TYPE 1 DIABETES MELLITUS: BEYOND THE INSULIN THERAPY

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Abstract

Type 1 diabetes mellitus (DM1) is an autoimmune disease that leads to the destruction of insulin-secreting pancreatic β cells. This usually leads to absolute insulin deficiency and requires insulin treatment for survival. In spite of remarkable progress made, like self-glucose monitoring, advances in insulin therapy (insulin analogs, insulin pumps) and higher standards of care, most people with diabetes continue to develop disabling complications.

Currently, the only reliable option for establishing durable normoglycemia in patients with DM1 is whole pancreas transplantation. In spite of excellent results, pancreas transplantation has become the therapy of choice only for a selected group of patients with end-stage renal disease (simultaneous pancreas-kidney transplantation or pancreas after kidney transplantation).

Islets transplantation, applied to the patients with labile DM1, cannot achieve insulin independence in majority of patients and suffers from lack of organ donors and life-long imunosupression.

A major goal of current diabetes research is to generate an abundant source of autologous glucose-responsive insulin-secreting cells that can replace the destroyed β cells and avoid immune rejection.

Keywords: type 1 diabetes mellitus, pancreas transplantation, gene therapy.

DIABETUL ZAHARAT TIP 1: DINCOLO DE TERAPIA CU INSULINĂ

Rezumat

Diabetul zaharat tip 1 (DZ1) este o boală autoimună, caracterizată prin distrucţia celulelor β pancreatice, secretoare de insulină. Aceasta duce la deficit absolut de insulină endogenă şi la nevoia terapiei cu insulină exogenă pentru supravieţuire. În ciuda progreselor remarcabile înregistrate în domeniu, cum ar fi: automonitorizare glicemică, insulinoterapie (analogi de insulină, pompe de insulină), standarde de îngrijire mai ridicate ale pacienţilor, majoritatea pacienţilor cu diabet dezvoltă complicaţii cronice debilitante.

În prezent, singura opţiune terapeutică care restabileşte normoglicemia de durată la pacienţii cu DZ1 este transplantul de pancreas. În ciuda rezultatelor excelente, transplantul de pancreas a devenit terapia de elecţie doar pentru pacienţii cu boală renală terminală (transplantul simultan de pancreas şi rinichi sau transplantul de pancreas după un transplant renal).

Transplantul de insule pancreatice, care se aplică în prezent pacienţilor cu DZ1 labil, nu asigură independenţa de insulinoterapie la majoritatea pacienţilor şi este limitat de lipsa donatorilor şi a necesarului imunosupresiei pentru toată viaţa.

Ţinta cercetărilor curente în diabet este generarea unei surse abundente de celule secretoare de insulină în răspuns la concentraţia glucozei plasmatice, care să înlocuiască celulele β distruse şi să evite rejecţia imună.

Cuvinte cheie: diabetul zaharat tip 1, transplantul de pancreas, terapie genică.

Type 1 diabetes mellitus (DM1) previously encompassed by the terms insulin-dependent diabetes, or juvenile-onset diabetes, results mostly from a cellular-mediated autoimmune destruction of insulin-secreting β-cells. Beta cells destruction leads to absolute insulin deficiency and require insulin treatment for survival.

Although the patient can survive with conventional insulin therapy, relatively poor blood glucose control leads to long-term complications for most diabetic patients. While intensive therapy effectively delays the onset and slows the progression of chronic diabetic complications it is associated with increased risk of hypoglycemia and low patient compliance [1].

Disposal of a glucose load after a meal requires a rapid increase in insulin levels. It is difficult to use subcutaneous insulin injections to match this rapid physiological insulin peak. There have been a number of developments that improve insulin delivery with better timing characteristics. The short-acting insulins are quickly absorbed from the subcutaneous tissue and disappearance rate is faster than the regular insulin. Thus, it is possible to give a dose much closer to mealtime than with regular insulin, and there is less risk of hypoglycemia at a later time. Furthermore, the administration of insulin by continuous subcutaneous infusion through insulin pumps permit preprogrammed delivery of *basal* insulin profiles as well as quick premeal infusion of *bolus* insulin doses.

Although we are much closer to the goal of physiologic insulin replacement, this goal remains elusive, owing to the inherent limitation of administering insulin at a non-physiologic site (subcutaneous tissue).

1. Pancreas and islet transplantation

Pancreas transplantation

The most obvious solution is to provide patients with the β-cells they are missing, witch can be done with pancreas or islets transplantation.

More than 35,000 pancreas transplantations have been reported worldwide to the International Pancreas Transplant Registry, until March 2011. The majority (75%) were performed simultaneously with kidney transplantation (SPK), while less were given after previous kidney transplantation (PAK) (18%) or pancreas transplantation alone (PTA) (7%). Patient survival reaches over 95% at one year post-transplant and over 83% after 5 years. The best graft survival was found in SPK with 86% pancreas and 93% kidney graft function at one year. PAK pancreas graft function reached 80%, and PTA pancreas graft function reached 78% at one year. In all groups, early technical graft loss rates decreased significantly to 8-9%, and immunological graft loss to 1.8-6% [2].

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Long-term studies of motor, sensory, and autonomic neuropathy have demonstrated that these complications stabilize after pancreas transplantation [3] and native-kidney biopsies have shown a dramatic reversal of mesangial accumulation and basement-membrane thickening 10 years after the establishment of normal glucose levels by pancreas transplantation [4].

Despite excellent outcomes and improvements in terms of patient survival, graft function and insulin independence, pancreas transplantation still has limited application since it requires complex surgery, with high risk of surgical complications, technical failure and long term immunosuppression. Thus, the risk-to-benefit ratio, in the context of a very expensive procedure, limits its applicability to a highly selected group of patients, those with advanced nephropathy or labile diabetes.

Diabetic patients with end-stage renal failure have a poor prognosis without transplantation. Transplantation with SPK provides a marked extension of the patient's life and freedom from insulin injections, being considered the "gold standard" for this group of patients. The 5- , 10- and 20- year patient survival rates were 89, 80, and 58%, respectively after SPK transplantation [5].

Islets trasplantation: Pancreatic islets are extracted from the healthy pancreas, from brain-dead donors and injected by a minimally invasive procedure percutaneously into the liver *via* the portal vein. Islet transplantation is a less demanding method of β cell replacement in that it does not involve major surgery, it permits a lesser degree of immunosuppression, and is potentially less expensive for the recipient. Unfortunately, the initial results were disappointing. In 2000 a group of investigators from Edmonton/Canada developed a new immunosuppressive regimen that excluded islet-toxic glucocorticoids (rapamycin, daclizumab and tacrolimus), which significantly improved the success rate. A successful transplant needs at least 10,000 islet equivalents per kilogram body weight, often obtained from a minimum of two donor pancreases [6]. Since the report from Edmonton, many transplantation centers throughout the world have initiated corticosteroid-free protocols with successful results. Data analysis of 458 islet cell transplants performed worldwide (1999-2004) and reported to the International Islet Transplant Registry, shows at 1 year a patient survival rate of 97%, a functioning islet graft in 82% of the cases, whereas insulin independence was meanwhile achieved in 43% of the cases. However, using the novel protocol established by the Edmonton Center, the insulin independence rates have improved significantly reaching 50-80% [7].

Insulin independence should not be seen as a primary goal, but good glucose control and avoidance of severe hypoglycaemia. The endogenous insulin production achieved by islet transplantation, combined with optimal insulin therapy, were sufficient for maintaining glucose levels comparable to those achieved with pancreas

transplantation. A higher rate of insulin independence following pancreas transplantation (after 1 year 96 vs 31% in islets group) was counterbalanced by a higher rate of serious adverse events (40% re-laparotomies vs 0% in islets group) [8].

Although islet transplantation is a viable future treatment for DM1, significant hurdles remain, including the limited islet supply and complications associated with both the procedure and the required lifelong immunosuppression. Therefore, this procedure can only be justified in a selected patient cohort (i.e., patients with severe metabolic instability despite compliance with an optimized diabetes regimen using best current dietary, blood glucose monitoring, and insulin delivery systems) [9].

2. Construction of surrogate β-cells

Demand for islet transplantation far exceeds the number of islets available. The success of Edmonton protocol depends on the use of two or more cadaver pancreases. Even if the procedure is limited to adults with type 1 diabetes who have recurrent hypoglycemia and poor symptom recognition, there are not enough pancreases to meet the need. This problem could be circumvented if an autologous cell type that is available in enough quantity could be engineered to secret insulin in response to glucose.

Expansion of beta cells: Normal adult beta cells are difficult to propagate in culture, ***in vitro***, without partial or complete loss of function.

Recent studies of *in vitro* expansion suggest that β-cells have a potential for dedifferentiation, expansion, and redifferentiation in appropriate culture conditions [10]. However, the potential of these cells in controlling blood glucose levels *in vivo* should be established.

In vivo expansion of β-cells is an atractive strategy. Few factors were shown to increase β cells mass in rodents and have potentialy similar effects in human, like: glucagon-like peptidul 1 (GLP1), epidermal growth factor (EGF), gastrin. Experimental studies have shown a beneficial effect of GLP-1 agonists on β-cell mass and function, improving glycemic control in diabetic animals. Increased β-cells mass occured by increasing β-cell proliferation and neogenesis and decreasing apoptosis [11]. However, human studies are missing meanwile. In a recent study on 16 patients with long-standing DM1 (21.3±10.7 years), exenatide (GLP-1 agonist) had no significant effect on β -cell function [12]. An important finding of this study was that 85% of individuals with long-standing type 1 diabetes who were screened in this trial, still had residual β-cell function (C-peptide levels ≥0.05 ng/ml). Future clinical trials should investigate the potential of GLP-1 agonist in recent onset DM1 or individuals with preclinical DM1 who still have a significant viable β-cell mass [13].

The results of this approach could be limited by the uncontrolled ongoing autoimmune process, which is why new strategies combine therapy addresed to β-cell expansion and immunomodulation. Preliminary clinical studies have shown favorable results for a few immunomodulatory strategies, like: GAD65, heat-shock protein peptide, anti-CD3 antibodies, autologous hematopoietic stem cells, autologous umbilical cord blood. Ongoing randomized, double-blind, phase II/III trials should confirm them and establish the best option.

Stem cells: An inadequate supply of islet tissue represents a major obstacle to the widespread implementation of islet transplantation. One promising solution to this problem is the induction of embryonic or adult stem cells to islet cell differentiation.

1) Embryonic stem cell lines are derived from the inner cell mass of a blastocyst. They possess the ability to differentiate *in vitro* into a variety of cell lineages and they have been shown to restore the function of injured organ upon their transplantation.

Recent diferentiation protocols, which mimic *in vivo* pancreatic organogenesis, have achieved *in vitro* conversion of embryonic stem cells in insulin secreting cells, with comparable amount of insulin content like adult human β cells. However, their secretory response to glucose stimuli was minimal. *In vivo* transplantation of these cells improved their response to glucose and prevented the streptozotocin-induced diabetes. The identification of these *in vivo* factors that lead to maturation of embryonic cells-derived β-cells could be the last step to generation of enough amounts of functional β-cells [14].

2) Adult pancreatic stem/progenitor cells. Stem cells have been identified in a variety of adult tissues, contributing to organ maintenance and repair. The expansion and differentiation of pancreatic stem cells or progenitors cells is an attractive approach for the generation of β-cells and avoids the controversy and technical problems associated with embryonic stem cells. However, the precise location and phenotype of these pancreatic precursors has not yet been established. Most of the data suggest that pancreatic stem cells may be ductal cells or localized in close relation with the ductal epithelium. Thus, the development of differentiated islets from pancreatic duct cells has been demonstrated in cell cultures of mouse and human pancreatic cells [15]. The low proportions of differentiating cells suggests that either the methods are as yet inefficient, or that only a specific subpopulation of ductal cells are true islet progenitors. Extensive research has been carried out to ascertain if other pancreatic cell types play a role in the birth of new islets. Several studies have reported *in vitro* differentiation of exocrine cells into ductal-like cells that may then be capable of differentiation into endocrine cells [16]. Finally, there is the possibility that islets themselves contain progenitor cells that allow their prolonged survival and continual cellular turnover [17].

In contrast, a recent report suggests that pre-existing β-cells, rather than stem cells, are the major source during

adult life and after pancreatectomy in mice [18].

3) Adult extra-pancreatic stem/progenitor cells. The existence of extra-pancreatic progenitor cells that differentiate into β-cells has been suggested in several reports. Due to common endodermic origin of the liver and pancreas, the liver was one of the first places examined to find these cells.

Rodent-liver progenitor cells and human fetal liver epithelial progenitor cells [19] have been differentiated *in vitro* into insulin-secreting cells.

Another tissue with similar embryological origins is the upper gastrointestinal tract. Expression of pancreatic genes encoding PDX-1 and Isl-1 in rat intestinal stem cells, in presence of betacellulin led to insulin secretion [20].

Bone marrow-derived stem cells have been well characterized as having the capability to differentiate into many other tissue types. Transplantation of adult bone marrow-derived cells initiates endogenous pancreatic tissue regeneration and reduces hyperglycemia in mice with streptozotocin-induced pancreatic damage [21]. Bone marrow derived cells were induced also *in vitro*, under defined conditions, to differentiate into insulin-producing cells and reduced hyperglycaemia upon their transplantation in STZ-induced diabetic mice [22].

Engineered β-cells: A gene-therapy–based treatment of DM1 requires the development of a surrogate β-cell that can synthesize and secrete functionally active insulin in response to physiologically changes in glucose levels.

One approach involves studies attempting to express insulin gene in various non-β-cells. Expression of either human proinsulin gene in neuroendocrine cells (ACTH-secreting cell, GIP-secreting cells) or of variants of insulin that do not require proconvertase expression, like a single-chain insulin analog in non-neuroendocrine cells (HepG2, C2C12, primary myoblast, fibroblast) enables them to secret bioactive insulin and correct streptozotocin induced diabetes in rodents [23-24]. However, co-secretion of antagonistic hormones such as ACTH by neuroendocrine cells and reduced capacity to achieve glucose-dependent insulin secretion, limit their application. Some researchers have also attempted to derive glucose-regulated insulin production by driving insulin gene expression with various glucose-sensitive promoter elements. However, the slow time course of transcriptional control by glucose makes synchronizing insulin production with the periodic fluctuations in blood glucose levels an extremely difficult task.

Transdifferentiation is defined as an irreversible switch in postnatal life of one type of already differentiated cell to another type of normal differentiated cell. At the molecular level, the cause of trans-differentiation is presumably a change in the expression of a master regulatory (master switch) gene whose normal function is to distinguish between the two tissues in normal development [25]. A number of genes exist that exemplify the definition of a master switch gene. For example, *MyoD* is the master switch gene for myogenesis, while PPARγ is involved in adipogenesis.

Pancreatic transcription factors such as PDX-1, Ngn3, and Hes1 have been suggested to act also as master switch genes in pancreatogenesis.

In vivo expression of pancreatic duodenal homeoboxe 1 (PDX-1) in mouse liver by a adenoviral vector [26] and *in vitro* expression into fetal human progenitor liver cells [19], were shown to turn them into insulin producing cells. However, implementing these techniques to humans has several drawbacks. Systemic administration of adenoviral vector renders the recipient vulnerable to systemic viral dissemination and acute toxicity [27], while using fetal human progenitor liver cells raises ethical issues.

The most efficient way of delivering therapeutic genes to primary cells is by viral vectors. The "ideal" gene therapy treatment should deliver therapeutic genes only to cells that require their beneficial actions. Targeted expression can be achieved by using *ex vivo* strategies. *Ex vivo* cell transdiferentiation before transplantation into the host avoids systemic dissemination of viral vectors and ensures a tissue-specific expression of the transgene. Moreover, the *ex vivo* approach has an important advantage in that the safety of genetically modified cells can be evaluated before they are administered to the patient.

A few studies have demonstrated the permanent engraftment of *ex vivo* transduced hepatocytes (viral vector-mediated gene transfer), delivered to the livers of different animal models, including non-human primates [28]; and a therapeutic effect has been reported in a human clinical trial of hypercholesterolemia after autotransplantation of hepatocytes oncoretrovirally transduced with the low-density lipoprotein receptor gene [29].

In a recent work, we demonstrated that primary hepatocytes can be engineered to function as β-cells [30]. The ex vivo expression of PDX-1 in primary hepatocytes, by a lentiviral vector, induced distinct regulatory β-cell–like functions: they secrete insulin in response to glucose and secretagogues in a dose dependent manner; are morphologically rearrange like β-cells in vitro as well as in rat liver in vivo; are able to normalize in vivo plasma glucose levels when transplanted in diabetic mice; and reverse the weight loss associated with the disease in diabetic animals.

Autotransplantation of genetically engineered hepatocytes avoids immune rejection and the concomitant side effects of lifelong immunosuppressive treatment.

Using the patient's own liver cells as a primary target for *ex vivo* gene therapy for diabetes has several advantages. The liver can easily be accessed surgically; liver cells can be isolated in large quantities and can be easily returned to the body. Furthermore, hepatocytes are the only cells that possess the same glucose-sensing apparatus as β-cells, i.e., Glut2, GK and a similar K_{ATP} channel. Moreover, many liver-specific genes are controlled at physiological

glucose concentrations. Finally, the location of the liver is privileged for controlling glucose homeostasis, as the portal vein carries glucose and other products absorbed after feeding.

The adipose-derived stromal vascular cells were recently reported to differentiate in vitro into adipogenic, chondrogenic, myogenic, osteogenic cells and even toward nonmesenchymal lineages of neuroectodermal origin. Over-expression of PDX-1 in human bone marrow mesenchymal cells was shown to differentiate them into insulin-secreting cells. Adipose tissue, like bone marrow, is derived from the embryonic mesenchyma and the expression of PDX-1 master gene in adipose precursor cells switched them to differentiate toward insulin secreting cells (our unpublished work).

References

1. DCCT. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N Engl J Med, 1993; 329(14): 977-986.
2. Gruessner AC. 2011 Update on Pancreas Transplantation: Comprehensive Trend Analysis of 25,000 Cases Followed Up Over the Course of Twenty-Four Years at the International Pancreas Transplant Registry (IPTR). Rev Diabet Stud, 2011; 8(1): 6-16.
3. Navarro X, Sutherland DE, Kennedy WR. Long-term effects of pancreatic transplantation on diabetic neuropathy. Ann Neurol, 1997; 42(5): 727-736.
4. Fioretto P, Steffes MW, Sutherland DE, et al. Reversal of lesions of diabetic nephropathy after pancreas transplantation. N Engl J Med, 1998; 339(2): 69-75.
5. Wai PY, Sollinger HW. Long-term outcomes after simultaneous pancreas-kidney transplant. Curr Opin Organ Transplant, 2010.
6. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med, 2000; 343(4): 230-238.
7. Bretzel RG, Jahr H, Eckhard M, et al. Islet cell transplantation today. Langenbecks Arch Surg, 2007; 392(3): 239-253.
8. Gerber PA, Pavlicek V, Demartines N, et al. Simultaneous islet-kidney vs pancreas-kidney transplantation in type 1 diabetes mellitus: a 5 year single centre follow-up. Diabetologia, 2008; 51(1): 110-119.
9. Hirshberg B, Rother KI, Digon BJ, et al. Benefits and risks of solitary islet transplantation for type 1 diabetes using steroid-sparing immunosuppression: the National Institutes of Health experience. Diabetes Care, 2003; 26(12): 3288-3295.
10. Ouziel-Yahalom L, Zalzman M, Anker-Kitai L, et al. Expansion and redifferentiation of adult human pancreatic islet cells. Biochem Biophys Res Commun, 2006; 341(2): 291-298.
11. Kwon DY, Kim YS, Ahn IS, et al. Exendin-4 potentiates insulinotropic action partly via increasing beta-cell proliferation and neogenesis and decreasing apoptosis in association with the attenuation of endoplasmic reticulum stress in islets of diabetic rats. J Pharmacol Sci, 2009; 111(4): 361-371.
12. Rother KI, Spain LM, Wesley RA, et al. Effects of exenatide alone and in combination with daclizumab on beta-cell function in long-standing type 1 diabetes. Diabetes Care, 2009; 32(12): 2251-2257.
13. Bosi E. Time for testing incretin therapies in early type 1 diabetes? J Clin Endocrinol Metab, 2010; 95(6): 2607-2609.
14. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol, 2008; 26(4): 443-452.
15. Gao R, Ustinov J, Pulkkinen MA, et al. Characterization of endocrine progenitor cells and critical factors for their differentiation in human adult pancreatic cell culture. Diabetes, 2003; 52(8): 2007-2015.
16. Rooman I, Heremans Y, Heimberg H, et al. Modulation of rat pancreatic acinoductal transdifferentiation and expression of PDX-1 in vitro. Diabetologia, 2000; 43: 907-914.
17. Guz Y, Nasir I, Teitelman G. Regeneration of pancreatic beta cells from intra-islet precursor cells in an experimental model of diabetes. Endocrinology, 2001; 142(11): 4956-4968.
18. Dor Y, Brown J, Martinez OI, et al. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. Nature, 2004; 429(6987): 41-46.
19. Zalzman M, Gupta S, Giri RK, et al. Reversal of hyperglycemia in mice by using human expandable insulin-producing cells differentiated from fetal liver progenitor cells. Proc Natl Acad Sci USA, 2003; 100(12): 7253-7258.
20. Kojima H, Nakamura T, Fujita Y, et al. Combined expression of pancreatic duodenal homeobox 1 and islet factor 1 induces immature enterocytes to produce insulin. Diabetes, 2002; 51(5): 1398-1408.
21. Hess D, Li L, Martin M, et al. Bone marrow-derived stem cells initiate pancreatic regeneration. Nat Biotechnol, 2003; 21(7): 763-770.
22. Oh SH, Muzzonigro TM, Bae SH, et al. Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for the treatment of type I diabetes. Lab Invest, 2004; 84(5): 607-617.
23. Auricchio A, Gao GP, Yu QC, et al. Constitutive and regulated expression of processed insulin following in vivo hepatic gene transfer. Gene Ther, 2002; 9(14): 963-971.
24. Lee HC, Kim SJ, Kim KS, et al. Remission in models of type 1 diabetes by gene therapy using a single-chain insulin analogue. Nature, 2000; 408(6811): 483-488.
25. Tosh D, Slack JM. How cells change their phenotype. Nat Rev Mol Cell Biol, 2002; 3(3): 187-194.
26. Ferber S, Halkin A, Cohen H, et al. Pancreatic and duodenal homeobox gene 1 induces expression of insulin genes in liver and ameliorates streptozotocin-induced hyperglycemia. Nat Med, 2000; 6(5): 568-572.
27. Lozier JN, Csako G, Mondoro TH, et al. Toxicity of a first-generation adenoviral vector in rhesus macaques. Hum Gene Ther, 2002; 13(1): 113-124.
28. Andreoletti M, Loux N, Vons C, et al. Engraftment of autologous retrovirally transduced hepatocytes after intraportal transplantation into nonhuman primates: implication for ex vivo gene therapy. Hum Gene Ther, 2001; 12(2): 169-179.
29. Grossman M, Rader DJ, Muller DW, et al. A pilot study of ex vivo gene therapy for homozygous familial hypercholesterolaemia. Nat Med, 1995; 1(11): 1148-1154.
30. Fodor A, Harel C, Fodor L, et al. Adult rat liver cells transdifferentiated with lentiviral IPF1 vectors reverse diabetes in mice: an ex vivo gene therapy approach. Diabetologia, 2007; 50(1): 121-130.