Regeneration of insulin production by autologous bone marrow blood autotransplantation in patients with type 1 diabetes

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Received: 20 November 2009 / Accepted: 11 December 2009 / Published online: 26 January 2010
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Keywords Autotransplantation · Beta cell function ·
Bone marrow · Type 1 diabetes

To the Editor: Among the possible cellular therapies to replace insulin secretion in diabetes, bone marrow-derived stem cells have aroused interest because of their plasticity and ease of procurement. Indeed, some experimental reports suggest that the bone marrow harbours cells capable of differentiation into beta cells [1–3], the transplantation of which can correct hyperglycaemia [1, 4]. However, these findings have not been corroborated in other studies [5]. Nonetheless, the experimental data are not conclusive and point to three potential roles of bone marrow cells in the treatment of diabetes, namely as a source of stem cells for

beta cells, as a facilitator of beta cell regeneration and as an immune modulator.

The infusion of autologous bone marrow was recently reported to restore insulin secretion in type 1 diabetes [6]. To reproduce these results, we evaluated the effect of an intrapancreatic infusion of autologous bone marrow blood on insulin production in patients with type 1 diabetes.

The present pilot study was designed to include ten patients with type 1 diabetes of more than 5 years duration and the following characteristics: inadequate glycaemic control ($\text{HbA}_{1c} > 8.5\%$ or recurrent hypoglycaemia) with intensified insulin treatment; free C-peptide $< 0.033 \text{ nmol/l}$ (basal and 6 min after administration of 1 mg glucagon, as measured with GlucaGen, Novo Nordisk, Gentofte, Denmark). Patients gave informed consent and the study protocol

Trial registration: ClinicalTrials.gov NCT00821899

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was approved by the Research Ethics Committee of the Hospital Clinic of Barcelona.

After 3 days of treatment with 5 to 10 $\mu\text{g kg}^{-1} \text{ day}^{-1}$ of granulocyte colony-stimulating factor subcutaneously administered every 12 h, 70 to 100 ml of autologous bone marrow was obtained by multipuncture of the posterior superior iliac crest under general anaesthesia using heparin as anticoagulant. The material obtained was filtered and kept in heparin until infusion. The cells were stored overnight at 4°C in a Teflon bag (Life-cell; Baxter, Deerfield, IL, USA) and subjected the following morning to testing for lot-release criteria including sterility, viability, total nucleated and CD34⁺ (haemopoietic progenitor cells) cell content.

Once cells had passed the lot-release criteria, the material was infused the morning after it had been obtained. A selective arteriography of the coeliac trunk and the superior mesenteric artery through a transfemoral route (Simmons I 5F catheter; Terumo) was done. After obtaining a vascular map of the pancreatic area, superselective catheterisation of the most accessible pancreatic artery was performed (3.2 Progreat microcatheter; Terumo, Leuven, Belgium) to infuse the bone marrow.

After the procedure, the patients were observed for 24 h for possible procedure-related complications. The follow-up consisted of clinical visits every week during the 1st month and monthly thereafter, and of telephone contact if

necessary. At 1 week, and at 1, 3, 6 and 12 months, we planned to determine routine biochemistry, HbA_{1c}, anti-GAD antibodies and basal and glucagon-stimulated free C-peptide.

To date three patients have been treated, two of whom have been followed for 6 months. One patient died suddenly 3 months after treatment with the necropsy study showing myocardial infarction as the cause of death. Considering the time since the procedure, we do not believe that the infusion contributed to death. Unfortunately, we do not have information on pancreas status. The clinical characteristics of these three patients are detailed in Table 1.

The volume infused was 65 to 103 ml with a quantity of CD34⁺ cells of 0.15×10^6 to 0.30×10^6 per kg body weight. The only adverse event reported was an auto-limited epigastric pain during bone marrow infusion in one patient (Table 1).

In the ten glucagon tests carried out during follow-up, C-peptide levels remained undetectable ($<0.033 \text{ nmol/l}$), with no change in the insulin dose or metabolic control (Table 2), strongly suggesting that the treatment was ineffective in regenerating beta cell function. The Research Ethics Committee accordingly recommended suspending the trial and we decided to stop the study.

This study suggests that intrapancreatic autologous bone marrow infusion has no effect on insulin secretion in patients with type 1 diabetes. We decided to use total bone

Table 1 Baseline clinical characteristics and details of the procedure

Characteristic	Patient		
	1	2	3
Age (years)	57	41	56
Sex	Male	Male	Male
Duration of diabetes (years)	30	5	14
Weight (kg)	65	58	92
BMI (kg/m^2)	21.7	19.6	30.7
Medical history			
Retinopathy	No	Background	Proliferative
Nephropathy	Microalbuminuria	None	Macroalbuminuria
Stroke	Yes	No	No
Ischaemic heart disease	No	No	No
Peripheral vasculopathy	No	No	No
Procedure			
Bone marrow infused (ml)	65	103	101
Total nuclear cells/kg	0.59×10^8	0.75×10^8	0.46×10^8
CD34 ⁺ /kg body weight	0.30×10^6	0.32×10^6	0.15×10^6
Artery of injection	Pancreatico-duodenal	Pancreatic magna and transverse	Pancreatic dorsal
Procedure time (min)	120	210	90
Adverse events	None	Abdominal pain during infusion	None

Table 2 Pretreatment and follow-up variables after autologous bone marrow infusion

Variable	Baseline	1 week	1 month	3 months	6 months
Weight (kg)					
Patient 1	65	66	68	—	—
Patient 2	58	58	57	58	57
Patient 3	92	94	96	95	96
Insulin dose (U/day)					
Patient 1	24	24	25	—	—
Patient 2 ^a	31	34	29	49	48
Patient 3 ^a	81	83	83	83	82
C-peptide (nmol/l) ^b					
Patient 1	<0.033/<0.033	<0.033/<0.033	<0.033/<0.033	—	—
Patient 2	<0.033/<0.033	<0.033/<0.033	<0.033/<0.033	<0.033/<0.033	<0.033/<0.033
Patient 3	<0.033/<0.033	<0.033/<0.033	<0.033/<0.033	<0.033/<0.033	<0.033/<0.033
Anti-GADab ^c (U/ml)					
Patient 1	<0.1	<0.1	<0.1	—	—
Patient 2	48.6	41.0	79.0	30.0	28.2
Patient 3	3.5	3.0	3.8	4.1	4.0
Hypoglycaemia (n) ^d					
Patient 1	2	0	3	—	—
Patient 2	0	0	4	3	3
Patient 3	0	0	4	0	0
HbA1c (%)					
Patient 1	8.9	8.8	9.4	—	—
Patient 2	9.5	9.3	8.0	8.7	8.0
Patient 3	10.4	10.3	8.7	9.1	9.2

^a Treated with insulin pump;^b fasting/stimulated; ^c anti-GADab, anti-GAD antibodies;^d episodes per week

marrow cells rather than CD34⁺ purified cells in order to reproduce the previous report exactly [6] and because of the current lack of information on the efficiency of these two options. Although we only studied three patients, we think that the evidence obtained over the 6 months of follow-up, with repeated glucagon tests in which all the basal and stimulated C-peptide levels were below the sensitivity threshold of the method, is sufficient to support this affirmation.

Type 1 diabetes is a potential candidate for stem cell therapy and diabetic patients are strongly motivated to explore any way that may restore them to a non-diabetic condition. In this context, some private centres are offering treatment with autologous bone marrow to cure diabetes [7, 8].

Up to now, two papers on this technique have been published. One, mentioned above, was in type 1 diabetes patients, and its results are completely discordant with ours [6]. In the other, more recent study in patients with type 2 diabetes, Bhansali et al. [9] observed a moderate improvement in C-peptide levels in seven of ten patients treated with intrapancreatic autologous bone marrow transplantation. However, in this study patients were under an intensified regime of visits that could explain the improve-

ment in metabolic control and, secondarily, also that in beta cell capacity through an amelioration of glucotoxicity.

Based on our experience and on the inconsistency of the previous information, we believe that intrapancreatic autologous bone marrow transplantation does not induce any relevant effect on beta cell function in type 1 diabetes patients with undetectable C-peptide levels. Our data, obviously, cannot rule out the possibility that bone marrow transplantation could be effective in preserving or even restoring beta cell function in recently diagnosed type 1 diabetes patients. However, a recent report, in which autologous umbilical cord blood infusion failed to preserve C-peptide levels in young newly diagnosed children 1 year after infusion, does not support this hypothesis [10].

The potential of stem cell therapy is enormous, but its practical clinical application must be the consequence of robust scientific evidence. Diabetes is a heterogeneous disease and it is essential to define which kind of patients may benefit from receiving cell therapy. Taking this into account, the results of the present study, albeit based on only a few cases, with specific characteristics, provide additional information to patients considering this currently available treatment.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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