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Intramuscular islet allotransplantation in type 1 diabetes mellitus

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Abstract. – OBJECTIVE: Alternative sites to the liver for islet transplantation have been studied for a long time. Intramuscular islet transplantation appears to be an alternative site to the liver because of the ease of access. First islet autotransplantations were reported in patients after total pancreatectomies. The transplanted islets showed a proper revascularization and their function was observed for up to 2 years after the implant. However, only a few cases of autotransplantation and no allotransplantation have been performed. The aim of this study was to verify the feasibility of islet allotransplantation into muscles.

PATIENTS AND METHODS: In four patients affected by type 1 diabetes mellitus in which liver islet allotransplantation was contraindicated, human islets were transplanted into patients' arm muscle with local anesthesia.

RESULTS: The surgery was minimally invasive, without complications. In one patient a moderate local inflammatory reaction was observed at the site of the implant, which resolved spontaneously within 4 days. Islet graft function was observed after transplantation in all patients, but it progressively disappeared in 3 out 4 patients within a short time.

CONCLUSIONS: In this first ever-reported intramuscular pancreatic islet allotransplantation, the procedure appears feasible but new strategies must be envisaged to significantly improve islet engraftment and the long-term graft function.

Key Words:
Islet transplantation, Site of implant, Intramuscular transplantation, Engraftment.

Introduction

Pancreatic islets are usually transplanted through the portal vein into the liver where they engraft and replace beta cell function[1]. However, a large proportion of transplanted islets are lost a few hours after the transplant, principally due to an instant blood-mediated inflammatory reaction (IBMIR)[2,3]. Thereafter, islets into the liver are exposed to a high glucose concentration of immunosuppressive drugs that leads to chronic graft exhaustion and then graft loss[4]. In addition, there are some clinical conditions that contraindicate transplanting into the liver: portal hypertension, hepatic disease or thrombophilic disease. Therefore, the search for alternative sites for islet transplantation is one of the hot topics in this field. Most of the data available on alternative sites were achieved in animal models of islet transplantation and included islet transplantation in the pancreas, subcutaneous tissue, muscle tissue, omentum, bone marrow, gastric submucosa, genitourinary tract, the kidney capsule, testis, thymus and anterior eye chamber[5-7]. In some of these sites (omentum, bone marrow, muscle) clinical trials have been started and the first case studies have been reported[8-11]. A truly efficient implant site as an alternative to the liver has not been identified yet.

Skeletal muscle was proposed as an alternative site for islet implant, characterized by minimal blood contact (thus preventing IBMIR), easy access and an efficient islet revascularization[6,7]. Vasculature within islets engrafted in striated muscle is functional and restored within 2 weeks after transplantation[12] and the vascular system was similar to that in native islets[11]. In an animal model for both human and murine islets, equivalent or superior graft function was observed for transplantation into skeletal muscle compared with the portal vein[5]. The first clinical islet autotransplantations into the skeletal muscle after total pancreatectomy showed graft function up to 2 years later[10], and the islets were easily visualized through high-resolution magnetic resonance imaging[11] or through GLP-1 receptor scanning[13]. Indeed, no data about human islet allotransplantation into the skeletal muscle of patients affected by type 1 diabetes mellitus are available. Here,

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we report the results of the first 4 transplantations in patients affected by type 1 diabetes mellitus in which liver engraftment was contraindicated for different clinical reasons.

Patients and Methods

Patients

Informed consent was obtained from patients who underwent the procedure, according to the requirement of local Ethical Committee. The protocol was approved by the Local Ethical Committee. Inclusion criteria were: C-peptide ≤0.5 ng/ml in type 1 diabetes mellitus, age between 18 and 65 years, minimum disease duration of 5 years, brittle diabetes or in active immunosuppression therapy for another organ transplantation and contraindication to intra liver transplantation (thrombophilia, cardiovascular disease, portal hypertension, liver disease). Four patients were eligible for intramuscular islet transplant.

Patient #1 was transplanted 10 years ago with a liver transplantation, complicated by partial hepatic arterial thrombosis that contraindicated an intrahepatic invasive procedure.

Patient #2 was transplanted 1 year ago with intra hepatic islet transplantation, showing an increase of portal pressure up to 19 mmHg at the end of the procedure.

Patient #3 was submitted to a simultaneous islet and heart transplantation. In the first days after the heart transplantation, hemodynamic disfunction contraindicated islet transplantation into the liver. Patient received islet transplantation 2 days after heart transplantation.

Patient #4 showed a thrombophilic condition.

Islet Production

Human islets were isolated from pancreata of multiorgan cadaveric donors. Islet isolation and culture were performed in the Regional Tissue Bank authorized by CNT (Italian National Centre for Transplantation). Organs were harvested utilizing cold perfusion (Celsior, Genzyme, Naarden, The Netherlands), and islets were isolated using the automated method, as previously described[14,15]. Pancreata were digested using a cold enzymatic blend solution (Collagenase NB1, SERVA, Heidelberg, Germany) reconstituted in Hank's Balanced Salt Solution 25 mM of HEPES (Euroclone, Pero, Milan, Italy), supplemented with neutral protease (Protease NB, SERVA, Heidelberg, Germany) and islets were purified with discontinuous gradient. Purification was performed using polysucrose gradient solutions at 1.132, 1.108, 1.096, 1.060 e 1.037 g/L (Mediateech-Cellgro, Manassas, VA, USA). Collagenase activities were tested as previously described[16].

Islets were seeded in 75 cm^2 suspension flasks at the concentration of 20,000 equivalent islets/flask and cultured for 24-48 hours before transplantation at 24°C and 5% CO_2 in Miami Media (Mediatech-Cellgro, Manassas, VA, USA), supplemented with ciprofloxacin (Fresenius Kabi, Verona, Italy).

Islet viability was assessed with propidium iodide (PI) (Sigma-Aldrich, Milan, Italy) and calcein AM (Thermo Fisher, Waltham, MA, USA).

Islet Transplant

Islet preparation was suspended in Ringer Lactate (Fresenius Kabi, Verona, Italy) supplemented with bicarbonate (1 mEq/ml, S.A.L.F., Bergamo, Italy) and human serum albumin 20% (Kedrion, Lucca, Italy). Aliquots were loaded in 5 ml luer/lock syringes packaged in double wrap and transported to the operating room.

Islets were transplanted under local anesthesia in the brachioradialis muscle of the forearm (patients #1, #2 and #3) or in the bicep muscle (patient #4) (Figure 1). Briefly, skin was incised and the muscular band exposed. Small incisions were made in the inner muscle; syringes were equipped with a rounded needle, and islets seeded between fibers while the needle was slowly receded.

All the patients, with the exclusion of patient #4, were already under immunosuppression therapy. After islet transplantation, immunosuppression therapy remained the same. Patient #4 was treated as previously described[17].

Results

The characteristics of the transplanted patients are reported in Table I. Patient #2 showed a residual islet function from previous islet transplantation. The patients received an islet allotransplantation (Table II). In all patients, an inflammatory reaction was observed to different extents in the site of implant, particularly evident in patient #4, minimal in patient #3. The inflammation completely resolved after 6 days from the transplant. C-reactive protein increased after islet transplantation only in patient #4 (from 0.1 to the peak value of 4.9 mg/l 3 days after transplantation). After islet transplantation, c-peptide values increased

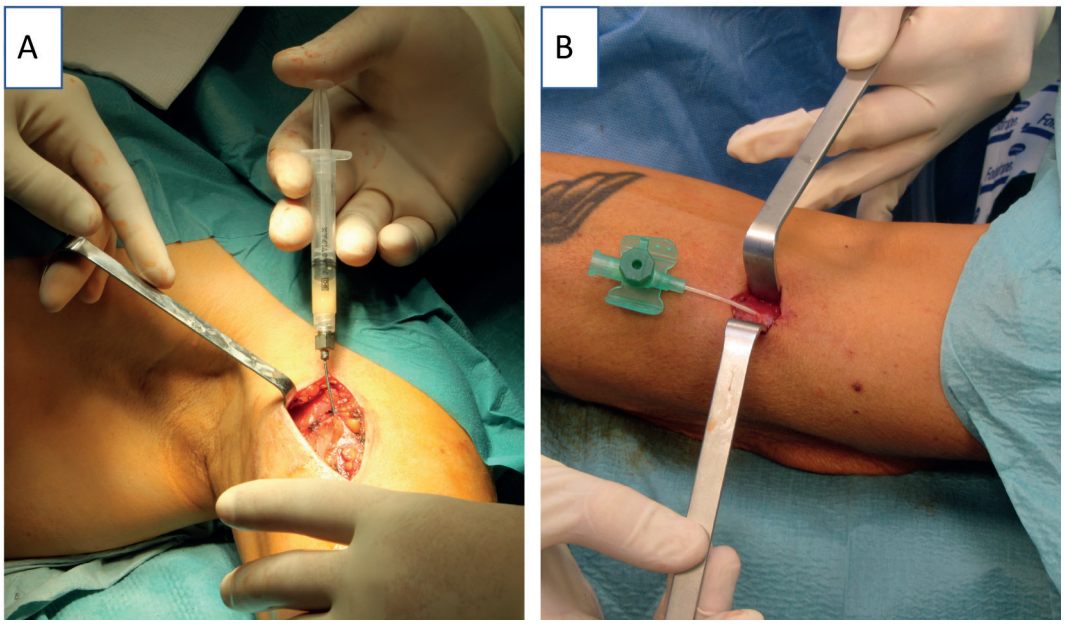

Figure 1. Islets transplantation under local anesthesia in bicep *(A)* or in brachioradialis *(B)* muscle in the forearm.

Table I. The characteristics of the transplanted patient.

Patient ID	Sex	Age (years)	Height (cm)	Weight (Kg)	Years of diabetes	C-pep (ng/ml)	HbA1c (%Hb)
#1	M	53	173	76	38	<0.1	7.2
#2	F	36	162	74	25	0.5	6.6
#3	F	45	159	53	37	0.1	8.1
#4	M	41	170	70	28	0.1	8.8

Table II. The characteristics of the transplanted islet preparations.

Patient ID	Number of Equivalent Islet (IE/kg b.w.)	Volume (ml)	Purity (%)
#1	396.000 (5.21)	1	95
#2	397.000 (5.36)	2	80
#3	243.260 (4.58)	4	30
#4	471.000 (6.72)	4	80

at different rates in the four recipients (Figure 2). Patient #3 showed C-peptide 0.3 ng/ml up to 24 months after transplantation. No significant decrease of insulin requirement was observed in patients #1, #2 and #4. Patient #3 halved the insulin requirement (from 70 to 35 u per day a year later). However, in the patient #3 the general inflammatory and hemodynamic conditions were significantly improved after the simultaneous islet and heart transplantation. Therefore, the reduction in insulin requirement may also be partially influenced by the positive outcome of heart transplantation. HbA1c during follow up was reported in Table III. Antibodies anti GAD increased after transplantation only in the patient #2 (from basal 0.9 to 9.0 U/ml 3 months later).

Table III. HbA1c values before and after transplantation.

Patients	Basal (%)	3 month (%)	12 month (%)
#1	7.2	6.5	7.1
#2	6.6	7.7	10.1
#3	8.0	6.2	8.2
#4	8.8	8.1	7.8

Discussion

This is the first clinical experience of human islet allotransplantation into the skeletal muscle in patients affected by type 1 diabetes mellitus.

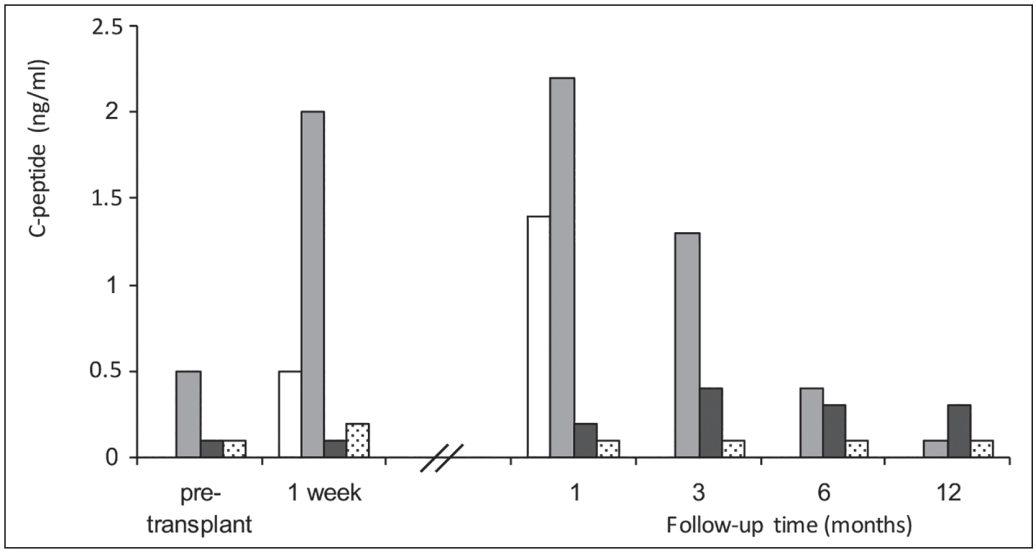

Figure 2. C-peptide values before and after transplantation of patient #1 (white bars), patient #2 (gray bars), patient #3 (black bars), patient #4 (dotted bars).

Previously, only islet autotransplantations in patients submitted to total pancreasectomy have been reported[10]. In the case of a 7-year-old girl, who underwent a total pancreatectomy followed by islet autotransplantation to the brachioradialis muscle of the right forearm, C-peptide increased up to 1.37 ng/mL and a decreased insulin requirement and a normal HbA1c were reported during the 2 year follow-up. In three patients submitted to autotransplantation into the brachioradialis muscle, islets were visualized using magnetic resonance imaging 3-6 months after surgery, although plasma c-peptide was not detectable[11]. In our set of patients, islet graft function was observed after transplantation in all the patients, from 3 up to 24 months after the transplant, albeit to a small degree. Indeed, the metabolic impact of graft function remained limited and in 2 out 4 patients rapidly decreased. With one exception, graft function was not able to significantly modify the recipient insulin requirement and HbA1c was not strictly controlled in all the patients. No definitive conclusion can be taken from this preliminary experience: this is not a controlled study but a pilot experience in four heterogenous patients. The number of transplanted islets in each patient was lower than we usually transplant, according to our protocol[17]. Many factors could impair islet engraftment into the skeletal muscle[18]. The injection of large clusters of islets causes substantial early islet cell death due to hypoxia. The revascularization process occurred within two weeks, but this is probably too late to prevent early cell death. Exocrine contamination could amplify islet hypoxia soon after transplantation[19]. The inflammatory reaction to the islet graft might trigger a rejection or an autoimmune reaction and subsequently to the loss of islets. Finally, the surgical technique might cause myofiber trauma, thus amplifying the inflammatory reaction[19].

Many strategies have been proposed to improve islet engraftment and function and most of them appeared to be particularly suited for a transplant into the skeletal muscle. The co-transplantation of mesenchymal stem cells reduced inflammation and supported endothelial cell interactions[20]; the co-transplantation of polymerized hemoglobin or the use of oxygen carriers reduced beta cell hypoxia[21]. Finally, the pre-transplant uses of medical devices to induce revascularization[22] and the use of growth factors, anti-inflammatory or anti apoptotic strategies might favor islet engraftment[23-28].

Skeletal muscle appeared to be a safe site for islet implant; in this procedure, complications are physically restricted to the arm used for the implant. This transplant is therefore feasible even in severely ill patients such as heart transplant patients.

Conclusions

Intramuscular islet transplantation is a safe and feasible procedure, but still needs substantial improvement to favor engraftment and, therefore, long-term islet graft function.

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F.B., participated in research design and in the writing of the article, G.C., L.DC and A.L. participated in the project and in the article revision.

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Conflict of Interest
The Authors declare that they have no conflict of interest.

References

1) Shapiro AM, Pokrywczynska M, Ricordi C. Clinical pancreatic islet transplantation. Nat Rev Endocrinol 2017; 13: 268-277.
2) Moberg L, Johansson H, Lukinius A, Berne C, Foss A, Källen R, Ostraat O, Salmela K, Tibell A, Tufveson G, Elgue G, Nilsson Ekdahl K, Korsgren O, Nilsson B. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. Lancet 2002; 360: 2039-2045.
3) Eich T, Eriksson O, Lundgren T; Nordic Network for Clinical Islet Transplantation. Visualization of early engraftment in clinical islet transplantation by positron-emission tomography. N Engl J Med 2007; 356: 2754-2755.
4) Delaune V, Berney T, Lacotte S, Toso C. Intraportal islet transplantation: the impact of the liver microenvironment. Transpl Int 2017; 30: 227-238.
5) Stokes RA, Cheng K, Lalwani A, Swarbrick MM, Thomas HE, Loudovaris T, Kay TW, Hawthorne WJ, O'Connell PJ, Gunton JE. Transplantation sites for human and murine islets. Diabetologia 2017; 60: 1961-1971.
6) Cantarelli E, Piemonti L. Alternative transplantation sites for pancreatic islet grafts. Curr Diab Rep 2011; 11: 364-374.
7) Svesson J, Lau J, Sandberg M, Carlsson PO. High vascular density and oxygenation of pancreatic islets transplanted in clusters into striated muscle. Cell Transplant 2011; 20: 783-788.
8) Baidal DA, Ricordi C, Berman DM, Alvarez A, Padilla N, Ciancio G, Linetsky E, Pileggi A, Alejandro R. Bioengineering of an intraabdominal endocrine pancreas. N Engl J Med 2017; 376: 1887-1889.
9) Maffi P, Balzano G, Ponzoni M, Nano R, Sordi V, Melzi R, Mercalli A, Scavini M, Esposito A, Peccatori J, Cantarelli E, Messina C, Bernardi M, Del Maschio A, Staudacher C, Doglioni C, Ciceri F, Secchi A, Piemonti L. Autologous pancreatic islet transplantation in human bone marrow. Diabetes 2013; 62: 3523-3531.
10) Rafael E, Tibell A, Rydén M, Lundgren T, Sävendahl L, Borgström B, Arnelo U, Isaksson B, Nilsson B, Korsgren O, Permert J. Intramuscular autotransplantation of pancreatic islets in a 7-year-old child: a 2-year follow-up. Am J Transplant 2008; 8: 458-462.
11) Christoffersson G, Henriksnäs J, Johansson L, Rolny C, Ahlström H, Caballero-Corbalan J, Segersvärd R, Permert J, Korsgren O, Carlsson PO, Phillipson M. Clinical and experimental pancreatic islet transplantation to striated muscle: establishment of a vascular system similar to that in native islets. Diabetes 2010; 59: 2569-2578.
12) Christoffersson G, Carlsson PO, Phillipson M. Intramuscular islet transplantation promotes restored islet vascularity. Islets 2011; 3: 69-71.
13) Pattou F, Kerr-Conte J, Wild D. GLP-1-receptor scanning for imaging of human beta cells transplanted in muscle. N Engl J Med 2010; 363: 1289-1290.
14) Ricordi C, Goldstein JS, Balamurugan AN, Szot GL, Kin T, Liu C, Czarniecki CW, Barbaro B, Bridges ND, Cano J, Clarke WR, Eggerman TL, Hunsicker LG, Kaufman DB, Khan A, Lafontant DE, Linetsky E, Luo X, Markmann JF, Naji A, Korsgren O, Oberholzer J, Turgeon NA, Brandhorst D, Friberg AS, Lei J, Wang LJ, Wilhelm JJ, Willits J, Zhang X, Hering BJ, Posselt AM, Stock PG, Shapiro AM. Purified Human Pancreatic Islets (PHPI) master production batch record – a standard operating procedure of the NIH clinical islet transplantation consortium. CellR4 2014; 2: e891.
15) Ricordi C, Goldstein JS, Balamurugan AN, Szot GL, Kin T, Liu C, Czarniecki CW, Barbaro B, Bridges ND, Cano J, Clarke WR, Eggerman TL, Hunsicker LG, Kaufman DB, Khan A, Lafontant DE, Linetsky E, Luo X, Markmann JF, Naji A, Korsgren O, Oberholzer J, Turgeon NA, Brandhorst D, Friberg AS, Lei J, Wang LJ, Wilhelm JJ, Willits J, Zhang X, Hering BJ, Posselt AM, Stock PG, Shapiro AM. National institutes of health-sponsored clinical islet transplantation consortium phase 3 trial: manufacture of a complex cellular product at eight processing facilities. Diabetes 2016; 65: 3418-3428.
16) Salamone M, Seidita G, Cuttitta A, Rigogliuso S, Mazzola S, Bertuzzi F, Ghersi G. A new method to value efficiency of enzyme blends for pancreatic tissue digestion. Transplant Proc 2010; 42: 2043-2048.
17) Bertuzzi F, Marazzi M, De Carlis LG, Rampoldi AG, Bonomo M, Antonioli B, Tosca MC, Galuzzi M, De Gasperi A, Colussi G. Sustained islet allograft function after peritransplant treatment using exenatide with and without everolimus. Transplantation 2016; 100: e117-e118.
18) Sakata N, Aoki T, Yoshimatsu G, Tsuchiya H, Hata T, Katayose Y, Egawa S, Unno M Strategy for clinical setting in intramuscular and subcutaneous islet transplantation. Diabetes Metab Res Rev 2014; 30: 1-10.
19) Sterkers A, Hubert T, Gmyr V, Torres F, Baud G, Delalleau N, Vantyghem MC, Kerr-Conte J, Caiazzo R, Pattou F. Islet survival and function following

intramuscular autotransplantation in the minipig. Am J Transplant 2013; 13: 891-898.

20) Yoshimatsu G, Sakata N, Tsuchiya H, Minowa T, Takemura T, Morita H, Hata T, Fukase M, Aoki T, Ishida M, Motoi F, Naitoh T, Katayose Y, Egawa S, Unno M. The co-transplantation of bone marrow derived mesenchymal stem cells reduced inflammation in intramuscular islet transplantation. PLoS One 2015; 10: e0117561.

21) Paraskevas S. Use of oxygen carriers to promote islet engraftment in extravascular sites. Transplantation 2015; 99: 2015-2016.

22) Pepper AR, Gala-Lopez B, Pawlick R, Merani S, Kin T, Shapiro AM. A prevascularized subcutaneous device-less site for islet and cellular transplantation. Nat Biotechnol 2015; 33: 518-523.

23) Noguchi H, Miyagi-Shiohira C, Kurima K, Kobayashi N, Saitoh I, Watanabe M, Noguchi Y, Matsushita M. Islet culture/preservation before islet transplantation. Cell Med 2015; 8: 25-29.

24) Tsuchiya H, Sakata N, Yoshimatsu G, Fukase M, Aoki T, Ishida M, Katayose Y, Egawa S, Unno M. Extracellular matrix and growth factors improve the efficacy of intramuscular islet transplantation. PLoS One 2015; 10: e0140910.

25) Zoso A, Serafini P, Lanzoni G, Peixoto E, Messinger S, Mantero A, Padilla-Téllez ND, Baidal DA, Alejandro R, Ricordi C, Inverardi L. G-CSF and exenatide might be associated with increased long-term survival of allogeneic pancreatic islet grafts. PLoS One 2016; 11: e0157245.

26) Lombardo MF, De Angelis F, Bova L, Bartolini B, Bertuzzi F, Nano R, Capuani B, Lauro R, Federici M, Lauro D, Donadel G. Human placental lactogen (hPL-A) activates signaling pathways linked to cell survival and improves insulin secretion in human pancreatic islets. Islets 2011; 3: 250-258.

27) Najjar M, Manzoli V, Abreu M, Villa C, Martino MM, Molano RD, Torrente Y, Pileggi A, Inverardi L, Ricordi C, Hubbell JA, Tomei AA. Fibrin gels engineered with pro-angiogenic growth factors promote engraftment of pancreatic islets in extrahepatic sites in mice. Biotechnol Bioeng 2015; 112: 1916-1926.

28) Cadario F, Savastio S, Rizzo AM, Carrera D, Bona G, Ricordi C. Can Type 1 diabetes progression be halted? Possible role of high dose vitamin D and omega 3 fatty acids. Eur Rev Med Pharmacol Sci 2017; 21: 1604-1609.