 0.263 and 0.287 respectively and the *P* values for stimulated C-peptide were 0.989, 0.395, 0.325 and 0.408 respectively for groups Ⅰ to Ⅳ); (2) the Insulin sensitivity indices of HOMA IR and HOMA B also did not show any significant differences (*P* values for HOMA IR were 0.368, 0.223, 0.918 and 0.895 respectively and P values for HOMA B were 0.183, 0.664, 0.206 and 0.618 respectively for groups Ⅰ to Ⅳ); (3) Group Ⅰ showed a significant reduction in Insulin dose requirement ($P < 0.01$). Group Ⅱ patients also achieved a significant reduction in Insulin dosages ($P = 0.01$). The Group Ⅰ and Group Ⅱ patients together constituted the targeted group wherein the feeding arteries to pancreas were used for infusing stem cells. Group Ⅲ, which was the intravenous group, showed a non-significant reduction in Insulin dose requirement ($P = 0.137$). Group Ⅳ patients which comprised the control arm also showed a significant reduction in Insulin dosages at the end of six months ($P < 0.05$); and (4) there was a non-significant change in the Hb A1c levels at the end of 6 mo across all groups ($P = 0.355$, $P = 0.351$, $P = 0.999$ and $P = 0.408$ respectively for groups Ⅰ to Ⅳ).

CONCLUSION

Targeted route showed a significant reduction in Insulin requirement at the end of six months of study period whereas the intravenous group failed to show reduction.

Key words: Autologous bone marrow derived stem cell therapy; Type 2 diabetes mellitus

Core tip: Homing of stem cells to pancreas is an important pre-requisite for achieving therapeutic efficacy in type 2 diabetes mellitus patients. Homing of stem cells was demonstrated when targeted infusion was carried out. No discernible homing was there when intravenous infusion route was employed. It was only in the targeted group that a 50% reduction in Insulin dosage was observed, establishing a relationship between homing and therapeutic efficacy.

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INTRODUCTION

Type 2 diabetes mellitus accounts for 90%-95% of all cases of diabetes mellitus and is caused due to insulin deficiency superimposed on insulin resistance[1,2]. The former is the major defect resulting in a blunted response of pancreatic beta cells to secrete Insulin in response to increased blood glucose levels. The resultant hyperglycaemic state is responsible for the microvascular and macrovascular complications seen in diabetic patients. Many patients require exogenous Insulin to control their blood glucose levels but even then it is difficult to achieve euglycemic state with both hyperglycemia and hypoglycaemia observed for variable times in a single day[3]. To circumvent this problem beta cell replacement therapy was sought as a viable alternative. It was hoped that this would provide a more physiological response of Insulin secretion to blood glucose levels. Islet cell transplantation was first tried on patients with type 1 diabetes mellitus. Despite the progress made in the ensuing time, this method suffered from inherent shortcomings like limited supply of pancreatic islets as 2-3 donors are needed for a single transplant, the need to give lifelong immuno-suppression and the progressive loss of implanted cells which necessitated the reintroduction of Insulin in these patients[4-7]. Also the utility of islet transplants in type 2 diabetics is not own. Because of the above reasons the adult stem cells taken from the patients' own bone marrow became the automatic choice for clinical trials and much of the current research has been based on utilizing the autologous cells for treating diabetics[8]. Various factors secreted by these cells

have been postulated to affect the angiogenesis and create a tissue micro environment that is conducive for beta cell regeneration and survival[9,10]. Though embryonic stem cells are more plastic as compared to adult stem cells, their use is limited because of the ethical concerns and safety issues.

The pertinent questions in regenerative medicine at present are to find out optimal routes for delivery of stem cells, optimal cell types and their numbers, and lastly to know how homing characteristics affect therapeutic efficacy. Aim of the present study was to carry out a clinical trial for evaluating the effects of autologous bone marrow derived stem cell therapy (ABMSCT) when they were infused through different routes and comparing them with a control arm. Analysis was carried out to find any association between the homing characteristics and therapeutic efficacy of infused stem cells.

Stem cells can be potentially transplanted utilising one of these three routes: (1) through targeted approach by injecting cells into the artery supplying the organ; (2) by putting cells into the peripheral vein; and (3) through direct injection into the organ. In the context of diabetic patients the first two approaches are commonly used for infusing stem cells[11]. There is risk of pancreatitis with direct injection into the pancreas and hence this approach has been used sparingly in clinical trials. In our study the labelled stem cells were tracked *in vivo* to see their biodistribution and to establish relationship between homing characteristics and therapeutic efficacy. *In vivo* tracking assumes importance because it is believed that the therapeutic efficacy of the infused cells will profoundly depend on delivery of these cells to the target organ, a process called homing[12] Subsequent maintenance of viability and functionality of these cells is also important for achieving the clinical goals.

It is imperative to know the vascular supply of pancreas for the targeted delivery of stem cells. The endocrine pancreas (body and tail of pancreas) in most parts is supplied by the branches of splenic artery[13]. The exocrine part of pancreas (pancreatic head) is supplied by the pancreatico-duodenal arteries[14].

Among the current imaging modalities PET is considered the preferred modality because of the advantages it offers; like convenience of labelling stem cells, minimal toxicity, accessibility, better sensitivity and resolution, signal being directly related to cell viability and its use in any model. Direct cell labelling with a radiotracer has been used for many years to track cells *in vivo*. Typically the cells in a solution are incubated with a radiotracer for a defined period during which the radiotracer gains access inside the cells. After the incubation the supernatant containing the unbound activity is removed. The labelled cells are injected into the host and tracked over time. Radio labelling with PET tracer F18-FDG is easily accomplished and does not require any cellular modification except for the need to fast the cells.

The cell survival and function is generally inferred by the measures of clinical outcome[15]. In case of diabetic patients these parameters are easily quantifiable and give objective evidence of therapeutic efficacy. Theoretically a targeted approach utilising the feeding arteries of the organ would ensure optimal delivery of these cells whereas the intravenous route infusion, though easily accomplished, is likely to fail in this endeavour[16-19].

MATERIALS AND METHODS

The subjects meeting the below mentioned inclusion and exclusion criteria were enrolled. Patients were randomized to different arms using computer generated randomisation table. All procedures were carried out according to the Institute's ethical guidelines. Statistical review of the study was performed by a biomedical statistician before the submission of draft. The statistical methods of this study were reviewed by Dr. Raman Chauhan from the department of Preventive and Social Medicine, IGMC, Shimla, Himachal Pradesh, India.

Inclusion Criteria: (1) Patients with T2DM between 30 and 70 years of age; (2) failure to triple OHA (Oral hypoglycaemic agents) and on stable doses of Insulin for at least 3 mo; (3) On Vildagliptin, Pioglitazone and Metformin for at least 3 mo along with Insulin to maintain euglycemia; (4) HbA1c of 6.5%-7.5%; (5) Insulin requirement $\geqslant$ 0.4 IU/kg per day; and (6) Glutamic acid decarboxylase (GAD 65) antibody negative status.

Exclusion Criteria: (1) Patients with T1DM or secondary diabetes; (2) Patients with serum creatinine > 1.5 mg/dL; (3) Abnormal liver function tests (defined as value of transaminases > 3 times the upper value of normal or serum bilirubin higher than normal for the reference value of the laboratory); (4) History of pancreatitis; (5) Seropositivity for HIV, HBsAg and HCV; (6) History of myocardial infarction or unstable angina in the previous 3 mo; (7) History of malignancy; (8) Patients with active infections; and (9) Female patients who are pregnant or lactating.

Design of the study: A total of 130 patients were screened from June 2010 to May 2012, out of which 42 were eligible for the study. They were randomly divided into four groups. A total of 28 cases were included in the final analysis. The cases were assigned to these four groups comprising 7 patients in each group. In Arm Ⅰ, 7 patients received stem cell infusion in the superior pancreatico-duodenal artery (Group Ⅰ), In Arm Ⅱ, 7 patients received stem cell infusion in the splenic artery (Group Ⅱ). In Arm Ⅲ, 7 patients were given stem cells through the peripheral intravenous route (Group Ⅲ). Another 7 patients acted as controls (Group Ⅳ).

Bone marrow aspirate was taken from the iliac crest of patients under aseptic precautions. Bone marrow mononuclear cells were separated and purified using centrifugation. The 2-3 mL of stem cell

concentrate obtained was anti-coagulated with heparin and then used for labelling with PET tracer F18-FDG.

The targeted injections were carried out under fluoroscopic guidance by the interventional radiologist employing digital subtraction angiography technique. In Group Ⅰ, a 5F catheter was selectively navigated through trans-femoral route into superior pancreatico-duodenal artery beyond the origin of cystic artery and super-selective injection of stem cells was carried out. Post stem cell transfer a diagnostic run was taken to look for the patency of superior pancreatico-duodenal artery. Similarly Group Ⅱ patients received super-selective injection in the proximal splenic artery at the origin of dorsal pancreatic artery. An F18-FDG PET scan was done at 30 min and 90 min after the infusion of F18-FDG labelled stem cells. One patient was imaged up to 16 h after the infusion; however there was no discernible tracer activity at the targeted region at that time. The Standardized uptake values (SUVs) in pancreas and other organs were determined for semi-quantitative analysis. Group - Ⅲ patients were imaged after peripheral intravenous injection of labelled stem cells was given in the ante-cubital vein.

Keeping the primary objectives in consideration at a power of 80% with confidence interval of 95%, the sample size for the study was calculated as 6 cases in single arm. The measures of clinical outcome were expressed as Mean ± SE. Baseline and post treatment data was compared at the end of six months, using paired *t* test. Cases and controls data were analyzed using independent *t* test. A probability (*P*) value of < 0.05 was regarded as statistically significant. Measures of clinical outcome were taken as the change or improvement in the following parameters: (1) C-peptide assay; (2) HOMA-IR and HOMA-B; (3) Reduction in Insulin dose - patients showing a reduction of more than 50% was regarded as responders; and (4) Reduction in HbA1c levels.

In all patients blood glucose was monitored intensively for 6 wk. Patients were asked to monitor at least two - 5 point profile blood sugar (defined as fasting, 2 h post breakfast, 2 h post lunch, 2 h post dinner and at 3 am) using glucometer every 2 wk. Dose escalation was done in first 2 visits. In the last 2 wk of run in period the patients were put on stable doses of Insulin which were taken as the baseline Insulin requirement. These patients received a fixed regime of three OHAs as follows: Vildagliptin 100 mg/d, Metformin 2 g/d and Pioglitazone 15 mg/d. The doses of OHAs were kept constant at this level and only Insulin dose was altered during the follow up period. Patients were evaluated at periodical intervals following stem cell therapy. Patients were urged to keep the five point profile of blood glucose levels and down titration of Insulin dosages was done on the basis of averaged out blood glucose values. HbA1c was measured at the end of 3 mo and at 6 mo. Maintenance or reduction of HbA1c values despite tapering of the insulin dosages was taken as significant. Insulin sensitivity indices of HOMA-IR and HOMA-B were calculated by the standard mathematical equations. After omitting Vildagliptin for 24 h and Insulin (NPH for 24 h and regular Insulin for 12 h) a baseline levels of: (1) fasting and Glucagon stimulated C-peptide and and (2) serum Insulin were done. HOMA-IR and HOMA-β was calculated using the following equations: - HOMA-IR C-peptide = fasting C-peptide × Fasting Plasma Glucose/22.5 - HOMA - β C-peptide = 20 × Fasting C-peptide/Fasting glucose - 3.5.

C-peptide is considered to be a good marker of Insulin secretion hence a marker of β - cell function because of its equi-molar secretion with Insulin, negligible hepatic extraction, and constant peripheral clearance at different plasma concentrations and in presence of alterations in plasma glucose concentrations. While fasting C-peptide alone is easy to obtain and correlates with stimulated C-peptide, it may be insufficient to detect subtle effects of therapy. After clinical diagnosis, the appropriate test may include the stimulated C-peptide response to intravenous glucagon. The stimulated C- peptide values were obtained at baseline and at the end of the study period. The area under C-peptide response curve was evaluated.

Comparison of baseline characteristics was carried out to check for fair randomisation and ensure that no bias creeps in at the time of reporting results (Table 1). Analysis of variance (ANOVA) was used to find p value. Procedural details in the three groups are shown in Table 2.

RESULTS

C-peptide assay and HOMA IR, HOMA B

The Insulin sensitivity indices among groups 1 to 4 are shown in Tables 3-6 respectively. The area under C-peptide response curve was increased at the end of 6 mo however the difference remained statistically non-significant across all groups (*P* values for fasting C-peptide were 0.973, 0.103, 0.263 and 0.161 respectively and the *P* values for stimulated C-peptide were 0.989, 0.395, 0.325 and 0.346 respectively).

The Insulin sensitivity indices of HOMA IR and HOMA B did not show any significant differences (*P* values for HOMA IR were 0.368, 0.223, 0.918 and 0.306 respectively). The *P* values for HOMA B were 0.183, 0.664, 0.206 and 0.242 respectively.

Insulin dose requirements

There was progressive and consistent decrease in fasting and post prandial plasma glucose leading to decrease in Insulin dosages after the targeted injection of stem cells into the feeding arteries of pancreas (superior pancreatico-duodenal and splenic arteries in Group 1 and Group 2 patients respectively). This reduction in Insulin dose remained statistically significant at 3 and 6 mo of follow up (*P* values of 0.011 and 0.003 respectively for Group 1 patients and

Table 1 Comparison of baseline characteristics

Parameter	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ	*P* value
Age	57.83 ± 5.84	49.85 ± 9.63	53.28 ± 7.29	55.7 ± 7.7	0.351
Sex	M-4, F-3	M-6, F-1	M-6, F-1	M-5, F-2	0.592
Duration of diabetes (in years)	19.5 ± 5.54	14.28 ± 6.77	14.28 ± 5.64	19.6 ± 6.4	0.213
Insulin requirement	43.66 ± 5.35	39.71 ± 3.81	45 ± 6.57	43.86 ± 4.50	0.893
Duration of insulin (in years)	3.75 ± 1.72	4.47 ± 4.1	6.66 ± 4.54	4.4 ± 2.7	0.742
Weight	79 ± 18.89	72.14 ± 8.41	75.78 ± 9.95	77.7 ± 13	0.75
BMI	28.83 ± 4.26	26.57 ± 2.63	26.85 ± 3.97	29.6 ± 1.9	0.349
Body fat	31.5 ± 8.57	29.57 ± 5.38	33.14 ± 7.26	35.1 ± 2.5	0.452
Waist circumference	98.5 ± 9.46	92.57 ± 6.10	98.42 ± 9.51	101.6 ± 4.1	0.152
FPG	106.66 ± 14.36	103.42 ± 16.89	89.71 ± 9.21	103.5 ± 6.0	0.112
HbA1c	6.8 ± 0.18	6.4 ± 0.16	6.7 ± 0.15	6.6 ± 0.24	0.75
FPI (uU/mL)	123 ± 92	57 ± 48	22 ± 5	14.1 ± 4.3	0.17
HOMA-IR%	11.72 ± 5.39	3.31 ± 1.11	5.92 ± 0.67	4.6 ± 1.5	0.446
HOMA-B%	146.26 ± 56.44	40.75 ± 12.02	129.57 ± 65.20	77.8 ± 22.4	0.451
Fasting C-pep (ng/mL)	2.97 ± 1.51	1.28 ± 0.14	1.41 ± 0.25	1.1 ± 0.2	0.221
Stimulated C-peptide (ng/mL)	4.26 ± 1.59	2.63 ± 0.23	2.37 ± 0.36	2.1 ± 0.3	0.192
Number of BMMNCs infused	$4.9 \pm 3.10 \times 10^8$	$12.04 \pm 4.84 \times 10^8$	$6.88 \pm 2.30 \times 10^8$	NA	NA

BMI: Body mass index; FPG: Fasting plasma glucose; HbA1c: Glycosylated hemoglobin; HOMA-IR: Homeostasis model assessment insulin resistance index; BMMNCs: Bone marrow mononucleated cells; HOMA-B: Homeostasis model assessment beta cell function.

Table 2 Procedural details in Group Ⅰ, Ⅱ and Ⅲ patients were as follows

Parameter	Group Ⅰ	Group Ⅱ	Group Ⅲ
Volume of marrow aspirated (mL)	149 ± 60	222 ± 22.97	199 ± 49.91
Aspiration - Unilateral/Bilateral	U/L-3, B/L-4	B/L-7	U/L-4, B/L-3
Bone marrow TNC count	$11.72 \pm 6.36 \times 10^8$	$20.28 \pm 8.55 \times 10^8$	$11.72 \pm 3.03 \times 10^8$
Bone marrow MNC count	$4.9 \pm 3.10 \times 10^8$	$12.04 \pm 4.84 \times 10^8$	$6.88 \pm 2.30 \times 10^8$
Bone marrow CD 34+ cell count	0.71% ± 0.45%	0.87% ± 0.35%	0.58% ± 0.30%
Procedure time (min)	57 ± 18	50 ± 8	38 ± 18
Artery cannulated	SPD-5, SMA-2	Splenic-7	IV route
Catheter change required	1 case- multiple times	Nil	NA
SUV max at Pancreas at 30 min (mean ± SD)	21 ± 38	3.4 ± 3.34	1.56 ± 0.34

NA: Not applicable.

Table 3 Insulin sensitivity indices in Group Ⅰ

Time	0 mo (mean ± SE)	6 mo (mean ± SE)	12 mo	*P* value
FPI (uU/mL)	123 ± 91.87	75.83 ± 58.69		0.707
HOMA-IR%	11.72 ± 5.39	6.96 ± 1.32		0.368
HOMA-B%	146.26 ± 56.44	63.33 ± 13.99		0.183
Fasting C-peptide (ng/mL)	2.97 ± 1.51	2.94 ± 0.80		0.973
Stimulated C-peptide (ng/mL)	4.26 ± 1.59	4.23 ± 0.84		0.989

HOMA-IR: Homeostasis model assessment insulin resistance index; HOMA-B: Homeostasis model assessment beta cell function.

Table 4 Insulin sensitivity indices in Group Ⅱ

Time	0 mo (mean ± SE)	6 mo (mean ± SE)	*P* value
FPI (uU/mL)	57.33 ± 48.19	71.04 ± 38.88	0.62
HOMA-IR %	3.31 ± 1.11	4.76 ± 0.45	0.223
HOMA-B %	40.75 ± 12.02	46.86 ± 2.62	0.664
Fasting C-peptide (ng/mL)	1.282 ± 0.14	1.896 ± 0.29	0.103
Stimulated C-peptide (ng/mL)	2.63 ± 0.23	3.008 ± 0.26	0.395

HOMA-IR: Homeostasis model assessment insulin resistance index; HOMA-B: Homeostasis model assessment beta cell function.

P values of 0.003 and 0.01 respectively for Group2 patients). The intravenous arm patients belonging to

Table 5 Insulin sensitivity indices in Group Ⅲ

Time	0 mo (mean ± SE)	6 mo (mean ± SE)	*P* value
FPI (uU/mL)	21.94 ± 5.12	16.45 ± 4.39	0.434
HOMA-IR%	5.92 ± 0.67	6.13 ± 1.5	0.918
HOMA-B%	129.57 ± 65.20	59.58 ± 22.96	0.206
Fasting C-peptide (ng/mL)	1.415 ± 0.25	2.49 ± 0.74	0.263
Stimulated C-peptide (ng/mL)	2.37 ± 0.36	3.48 ± 0.87	0.325

HOMA-IR: Homeostasis model assessment insulin resistance index; HOMA-B: Homeostasis model assessment beta cell function.

Table 6 Insulin sensitivity indices in Group Ⅳ

Time	0 mo (mean ± SE)	6 mo (mean ± SE)	*P* value
FPI (uU/mL)	14.1 ± 4.3	18.2 ± 4.3	0.525
HOMA-IR%	4.6 ± 1.5	4.9 ± 1.2	0.895
HOMA-B%	77.8 ± 22.4	91.7 ± 14.6	0.618
Fasting C-peptide (ng/mL)	1.1 ± 0.2	1.5 ± 0.4	0.287
Stimulated C-peptide (ng/mL)	2.1 ± 0.3	2.8 ± 0.8	0.408

HOMA-IR: Homeostasis model assessment insulin resistance index; HOMA-B: Homeostasis model assessment beta cell function.

Table 7 Insulin dose (U/d) before and after autologous bone marrow derived stem cell therapy in Group I

Patient	Baseline	3 mo	6 mo	12 mo	18 mo	24 mo
1	32	22	16	Nil	Nil	Nil
2	66	55	53	53	50	_
3	30	20	16	14	-	-
4	46	26	20	20	-	-
5	40	18	12	-	-	-
6	48	8	10	-	-	-
7	Lost to FU					
Mean ± SE	44 ± 5	25 ± 7	21 ± 7			
P value		0.011	0.003			

Table 8 Insulin dose (U/d) before and after autologous bone marrow derived stem cell therapy in Group II

Patient	Baseline	3 mo	6 mo	12 mo
1	40	27	16	12
2	46	44	38	34
3	25	18	8	5
4	30	20	20	18
5	40	34	30	-
6	55	40	-	-
7	45	27	-	-
Mean ± SE	40 ± 4	30 ± 4	22 ± 5	
P value		0.003	0.01	

Group 3 did not show significant reduction at 3 mo and 6 mo of follow up (*P* value of 0.087 and 0.137 respectively). The Group 4 patients showed a non significant decrease at 3 mo (*P* value of 0.999) but a significant decrease at 6 mo of follow up (*P* value 0.021). This was ascribed to the placebo effect. When

Table 9 Insulin dose (U/d) before and after autologous bone marrow derived stem cell therapy in Group Ⅲ

Patient	Baseline	3 mo	6 mo	12 mo	18 mo
1	53	48	36	27	18
2	47	34	15	12	-
3	51	25	Nil	Nil	-
4	75	78	86	70	-
5	26	26	18	24	-
6	25	20	18	-	-
7	38	32	30	-	-
Mean ± SE	45 ± 7	38 ± 8	34 ± 11		
P value		0.087	0.137		

Table 10 Insulin dose (U/d) before and after autologous bone marrow derived stem cell therapy in Group Ⅳ

Patient	Baseline	3 mo	6 mo
1	30	30	32
2	37	25	23
3	46	40	34
4	68	55	57
5	41	26	20
6	45	41	39
7	40	49	24
Mean ± SE	44 ± 5	38 ± 4	33 ± 5
P value		0.999	0.021

cases and controls were compared there were no statistically significant differences in the Insulin dosage requirements. However the patients achieving a target of a 50% reduction in Insulin dosages and hence falling in the category of responders were seen only in the targeted group. Among different groups the insulin dose requirement before and after ABMSCT is depicted in Tables 7-10.

Inter and intra group comparison

When inter-group and intra-group comparisons were made for the parameters of clinical outcome across all groups, there were no statistically significant differences. The results are depicted in Tables 11 and 12.

HbA1c levels

The HbA1c levels were reduced in Group 1 patients despite tapering of the Insulin dosages (Mean value of 6.51 at six months compared to 6.65 at baseline). This group also showed best homing and retention of stem cells. Group 2 showed a slight increase in mean value of HbA1c, (6.7 at six months compared to 6.41 at baseline). However in this group also there was a significant decrease of Insulin requirement at the end of 6 mo. The Group 3 patients showed a non-significant decrease in the HbA1c levels (Mean value of 6.61 at six months compared to 6.7 at baseline). The group 4 patients maintained their HbA1c levels at 6.6 despite tapering of the Insulin dosages. The change in HbA1c levels among different groups is depicted in

Table 11 Comparison of different case arms (Group Ⅰ, Ⅱ and Ⅲ) with control arm (Group Ⅳ) at zero month

Parameter	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ	*P* value Group Ⅰ	*P* value Group Ⅱ	*P* value Group Ⅲ
Insulin requirement	44 ± 5	40 ± 4	45 ± 7	44 ± 5	0.979	0.541	0.927
HbA1c	6.8 ± 0.18	6.4 ± 0.16	6.7 ± 0.15	6.6 ± 0.24	0.983	0.453	0.9
HOMA IR%	11.72 ± 5.39	3.31 ± 1.11	5.92 ± 0.67	4.6 ± 1.5	0.544	0.327	0.333
HOMA-B%	146.2 ± 56.44	40.75 ± 12.02	129.57 ± 65.20	77.8 ± 22.4	0.593	0.212	0.559
Fasting C-peptide (ng/mL)	2.97 ± 1.51	1.282 ± 0.14	1.415 ± 0.25	1.1 ± 0.20	0.174	0.087	0.139
Stimulated C-peptide (ng/mL)	4.26 ± 1.59	2.63 ± 0.23	2.37 ± 0.36	2.1 ± 0.3	0.144	0.132	0.324

HbA1c: Glycosylated hemoglobin; HOMA-IR: Homeostasis model assessment insulin resistance index; HOMA-B: Homeostasis model assessment beta cell function.

Table 12 Comparison of different case arms (Group Ⅰ, Ⅱ and Ⅲ) with control arm (Group Ⅳ) at 6 mo

Parameter	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ	*P* value Group Ⅰ	*P* value Group Ⅱ	*P* value Group Ⅲ
Insulin requirement	21 ± 7	22 ± 5	34 ± 11	33 ± 5	0.174	0.183	0.69
HbA1c	6.5 ± 0.16	6.7 ± 0.12	6.7 ± 0.21	6.6 ± 0.22	0.816	0.704	0.779
HOMA IR%	6.96 ± 1.32	4.76 ± 0.45	6.13 ± 1.5	4.9 ± 1.2	0.404	0.358	0.322
HOMA B%	63.33 ± 13.99	46.86 ± 2.62	59.58 ± 22.96	91.7 ± 14.6	0.096	0.29	0.213
Fasting C-peptide (ng/mL)	2.94 ± 0.80	1.896 ± 0.29	2.49 ± 0.74	1.5 ± 0.4	0.091	0.34	0.187
Stimulated C-peptide (ng/mL)	4.23 ± 0.84	3.008 ± 0.26	3.48 ± 0.87	2.8 ± 0.	0.14	0.566	0.412

HbA1c: Glycosylated hemoglobin; HOMA-IR: Homeostasis model assessment insulin resistance index; HOMA-B: Homeostasis model assessment beta cell function.

Table 13 Change in HbA1c % before and after autologous bone marrow derived stem cell therapy in Group Ⅰ

Patient	Baseline	3 mo	6 mo	12 mo	18 mo	24 mo
1	7.2	5.7	7.1	6.4	6.8	7.3
2	6.7	6.2	6.7	6.9	6	-
3	6	-	6.4	5.7	-	-
4	7	6.8	6.6	-	-	-
5	6.1	6.3	5.9	-	-	-
6	6.9	6.5	6.4	-	-	-
7	Lost to FU					
Mean ± SE	6.8 ± 0.18	6.3 ± 0.18	6.5 ± 0.16			
P value		0.164	0.355			

Table 14 Change in HbA1c % before and after autologous bone marrow derived stem cell therapy in Group Ⅱ

Patient	Baseline	3 mo	6 mo	12 mo	18 mo
1	7	7.1	7	7.4	6.5
2	6.2	6.4	6.5	6.7	7.7
3	6.6	6.5	6.5	6.5	-
4	5.6	7.5	7	6.4	-
5	6.6	6.7	6.5	-	-
6	6.4	6.3	-	-	-
7	6.5	5.7	-	-	-
Mean ± SE	6.4 ± 0.16	6.6 ± 0.21	6.7 ± 0.12		
P value		0.573	0.351		

Table 15 Change in HbA1c % before and after autologous bone marrow derived stem cell therapy in Group Ⅲ

Patient	Baseline	3 mo	6 mo	12 mo	18 mo
1	6.3	6.2	5.9	7.1	5.9
2	6.8	5.8	6.8	6.3	-
3	6.9	6.9	6.9	7.4	-
4	6.8	6.7	7.2	7	-
5	6.9	7.2	7.5	7.5	-
6	6	6	6.2	-	-
7	7.2	6.8	6.4	-	-
Mean ± SE	6.7 ± 0.15	6.5 ± 0.19	6.7 ± 0.21		
P value		0.28	0.999		

Table 16 Change in HbA1c % before and after autologous bone marrow derived stem cell therapy in Group Ⅳ

Patient	Baseline	3 mo	6 mo
1	6.6	6.3	5.5
2	7	7.1	6
3	7	7	6.7
4	7.5	7.8	7
5	6	6.9	7.1
6	6.8	7.2	6.8
7	5.6	5.5	7
Mean ± SE	6.6 ± 0.6	6.8 ± 0.7	6.6 ± 0.2
P value		0.258	0.408

Tables 13-16.

DISCUSSION

There has been a growing interest among the medical scientific community for utilizing cellular therapies in the treatment of Type 2 diabetes and its complications. An exponential rise in recent publications and clinical trials bears testimony to this fact. However despite the rapid transition from animal studies to clinical trials, many questions still remain unanswered in the field of regenerative medicine. Important ones being to find

the optimal cell delivery routes and techniques, to find optimal number and type of cells needed to achieve desired treatment effectiveness and also to find out the relationship between homing characteristics and therapeutic efficacy.

This is the first single blinded randomised case controlled study which evaluates the role of autologous bone marrow derived stem cells in patients with type 2 diabetes mellitus utilising different routes of administration and comparing them with a control arm. The study revealed that there was an increase in area under C-peptide response curve among all groups but the values remained statistically non-significant. No significant improvement in Insulin sensitivity indices of HOMA IR and HOMA B was noted. A progressive and consistent decrease in fasting and post prandial plasma glucose leading to decrease in Insulin doses was noted in group Ⅰ and group Ⅱ patients - the patients receiving targeted infusion of stem cells into the feeding arteries of pancreas. This reduction in Insulin dosages remained statistically significant at 6 mo of follow-up. The patients receiving intravenous infusion and belonging to group Ⅲ did not show any statistically significant reduction in Insulin requirements at the end of study period. The controls belonging to Group Ⅳ did have reduction in Insulin dose of 10 units (25%) at 6 mo. However none of them reached the primary objective of 50% reduction in Insulin dose. Though the reduction was statistically significant with a *P* value of 0.021, intensive lifestyle modifications after the sham procedure may have contributed to this reduction. HbA1c levels showed reduction or were maintained at the baseline level and this was observed despite tapering of Insulin dosages across all groups. However since the tapering of Insulin dosages were different across all groups and were not uniform, correlation coefficient for this parameter could not be calculated.

Maximum localization of labeled stem cells was seen in Group Ⅰ followed by Group Ⅱ patients. These groups included the patients which achieved the objective of 50% reduction in Insulin dose requirement at the end of study period. In the Group Ⅲ patients no discernible homing was there which corresponded to no significant change or improvement in the measures of clinical outcome. Hence it can be extrapolated that homing is tightly linked to therapeutic efficacy.

More studies are needed on this topic to validate the cost-effectiveness, durability, long term safety, molecular mechanisms involved and efficacy in varied population of type 2 diabetes patients.

Targeted route consistently showed an increase in area under C-peptide response curve. However no significant improvement in Insulin sensitivity indices of HOMA IR and HOMA B was noted. When the patients of this group were followed up for a period of six months, a significant reduction in the insulin requirement was noted. The HbA1c levels either showed reduction or were maintained at the baseline level despite tapering of insulin dosages.

On the other hand the intravenous group did not show any significant change or improvement in the parameters of clinical outcome. It was concluded that this route is not effective in type 2 diabetics.

Patients belonging to the control group showed a decrease in Insulin dose at 6 mo of follow up and this was ascribed to the placebo effect. However the probability of being a true responder was observed in the targeted group of patients only. Homing and retention of cells was documented when the targeted approach was used and was not seen when the cells were infused intravenously, lending credence to the theory that delivery of these cells to pancreas is an important prerequisite for achieving therapeutic efficacy.

COMMENTS

Background

Adult bone marrow mononuclear cells have shown promising therapeutic potential in various degenerative disorders including type 2 diabetes mellitus. However the optimal routes for infusing stem cells and their *in vivo* distribution have not been defined. The present study was designed to evaluate the effects of autologous bone marrow mononuclear cell therapy, when these cells were infused through different routes and comparing them with a control arm. Analysis was carried out to find any association between the homing characteristics and therapeutic efficacy of the infused cells.

Research frontiers

It is hard to imagine a more engaging contemporary research issue than stem cell research. Researchers believe that stem cell therapy has the potential to dramatically change the treatment of human disease. A number of adult stem cell therapies already exist, particularly bone marrow transplants that are used to treat leukaemia. The role of stem cell therapy is being probed in a host of other ailments and degenerative disorders including type 2 diabetes mellitus. The pertinent questions in regenerative medicine at present are to find optimal cell types, their dosages and the effective routes of administration.

Innovations and breakthroughs

A key issue to consider is the route of administration. A reliable stem cell delivery system is essential to the success of stem cell therapy. Most currently offered therapies are utilizing the intravenous delivery route for adult stem cells infusion. However there is a concern that stem cells might fail to reach to their targeted destination because they might get trapped in the lung. This is the first study of its kind which has sought to find out treatment outcome of autologous bone marrow mononuclear cell therapy by targeted approach vis-a-vis peripheral intravenous route and comparing them with a control arm.

Applications

The study demonstrated that the probability of being a responder was there when cells were delivered through intra-arterial route. Homing and retention of cells was seen when the targeted approach was used and was not seen in the intravenous approach. Hence it was concluded that delivery of these cells to pancreas is an important prerequisite for achieving therapeutic efficacy.

Terminology

Bone marrow derived stem cells: Bone marrow derived stem cells are multi-potent stem cells that are capable of trans-differentiating into cell types other than cells of hematopoetic lineage. These cells can migrate towards the site of damage and differentiate under the influence of factors from the local micro environment.

Peer-review

In the opinion very good piece of work - bone marrow derived stem cell therapy

well done Randomized Controlled Trial performed well.

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