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·综 述 General review·

Islet transplantation in multicenter networks: the GRAGIL example

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【Abstract】 **Purpose of review** The enthusiasm generated by the results of the Edmonton protocol of islet transplantation is inciting a great number of institutions to start such programs. However, the procedure of islet isolation and purification is costly, complex and technically challenging. In order to share costs and to avoid facing the steep learning curve of the procedure, many centers interested in islet transplantation have looked into collaborating with experienced groups serving as core islet isolation facilities. **Recent findings** The proof of principle that remote islet processing and shipment could be successfully implemented with obtainng the Portland/Minneapolis, Huddinge/Giessen and Houston/Miami partnerships. Moreover, in order to increase both the donor pool and the number of patients gaining access to islet transplantation, multicenter networks, such as the Swiss-French GRAGIL consortium and the 4-country Nordic Network in Scandinavia have been built. The GRAGIL group has been fully operational since 1999, allowing the transplantation of 27 islet preparations processed in Geneva, Switzerland into 20 recipients in France over the course of 4.5 years. Organizational issues in the design of such networks are discussed based on the example of the GRAGIL experience. **Summary** The feasibility and the efficiency of islet transplantation in multicenter networks have been demonstrated. This strategy allows to increase the donor pool and the accessibility to islet transplantation in an extended population area. (J Intervent Radiol, 2006, 15: 626-631)

【Key words】 Islet of Langerhans transplantation; Distant islet processing; Collaborative networks; Costs; Islet shipping

The unprecedented success of the Edmonton protocol for clinical islet of Langerhans transplantation in patients with "brittle" forms of type 1 diabetes [1] has fueled a great deal of enthusiasm for the procedure and prompted increasing numbers of centers worldwide to offer islet transplantation to their patients [2]. However, the islet isolation and purification procedure is technically challenging[3] and is associated with a steep learning curve. For this reason, some programs have elected to perform islet transplantation procedures with islets processed at another center with previous experience in the field. Such collaborative efforts have been successfully attempted either as bilateral efforts or within the framework of multicenter networks. The Swiss-French GRAGIL ("Groupe Rhin-Rhne-Alpes-Genève pour la Transplantation d'lots de Langerhans")consortium was the first such network to be launched and reported very encouraging initial results [4] prior to the breakthrough Edmonton study. In this review, we look at the rationale for islet transplantation networks and the feasibility of remote islet processing, we discuss islet shipment issues, and we report on the logistics and results of the GRAGIL consortium.

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Rationale for islet transplantation in multicenter networks

Reasons for establishing collaborative multicenter networks of islet transplantation, in which one established institution serves as a core facility for islet of Langerhans isolation and processing, are twofold. First, successful islet transplantation rests not only on efficient immunosuppression (IS) and post-transplant management protocols, which can be easily transferred, but also on the expertise required to produce high quality islets, using a technically challenging islet isolation and purification procedure under ongoing improvement [3]. The steepness of the learning curve of the method was recently emphasized by disparities in the success rate of islet transplantation in a multicenter trial sponsored by the Immune Tolerance Network [5,6]. Second, cost issues are of increasing importance. In the United States, the Food and Drug Administration (FDA) considers islet preparations for transplantation into humans both as "drugs"and "biological products"that must be produced with strict enforcement of current good manufacturing practice (cGMP) rules. The situation is similar in European countries. These requirements imply additional equipment, personnel and procedures aimed at guaranteeing the purity, potency and safety of the islets released for transplantation, which adds up to the already substantial costs of the islet isolation process by itself. The costs for building a new, state-of-the-art islet facility in compliance with cGMP have been estimated at 1 ~ 2 million US$. The costs for one islet isolation/purification procedure vary between 10,000 ~ 20,000 US$. Considering that approximately 50% of processed pancreata result in a transplantable preparation, and that most patients require two islet infusions in order to achieve insulin-independence[7,8], this brings up the isolation-related costs for one patient to 40,000 ~ 80,000 US$.

In brief, considerations of avoiding the initiation of an islet transplantation program at the bottom of the learning curve and of sharing costs provide the rationale for the organization of multicenter islet networks.

Feasibility of remote islet processing

Islet isolation performed at a remote center prior to shipment of the islets for transplantation was first reported by a group in Oregon [9,10]. This experience took place in a setting of autologous islet transplantation in 5 patients undergoing total pancreatectomy for chronic pancreatitis. Surgeries were done in Portland, OR and pancreata were shipped to Minneapolis, MN, where islets were isolated, but not purified in order to maximize yields. Islets were then shipped back to Portland for intraportal infusion, which occurred 16 to 24 hours after pancreatectomy. Three of five patients had minimal or no insulin requirements after autotrans-plantation.

Although this experience was obtained with a very different patient population than type 1 diabetic subjects, and with specific technical issues regarding islet isolation, it provided a strong basis for further attempts in an allogeneic transplantation setting. A Swedish-German collaborative program was initiated in 1996, in which the well established group at the Justus Liebig University in Giessen isolated islets from pancreata harvested in and shipped from Sweden. After 1 to 4 days in culture, islets were shipped to Huddinge University for allogeneic transplantation. Although none of the 7 patients, who received 1 to 3 islet infusions, became insulin independent, all had initial islet graft function (C-peptide positivity), and 3 had long-term functioning grafts, with steroid-containing immunosuppressive protocols [11].

More recently, a similar collaboration was established between the transplantation center at the Baylor College of Medicine in Houston, TX and the Diabetes Research Institute at the University of Miami, FL, serving as the core islet isolation center [12,13]. In opposition to the two previously described experiences, in which pancreata and islets were shipped by commercial airliner, this collaborative effort elected to use charter jets. All 3 initial recipients achieved insulin independence after 1 ~ 2 islet infusions, representing 10,240 to 19,703 islet equivalents (IEQ)/kg body weight [12]. In a recent

update of this trial, 5 of 9 patients were off insulin, the 4 remaining patients expecting a second islet infusion[14].

These 3 examples can be seen as a proof of principle of the feasibility of the concept of remote islet isolation. Of course, it can be noted that in all 3 cases, highly experienced centers (Minneapolis, Giessen, Miami)were chosen as islet processing facilities.

Pancreas and islet shipping issues

To minimize cold ischemia time, pancreata should reach the isolation center within 8 hours of aortic cross clamp, a factor that was strictly enforced in the Houston/Miami collaboration and certainly accounts for their high rate of success[12]. However, the recent validation of the two-layer method, in which pancreata are oxygenated during transportation by oxygen-saturated perfluorochemical, can prolong the 8 hour time limit by an as yet undefined extent [7], and should allow to cut shipping costs (airliner vs chartered jet, road vs air). The two-layer method can prolong preservation time and salvage pancreata with prolonged cold ischemia times or harvested from marginal donors, allowing the recovery of higher numbers of islets with improved viability and function, and thus increasing the rate of transplantability of islet preparations [7,15,16].

Islets should be shipped in CMRL-based endotoxin-and xenoprotein-free culture medium. If islets are shipped in gas-permeable culture bags, room temperature (22 ~ 26℃) is preferred, since this is the optimal culture temperature for islets[17]. If islets are shipped in air-tight containers, such as transfer bags or syringes, cold storage (4℃) or room temperature are equally acceptable. We have observed that 8 hours of islet storage at 4℃ or room temperature in closed syringes had no impact on islet cell viability, apoptosis or in vitro function, whereas these parameters were altered after storage at 37℃ (Zeender et al, unpublished observation).

Quality control/quality assurance tests should be done before shipment and repeated after reception prior to transplantation. These tests include viability assessment (by staining with fluorescein diacetate and propidium iodide for example), microbiological sterility assessment (Gram stain, bacterial and fungal cultures, endotoxin detection) and insulin release in response to in vitro glucose challenge in static incubation assays [12]. In the interest of time, only results of Gram staining and viability are required before releasing islets for transplantation.

To study the impact of the duration of islet transportation, we have analyzed the in vivo function of 27 islet preparations transplanted into 21 patients in 5 transplantation centers within the GRAGIL network including Geneva, according to shipping time from the isolation laboratory in Geneva to the transplantation center. Islet preparations were classified into 1 of 3 groups: Group 1, shipping time < 1 hour; Group 2, shipping time = 2 hours; Group 3, shipping time > 5hours. C-peptide levels, HbA1c and insulin requirements were analyzed and compared at 1 month post-transplant. No detrimental effect of prolonged shipping time could be demonstrated as shown on Table 1 [18].

Table1 In vivo islet function after clinical transplantation according to shipping time in the GRAGIL network

Parameters	Group1	Group2	Group3
Number of patients	5	10	6
HbA1c pre-Tx[%]	8.2	9.0	8.9
HbA1c 1 month post-Tx[%]	7.0	7.0	6.9
Insulin needs pre-Tx[U/day]	43	45	45
Insulin needs 1 month post-Tx[U/day]	41	31	21
C-peptide > 0.5 ng/ml at 1 month	5/5	9/10	5/6
Insulin independence at 1 month	1/5	3/10	2/6
IEQ/kg transplanted	9,747	9,595	8,240

In Group 1, islet shipping time was < 1 hour, in Group 2 approximately 2 hours, and in Group 3 > 5 hours. Mean values or proportion of patients are shown. Adapted from [16].

Multicenter network organization: the GRAGIL example

The GRAGIL network is a Swiss-French collaborative effort that was initiated in 1997. The network was initially composed of 5 University centers, namely Besanon, Grenoble, Lyons, and Strasbourg in France, and Geneva in Switzerland [4]. Since the start of the project, 3 additional centers in

France (Dijon, Marseilles, and Nancy) have elected to join. Islet isolation procedures are performed for the network in a core islet processing facility located at the Cell Isolation and Transplantation Center of the University of Geneva, where clinical islet transplantation has been performed since 1992 [19]. The first patient was transplanted in 1999, and since then, 27 islet preparations have been shipped from Switzerland to the various centers in France for transplantation into 20 patients.

The 2 populations of patients with type 1 diabetes considered for islet transplantation within the network are patients already transplanted with a kidney, with a stable graft function (GRAGIL 1 protocols), or non-uremic patients with brittle forms of type 1 diabetes (GRAGIL 2 protocol). All pre-transplant workup is done locally by each participating center. Candidates are then discussed and inscribed on a common waiting list in multicenter conferences held on a regular basis. The waiting list is centrally administered at the University of Geneva, where a serum library of all patients is held. Sera from patients on the waiting list are sent to Geneva every 3 months for prospective crossmatches. At the end of each successful islet isolation procedure, islets are attributed to the best patient on the waiting list according to the following criteria: ABO compatibility, need for a second islet infusion, time on the waiting list, absence of HLA antigen repeats with respect to transplanted kidney, HLA matching, and body weight (a minimum of 5,000 IEQ/kg is required for each transplant). A crossmatch is always done prior to transplantation on 2 ~ 3 prospective recipients in order to have a backup in case of a positive crossmatch. Islets are conditioned in syringes and shipped by ambulance in transplantation medium at 4℃, for immediate transplantation upon arrival, as described previously [4].

This collaboration is approved by the French and Swiss organ sharing systems and regulatory authorities, .and transplantation/immunosuppression protocols have passed the IRBs of Grenoble and Geneva University Hospitals.

Another ambitious multicenter project was recently initiated in Scandinavia, after the initial success of the German-Swedish collaboration [11*]. In this Nordic Network, pancreata harvested throughout the 4 Scandinavian countries (Sweden, Norway, Denmark, and Finland) are shipped to a core islet processing facility located at the University of Uppsala, Sweden [20]. This network has been very active, and has already transplanted 16 recipients of islet-after-kidney (IAK) grafts since April 2001 (O. Korsgren, personal communication).

Pancreas exchange in the GRAGIL network

In the first 4 years of the GRAGIL collaboration (1999 ~ 2002), 191 pancreata were processed at the Cell Isolation and Transplantation Center in Geneva. As shown on Fig. 1A, the number of pancreata shipped from harvesting centers in France and proce-ssed in Geneva has been steadily on the increase, denoting a growing confidence in the functionality and efficiency of the network organization. In contrast, numbers of processed organs procured in Switzerland, where the program had been established for several years, remained stable. The success rate of islet isol-ation, and thus the number of transplanted islet pre-parations also increased over the first 4 years (Fig.1B). The distribution of islet preparations for transp-lantation has been very satisfactory in terms of allocation equity, since overall 52% of processed pancreata had been shipped from France, and 53.5% of islet preparations were transplanted in French centers.

Results of the GRAGIL clinical trials

The first GRAGIL clinical trial was initiated in 1999 for recipients of islet-after-kidney (IAK) grafts. The first 10 patients transplanted with an IS protocol associating cyclosporin microemulsion, mycophenolate mofetil and steroids, and anti-IL-2 receptor induction with basiliximab (GRAGIL 1A), have been previously reported[4]. Islet graft recipients received a mean of 9,000 IEQ/kg isolated from 1 or 2 pancreata. All patients had immediate graft function, as assessed by basal C-peptide levels > 0.5 ng/ml, but 5 (50%) gradually lost islet function 2 to 10 months after transplantation. In the 5 patients with an ongoing

functioning graft, HbA1c decreased from 8.6% (7.0 ~ 10.5%) pre-transplantation to 6.4% (5.8 ~ 7.0%) 1 year post-transplantation, indicating a marked improvement in blood glucose control. Two patients (20%) achieved sustained insulin indepen-dence. Unsurprisingly, they were the recipients of the 2 largest grafts in terms of islet mass per body weight, both well above the mark of 10,000 IEQ/kg[4].

In the immediate aftermath of the initial report of the Edmonton trial[1*], the GRAGIL group decided to implement a steroid-free IS regimen in recipients of IAK grafts (GRAGIL 1B). A novel protocol, inspired by the Edmonton protocol, was designed for this trial, associating low-dose cyclosporin microemulsion, the rapamycin analogue everolimus, and anti-IL-2R induction with basiliximab, in the absence of steroids. Cyclosporin was preferred over tacrolimus because of its lower islet toxicity [21]. Nine patients were transplanted with this protocol, all of them having graft function, as assessed by C-peptide positivity. Graft function was lost in 3 patients to app-arent acute rejection ($N = 2$) and recurrence of autoim munity ($N = 1$). One patient died 1 month post-transplant of pneumonitis of unclear causes. Six patients became insulin independent, but only one patient was still off insulin at 1 year post transplant [22*]. Reasons for this lack of long-term success could be related to islet shipment, the particular features of the IAK recipient population or the immunosup-pressive regimen, all 3 of which were significant variations from the Edmonton protocol. As discussed above and as demonstrated by the recent Miami/Houston experience, islet shipment does not seem to be detrimental to the success of islet transplantation. The patient population is indeed different, but islet transplantation in Geneva in IAK recipients, using the original Edmo-nton protocol has resulted in a high rate of insulin independence[23]. Worth mentioning, everolimus trough levels were consistently in the lower half (3~ 7 ng/ml) of the recommended therapeutic range (3 ~ 15 ng/ml), and it is likely that these patients were in fact underimmunosup-pressed. The everolimuscyclosporin association should still be viewed as a good combination, but guidelines for everolimus trough levels in a high everolimus-low cyclosporin regimen have to be refined. In order to pinpoint the element most likely to be responsible for this relative lack of success, the GRAGIL group has started a new study scheduled for 5 patients, who will receive islet transplant alone grafts (ITA) with the original Edmonton regimen.

Conclusion

The Edmonton success has generated a lot of enthusiasm and incited several centers to offer the therapeutic option of islet transplantation to their patients with type 1 diabetes. As efforts to standardize the procedure are still under way, under the leadership of the most experienced centers, we feel that the difficult process of islet isolation and purification should be maintained in a limited number of centers for the time being, in order to achieve optimal results. In this regard, the GRAGIL network allowed participating centers and their patients to benefit from islet transplantation, without the pros-pects of building a costly facility and facing a steep learning curve. Institutions willing to eventually harbour their own islet facility can also take advantage of a collaboration of this kind, by receiving advice from the core facility, and gaining experience in islet recipient management. This collaborative effort has been a mutual benefit as it has allowed to increase both the donor pool and the accessibility to islet transplantation for selected patients with type 1 diabetes in an extended population area.

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