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Research Paper

Therapeutic potential of Mesenchymal Stem Cells for the treatment of diabetic peripheral neuropathy

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ABSTRACT

Type-1 Diabetes is generally treated with exogenous insulin administration. Despite treatment, a very common long term consequence of diabetes is the development of a disabling and painful peripheral neuropathy. The transplantation of pancreatic islets is an advanced alternative therapeutic approach, but its clinical application is still very limited, mainly because of the great number of islets required to complete the procedure and of their short-term survival. An intriguing method to improve the performance of pancreatic islets transplantation is the co-transplantation of Mesenchymal Stem Cells (MSCs), adult stem cells already known to support the survival of different cellular populations.

In this proof-of-concept study, we demonstrated using an in vivo model of diabetes, the ability of allogenic MSCs to reduce the number of pancreatic islets necessary to achieve glycemic control in diabetic rats, and overall their positive effect on diabetic neuropathy, with the reduction of all the neuropathic signs showed after disease induction.

The cutback of the pancreatic islet number required to control glycemia and the regression of the painful neuropathy make MSC co-transplantation a very promising tool to improve the clinical feasibility of pancreatic islet transplantation for diabetes treatment.

1. Introduction

Diabetes is a metabolic disease with increasing incidence worldwide and with important social and economic effects. The disease is characterized by hyperglycemia, which may be caused by an alteration of insulin production secondary to degeneration of pancreatic beta cells (type-1 diabetes), or by an altered body response to insulin (type-2 diabetes) (Ezquer et al., 2012). The first consequence of the altered insulin production is the inability of the cells to collect and store glucose as energetic substrate, and therefore to produce Adenosine TriPhosphate (ATP). In addition, the high blood glucose concentration produces severe alterations such as protein glycosylation, increased lipid peroxidation, and production of high levels of free radicals (Davey et al., 2014; Kolka and Bergman, 2013; Premkumar and Pabbidi, 2013). These changes are hypothesized to be responsible for long term side effects of diabetes such as microcirculation damage and the onset of peripheral neuropathies (Davey et al., 2014). In fact, at least 1/3 of diabetic patients develop a peripheral neuropathy that even strict glycemic control can only partly prevent (Zenker et al., 2013). Current therapy based on exogenous insulin administration fails to properly adjust blood glucose, often producing peaks of hypo- and hyper-glycemia. Improvements to conventional insulin administration are under investigation, including the use of microinfusion pumps to achieve real time blood glucose, adjustment, but another promising therapeutic approach is based on the replacement of pancreatic islets (Ryan et al., 2005). The first results of pancreatic islets transplantation were encouraging, since this method allowed a better glycemic control with respect to traditional therapies and allowed the reduction of long term side effects

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(Warnock et al., 2008). However, at least two serious limitations are blocking the path to the diffusion of this method into clinical practice: i) the high number of pancreatic cells required for an effective replacement and ii) the short-term duration of transplanted tissue (Warnock et al., 2008). To obtain a good glycemic control at least 12,000–16,000 islets/kg are required for each patient, and this implies the use of more than one cadaveric donor (Warnock et al., 2008). Among the causes for the short term survival of transplanted pancreatic islets, immune response against the transplanted tissue and insufficient vascularization are the most prominent (Tjernberg et al., 2008). Theoretically, the use of immune suppressive drugs could allow to prolong the transplanted tissue survival, but this treatment is potentially associated with severe side effects (Mallett and Korbutt, 2009).

A possible strategy to improve the outcome of pancreatic islet transplantation is to partner them with Mesenchymal Stem Cells (MSCs), exploiting their capacity to down-regulate immune cell activation (Waterman et al., 2012) and thus limiting the rejection of transplanted tissue (D'Addio et al., 2014). Moreover, MSCs are able to increase the survival of several cell populations, including neurons, both by direct contact (Scuteri et al., 2006) and by release of trophic factors (Crigler et al., 2006). This ability to increase cellular survival and to provide trophic support might also improve the function of organs and systems typically affected by diabetes, such as the kidney and the peripheral nerves.

So far, we have already demonstrated in a syngenic model of diabetes that 2000 pancreatic islets co-transplanted with MSCs have the same effect of 3000 pancreatic islets, thus reducing the number of pancreatic islets necessary to achieve normoglycemia (Figliuzzi et al., 2009). However, this syngenic model does not reproduce the real clinical setting, where pancreatic islets derive from cadaveric donors, and it could be difficult to obtain a sufficient number of MSCs from the same patients candidates for transplantation (Berman et al., 2010; Wu et al., 2013). Therefore, in order to mimic the most unfavorable clinical situation, it is necessary to explore the potential of an allogenic model co-transplanted with third party MSCs.

For all these reasons, besides checking if allogenic MSCs co-transplantation has the same effect on glycemic control achieved in the syngenic model, the aim of the present proof-of-concept study is to verify their effect on diabetic neuropathy and nephropathy. Moreover, we also verified if MSCs alone, without pancreatic islets, have any effect on diabetic animals. To avoid the use of immune-suppressive drugs, both islets alone and islets with MSCs were encapsulated before the transplantation into a microalginate matrix (Mallett and Korbutt, 2009).

Table 1
Evaluation of Armanni-Epstein lesions in distal tubules.

	Armanni-Epstein lesions distal tubules
Group A	
a1	Absent
a3	Absent
a4	Absent
a5	Absent
a6	Absent
a7	Absent
a8	Absent
Group B	
b9	Severe, diffuse
b11	Severe, diffuse
b12	Severe, diffuse
b13	Severe, diffuse
b14	Severe, diffuse
b15	Severe, diffuse
b17	Severe, diffuse
Group C	
c18	Moderate, diffuse
c19	Absent
c20	Absent
c21	Moderate, diffuse
c22	Slight, diffuse
c23	Moderate, diffuse
c24	Moderate, diffuse
Group D	
d25	Absent
d26	Moderate, diffuse
d27	Slight, diffuse
d28	Slight, diffuse
d29	Slight, diffuse
d30	Absent
d31	Slight, diffuse
Group E	
e33	Severe, diffuse
e34	Severe, diffuse
e35	Severe, diffuse
e36	Severe, diffuse
e37	Severe, diffuse
e38	Severe, diffuse
e39	Severe, diffuse
e40	Severe, diffuse

2. Materials and methods

The study was approved by the Milano-Bicocca University ethics committee (N 0012732/13) and it was performed in conformity with the institutional guidelines, in compliance with national (DL n. 26/2014), international (EEC Council Directive 2010/63/EU, OJL 358, Dec. 1987; NIH Guide for the Care and Use of Laboratory Animals, US NRC, 1996) laws and policies and with the ARRIVE guidelines. Unless otherwise indicated, all reagents and materials were obtained from Sigma-Aldrich (Saint Louis, MO, USA).

2.1. Animals

Male 8 week-old Lewis rats (175–200 g, Envigo, Udine, Italy) were randomly divided into 5 groups of 8 animals: a) healthy controls, b) diabetic rats, c) diabetic rats transplanted in the peritoneal cavity with microencapsulated allogeneic islets (3000 islets/rat) (Remuzzi et al., 2009), d) diabetic rats transplanted with microencapsulated islets (2000 islets/rat) and 10^6 MSCs/rat, e) diabetic rats intravenously injected with 10^6 MSCs/250 μl saline solution/rat. Diabetes was induced by intraperitoneal injection (i.p.) with 60 mg/kg Streptozotocin (STZ). Both body weight and glucose blood level were evaluated weekly. For an automated quantification of blood glucose level a commercial kit was used. In particular, after a 8-h fast, a drop of blood from the caudal vein was placed on a test stick and inserted in the Accu-Chek® Compact Plus device (Roche Diagnostics, Italy). Blood glucose concentration was immediately calculated as mg glucose /dl blood.

Whole blood from tail vein was also used and serum obtained by the centrifugation of clotted blood at 2.500 g for 15 min at 4 °C for determination of the levels of Creatinine and Blood Urea Nitrogen in order to evaluate kidney function with an automatic MIRA PLUS system (Horiba ABX Diagnostic, Montpellier, France, Chiorazzi et al., 2012).

The transplantation with islets alone or islets-MSCs was performed two months after the diabetes induction, after the assessment of an established neuropathy, detectable by a decrease in Nerve Conduction Velocity (NCV) and impaired nociceptive thresholds. Animals were sacrificed 2 months after transplantation (i.e. 4 months after diabetes induction).

2.2. Pancreatic islets

Pancreatic islets were isolated from Wistar rats (Envigo, Udine, Italy, body weight 250-300 g), using an automatic procedure already reported (Scuteri et al., 2014). Briefly, the pancreas of anesthetized rats were distended by injecting a collagenase P solution (Bushranger-Mannheim, Mannheim, Germany), removed and then loaded into a digestion chamber at 37 °C. When optimum digestion time was reached, the chamber was flushed with 4 °C Hanks' balanced salt solution (HBSS, Gibson Nitrogen Corporation, Paisley, Scotland) and digested tissue was purified