Cell-based Therapy for Treatment of Diabetes Mellitus: Can the Agonists of Growth Hormone-releasing Hormone Make a Contribution?

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Abstract

Beta cell replacement, to supply the body with cells producing insulin, is considered as one of the most important alternative approaches to the treatment of diabetes. Transplantation of human islets and the resulting progressive improvement of clinical results confirm the approach as a positive trend in this field. Recent progress in beta cell differentiation, deriving from many types of pluripotent stem cells, has potentially provided an unlimited source of β cells for research and clinical applications. Novel approaches are needed to make cell-based therapy more safe, reproducible and long-lastingly efficient. As an example, pretreatment of the islet cells with agonists of growth hormone-releasing hormone improves cell proliferation and metabolic functions and, facilitates engraftment of islets after transplantation in rodents. Here, we review current progress in islet transplantation and the studies using stem cell-derived insulin-producing β cells as therapeutic options in the treatment of diabetes.

Keywords

Diabetes, Islet transplantation, Mesenchymal stem cells, Embryonic stem cells, Induced-pluripotent stem cells, Beta cells, Growth hormone-releasing hormone agonists

Introduction

Diabetes currently affects more than 300 million people worldwide; this number is set to rise dramatically [1]. Permanently elevated blood glucose levels are the key indicator of this metabolic disorder. Type 1 diabetes (T1D) is an autoimmune disease in which insulin-producing β cells within the pancreatic islets are irreversibly destroyed, resulting in deficient insulin production. Type 2 diabetes (T2D) is a disease in which the pancreas produces insufficient amounts of insulin, due to progressive loss of β cells; systemic tissues may also become resistant to normal or even high levels of insulin. Severe complications, such as blindness, kidney failure, limb amputation and heart attack arise as a consequence of the long-term damage of tissues, caused by high levels of blood sugar. In both T1D and T2D the supply of insulin-producing tissue/cells is inadequate. Cell-based therapies are strategies to overcome this and thus reduce the dependence on exogenous insulin in diabetic patients. The main strategy is thus the development of such cell therapies to treat diabetes by the production of sufficient numbers of pancreatic endocrine cells that can function as primary islets.

The progress in pancreatic islet transplantation achieved over the past decade suggests that diabetes can be improved by replacing deficient β cells with new, functional, insulin-producing cells [2-4]. These techniques, while effective, are hindered by immune rejection as well as by the lack of adequate supplies of primary tissues for such transplantation. Generation of functional β cells from other sources is needed, therefore, to overcome the islet shortage. Over the past several years, stem cell therapy, especially that using mesenchymal stem cells (MSCs) [5,6], embryonic stem cells (ESCs) [7,8] and induced pluripotent stem cells (iPSCs) [9,10], as sources of engineered insulin-producing cells, has provided alternative approaches to islet transplantation. Recent findings of the beneficial effects of agonists of growth hormone-releasing hormone (GHRH) on the functions of β cells may also provide new approaches to their application in T1D and T2D diabetes [11-14].

Herein, we outline the progress, in animal studies and in human clinical trials, in the generation of β cells from different types of stem cells and also in the progress in methodology for infusion of islets and stem cell derived β cells intra-corporeally for the treatment of diabetes.

Islet Transplantation

One emerging alternative to whole organ pancreatic transplantation is transplantation of isolated pancreatic islet cells. This process is based on the enzymatic isolation of pancreatic islets...
from pancreata procured from cadaver donors. The islets obtained are infused into the liver of the recipient through a percutaneous catheterization of the portal venous system. This procedure for islet cell transplantation (ICT) is considered to be minimally invasive, and allows the selective transplantation of a population of insulin-producing cells. ICT can be considered an alternate option in the restoration of glucose homeostasis in a subset of T1D patients with unstable glycemic control and with frequently severe hypoglycemia which has failed correction by standard intensive insulin therapy [3].

The ICT method was first developed in the 1970s. Initial efforts with ICT showed that the treatment reduced the occurrence of diabetic complications, however, the long-term outcome was unsatisfactory. The results were improved remarkably, in 2000, by the Collaborative Islet Transplant Registry. In this setting the key to the success of the islet cell transplantation lay in the quality and the mass of the islets used, as well as the immunosuppressive regimen. By using an improved, “Edmonton Protocol”, the destruction of islets, caused by rejection and recurrent autoimmune disease, was reduced. Further, the report by the Collaborative Islet Transplant Registry for allogeneic islet transplantation performed during the years 1999 to 2010, indicated that the rate of recipients achieving insulin-independence for 3 years showed increasing stability, from only 10% before 1999 to 44% in the years from 2007-2010 [16,17]. Using potent application of immunotherapy, a higher rate of insulin-independence can thus be achieved [18,19]. Increased levels of C-peptide and reduction of glycated hemoglobin, (Hb-A1c), attested to the increasing durability of islet graft function. However, the limitation of transplantable pancreatic donor material is still a major hurdle. To meet the demand for islet transplantation, a potential alternative is the use of animal sourced islets. The use of pancreatic islets obtained from pigs has emerged as a practical alternative to the use of human tissues, due to their greater availability and physiologic similarities to human islets. Long-term graft survival, in non-human primates, of porcine islets isolated from adult, neonatal or genetically engineered pigs has been reported [20-22]. Infusion of adult porcine islets resulted in normoglycemia in immunosuppressed, diabetic, non-human primates [23].

Minimization of attack by the host's immune system is a critical issue in achieving efficient islet engraftment. Immunosuppressive therapy, however, itself causes undesirable side effects. Despite many clinical and clinical trials, there is still not a single standard immunosuppressive regimen that can be used to suppress acute and chronic immune reactions, with lower toxicity, to grafted islets.

An islet encapsulation technology to treat diabetes, another conceptual option, has been developed. By trapping islets into man-made devices, a physical barrier between the islet cells and the immune system is created, thus allowing normal physiologic function of encapsulated islet cells. The system has been tested in several experimental models [24]. A pilot trial for safety and efficacy to treat patients with T1D is in progress [3]. A “bioartificial pancreas” has also been developed which consists of macrochambers specifically engineered for islet transplantation and survival. The subcutaneously implantable device allows for a controlled and adequate oxygen supply and is specially designed to afford immunologic protection of its contained donor islets against the host's immune system. This has made possible long-term glycemic control in diabetic rats and minipigs [12,25]. A breakthrough was reported in that a human patient, suffering from T1D, received an implanted bioartificial pancreas and experienced persistent graft function, with regulated insulin secretion and preservation of islet morphology and function, without the need for immunosuppression, for ten months [26]. This system/concept opens up an entirely new, fundamental strategy for the therapy of diabetes, by providing an avenue for future approaches using xenotransplantation. A clinical trial, using a “DIABECELL” device, which incorporates neonatal porcine islets encapsulated in alginate microcapsules, is also in progress [3]. DIABECELL has been safely transplanted in both healthy and diabetic animals. Following DIABECELL transplants, the requirement for daily insulin was significantly reduced in diabetic mice, rats, rabbits, dogs and non-human primates.

Designing a procedure to specifically isolate islets, with high cellular yields and minimal damage, is another critical issue in the spectrum of transplantation [2]. Strategies to retain the functionality of pancreatic islets, such as promotion of cell growth and metabolic function, are particularly important [27,28]. Numerous studies have reported efforts to improve the survival of islets by preventing the loss of islet cell viability and function during and following the transplantation period, in animal diabetic models. To this end growth hormone (GH), and various growth factors such as insulin-like growth factor-1 (IGF-1) and, glucagon-like peptide (GLP-1) were studied for their ability to stimulate proliferation and survival of pancreatic β cells [29-32]. Pharmaceutical screening to identify new drugs that can improve β cell function, survival, proliferation, or all of those, is another important possibility for improving the maintenance of functional islet cells for transplantation. For example, in mouse models of diabetes, the efficacy of islet transplantation can be substantially improved by the preconditioning of islets with kinase Ce (PKCe) activator [33]. Recently, agonists of growth hormone-releasing hormone (GHRH) were found to significantly improve β cell survival, growth and metabolic function, and to increase expression of cellular insulin, IGF-1 and vascular endothelial growth factor (VEGF); they also stimulated insulin secretion in response to glucose [34]. GHRH agonists also improves the in vivo engraftment and the metabolic function of islets following the transplantation into streptozotocin (STZ)-induced diabetic mice [11,13,14]. Pretreatment with GHRH agonists also significantly enhanced function of rat islets encapsulated in bioartificial macrochambers after implantation into diabetic rats [12]. The pretreatments led to a reduction of the islet mass necessary for normoglycemic metabolic control in diabetic animals [12,14]. VEGF has been reported to play a critical role in development of β cells, and is itself also associated with the survival of islets in vivo following transplantation [34,35]. We can speculate that the beneficial effects of GHRH agonists on the functions of β cells may provide an improved approach to ICT. Clinical trials of oral caspase inhibitor to prevent apoptosis of islets, and Sitagliptin, a drug to increase the amount of GLP-1, are already in progress in T1D patients after islet transplantation [3].

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) represent a stem cell population that can be isolated from a variety of adult tissues. MSCs exhibit a great capacity of self-renewal in culture, and also the potential of multipotent differentiation. Human MSCs exhibit low immunogenicity, thereby making them important and promising candidates for allogeneic cell therapy. MSCs have the potential to supply growth factors and cytokines, and the ability to selectively target into those injured tissues requiring repair [36,37]. Furthermore, MSCs are an abundantly available cell source and can be obtained from patients for use in autologous transplantation. MSCs are currently being evaluated in various pre-clinical and clinical studies and offer significant potential as a novel cellular therapy for tissue regeneration and repair, immune disorders, diabetes, and related complications [38].

In vitro differentiation of MSCs into insulin producing cells is well documented. Using multistep differentiation protocols, MSC-derived insulin-producing cells can be obtained from a variety of human adult tissues [5,39] including bone marrow [40,41], adipose tissue [42,43], umbilical cord or its blood [44,45], endometrium [46]. Numerous studies have reported the potential improvement of diabetes by transplantation of MSCs in T1D diabetic mice [6,47-50], rats [51] and miniature pigs [52]. Infusion of MSCs derived from bone marrow or umbilical cord improved the hyperglycemia and raised blood insulin levels in T2D mice [53]. The infusion of MSCs not only promoted β-cell function, but also ameliorated insulin resistance in T2D rats [53]. MSCs can also improve the secretion of a variety of trophic factors such as IGF-1 and VEGF. VEGF is known to play a key role in cell engraftment and MSC-mediated vasculogenesis [54,55]. Co-transplanting bone marrow cells concurrent with islet transplantation also significantly improves islet engraftment in
diabetic mice [56,57]. Transplantation with MSCs also ameliorated damages in cardiac dysfunction [54,58,59], renal failure [49,60,61], dysfunctional wound healing and limb ischemia [62,63], in diabetic animal models.

Preliminary and preclinical studies of infusions, using human MSCs derived from bone marrow, umbilical cord, cord blood or placenta, to treat patients with T1D and T2D have yielded promising data. The treatment is shown to be safe and well tolerated; reduction of insulin dependence is observed. Studies have further indicated that autologous bone marrow MSCs preserve β cell function in patients with recent-onset T1D [64,65]. In the management of T2D, they partially restore the function of islet beta-cells, maintaining blood glucose homeostasis, increasing levels of C-peptide [66,67] and improving wound healing in diabetic patients with critical limb ischemia [68]. The infusion of multipotent stem cells, derived from umbilical cord blood, remarkably improves C-peptide levels, reduces Hb-A1c values, decreases the required median daily doses of insulin in patients with T1D [69]; it also ameliorated metabolic control and reduced inflammation markers in patients with T2D [70]. Wharton’s jelly-derived MSCs (WJ-MSCs, isolated from the umbilical cord with a high yield of “young cells”) or MSCs derived from adipose tissue or placenta can also be used in the treatment of new-onset T1D [71,72], and T2D [73,74].

The abundantly available sources of MSCs for transplantation, and their low immunogenicity and immune-modulatory properties, give MSCs advantages over islets in cell based therapy. Animal studies and pilot clinical trials have demonstrated the effectiveness of MSCs in the treatment of T1D and T2D. However, despite the success of differentiation of human MSCs in vitro into functional pancreatic β cells, the rate of trans-differentiation was considered low [75,76], and the duration of functional maintenance in vivo is difficult to evaluate. The absence of standardized protocols for the expansion and generation of insulin secreting cells still leads to inconsistent clinical outcomes. MSCs do not pose the risk of producing teratomas, but their substantial expression of chemokines may have hidden risks for the promotion of tumor growth and metastasis. Karyotypic changes might also appear after long-term continuation in culture [37]. Nevertheless, transplantation of MSCs ameliorates the progress of diabetes and, perhaps, may have unique potential when used in combination with ICT [36,56,57]. Recently it has been reported that agonists of GHRH promote survival of cardiac myocytes and cardiac stem cells in vitro [77-79], reverse remodeling after myocardial infarction in vivo [77,78], accelerate wound healing [80] and augment the production of VEGF in mouse or human MSCs [53]. These interesting finding may provide a new insight into the use of agonists of GHRH for the potential tissue repair function of MSCs. The use of GHRH agonists may improve the efficacy of MSCs based therapy of diabetes.

Embryonic Stem Cells

The most significant aspect of the use of cell therapies to treat diabetes is the potential for the production of sufficient numbers of pancreatic endocrine cells that can function similarly to primary islets. Human embryonic stem cells (ESCs) derived from the inner cell mass of a blastocyst can proliferate extensively in vitro, be maintained indefinitely as an undifferentiated cell line, and have the potential to differentiate into derivatives of any of the three germ layers [81,82]. One advantage of applications using ESC-derived cells is that ESCs are not as immunologically potent as allogeneic adult cells; the use of ESCs therefore provides a promising alternative cell source for the cellular treatment of diabetes. Many studies have been reported wherein both mouse and human ESCs may differentiate into insulin-secreting cells [83-85]. By using strategies mimicking embryonic pancreatic organogenesis, human ESCs can be induced to differentiate in vitro into endocrine cells capable of synthesizing pancreatic hormones [86]. After implantation into diabetic mice, these cells efficiently generate glucose-responsive cells, exhibit the properties of functional β cells after engraftment, and prevent STZ-induced hyperglycemia [87]. Furthermore, by using defined cell surface markers, enriched populations of pancreatic endoerdrm cell types can be differentially separated, and give rise to all pancreatic endocrine cell lineages after transference into [88]. The success of the production of functional pancreatic progenitors from human ESCs has been developed; these cells, upon implantation, efficiently protect against diabetes in mice [89,90]. This system has provided a robust methodology for manufacturing pancreatic progenitors for use in clinical trials. The very first-in-human trial using human ESC-derived pancreatic precursor cells to treat patients with T1D is now under way [7,91]. The cells are encapsulated in a drug delivery system developed by the Encaptra Company. The system is placed under the patient's skin to protect from the recipient’s immune cells [91]. Recently, two groups independently reported that, by using a multiple-stage induction protocol, they efficiently converted human ESCs into insulin producing cells in vitro [92,93]. The cells displayed glucose-stimulated insulin secretion similar to that of human islets [92]. Xenotransplantation of these cells efficiently reverses diabetes in mice. In vitro expansion of these cells provides a promising alternative to using pancreatic progenitor cells and would overcome donor islet shortages. ESCs are a favorable source for cell based therapy, however, in addition to ethical issues, safety is a major concern because the possible contamination by undifferentiated cells is a hidden risk for the formation of teratomas or other tumors [94]. There is now a growing recognition that differentiated cells derived from ESCs are mostly immature [95]. These cells thus can mimic embryonic development and adopt phenotypes that resemble fetal or neonatal cells which, with the hidden danger of genetic mutagenesis, could lead to tumor formation. More studies, with long-term observation, are required to understand the mechanisms involved and their significance.

Induced Pluripotent Stem Cells

The exciting discovery of induced pluripotent stem cells (iPSCs), in 2006, opened a new possibility in generating replacement cell based therapy for disease treatment [96]. Forced expression of four defined key transcription factors can program mouse somatic cells, such as fibroblasts, into iPSCs. iPSCs resemble ESCs with their infinite self-renewal capacity and great potential to differentiate into a wide variety of cell types. Success in generation of iPSCs from human somatic cells was soon reported [97,98]. Un-differentiated iPSCs can be maintained as cell lines; this, therefore, provides great promise for disease modeling and for allowing the generation of personalized stem cells for autologous cell therapies [9,99].

Mouse skin fibroblast-derived iPSCs were able to differentiate into β-like cells, similar to normal, endogenous insulin-secreting cells, and thereby reverse hyperglycemia in diabetic mice [100]. iPSCs have been generated from patients suffering from T1D and T2D [101-103]. Insulin-producing cells have then been generated in vitro from iPSCs by directed differentiation, using small molecules and growth factors in culture [104,105]; these produced a nearly 25% yield of insulin-positive cells [106]. Recently, a stratagem for large-scale production of functional human β cells from human iPSCs in vitro has been demonstrated. By using sequential modulation of multiple signaling pathways found in the development of the pancreas, in a three-dimensional cell culture system, mono-hormonal insulin-producing and glucose-responsive cells were generated [105,107]; with reproducible protocols an approximately 50% yield could be obtained [93,108]. These cells also responded to multiple, sequential high-glucose challenges and thus functionally protected mice from diabetes. Therefore, this technique may allow us to produce large numbers of β cells in vitro for therapeutic application.

An advantage of using iPSCs is the absence of ethical concerns. The technology allows the generation of autologous cells for cell-replacement therapy. The somatic origin of iPSCs has minimized, but however, not eliminated, some of the challenges that have hampered the development of human ESC-based therapies. As with ESCs, a major obstacle to the use of iPSCs is the safety issue; the risk of teratoma formation can be substantial [109,110]. An improved understanding of the cellular healing mechanisms of cellular reprogramming is necessary in order to overcome these barriers before the so-called “next-generation stem cells” can be safely applied in patients with diabetes.
Transdifferentiation

Several studies have shown that β cells can be generated from other cell types of endodermal origin, without requiring transit through a pluripotent stage. Introduction of pancreatic transcription factor in vivo induced liver cells to express pancreatic endocrine-related genes including those for insulin production, without affecting normal hepatic function, and resulted in prevention of chemically induced hyperglycemia in mice [111,112]. A strategy of re-expressing key developmental regulators in vivo can reprogram differentiated pancreatic exocrine cells into cells resembling β cells in respect to size, shape, ultrastructure and expression of those genes essential for β cell functions [113-115]. Pancreatic ductal structures may also contain precursor cells that can yield insulin-producing cells. Ductal tissue from human pancreas can be isolated in large scale, expanded and precursor cells that can yield insulin-producing cells. Ductal tissue from human pancreas can be isolated in large scale, expanded and precursor cells that can yield insulin-producing cells.

The application of the GHRH agonists in the Treatment of Diabetes

Hypothalamic GHRH stimulates production and release of growth hormone (GH) from the pituitary gland, exerts some of its effects through the GH/IGF-1 axis, and also directly affects extra-pituitary cells expressing GHRH receptors by activating them. GHRH receptor(s) have been detected in pancreatic β cells, cardiac stem cells and MSCs from different species [11-14,55,77-80]. It has been proposed that the activation of signal pathways (such as MAPK/ERK, PI3K/AKT, and cAMP/PKA), triggered by the interaction between GHRH agonists and the receptors on β cells, plays an important role in the stimulation of metabolic function of pancreatic β cells [14]. The GHRH-GHRH receptor complex may enable signal transduction either or in cooperation with other pathways, likely the IGF-1 signaling pathway, in the regulation of development and function of pancreatic β cells. The beneficial effects of GHRH agonists on the metabolic function of pancreatic β cells may provide approaches to cell based therapy for treatment of diabetes.

Conclusion

In conclusion, strategies to supply the body with cells producing insulin are considered as the most important alternative approaches to the treatment of diabetes. The progress in the transplantation of pancreatic islets that has been achieved suggests that clinical diabetes can be improved by the replacement of deficient beta cells with new, functional cells. Generation of functional β cells from stem cells offers an attractive method of restoring islet cell mass. The use of MSCs, ESCs and iPSCs as sources for engineered insulin secreting cells, has provided an alternative approach to the use of islet transplants. Recent success in the generation of mono-hormonal, insulin-producing and glucose-responsive cells from human ESCs and iPSCs provides an enormous potential source of β cells for therapeutic usage. Beneficial effects of GHRH agonists and other hormonal and growth agents on the proliferation and function of β cells and on the engraftment of islets after transplantation, suggest that these classes of compounds might also improve the function of the insulin-producing-cells derived from ESCs, iPSCs and other cell types of endodermal origin, but further studies are required. The development and application of encapsulation technology to circumvent immune rejection by recipients may enable us to reduce or eliminate the necessity of immunosuppressive drugs. Progress in these areas opens up new avenues for the treatment of both T1D and T2D.

References


