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Pancreatic islets transplantation in the treatment of diabetes mellitus: present and future

Daniel Casanova

Servicio de Cirugía General y Digestiva, Hospital Universitario Marqués de Valdecilla, Universidad de Cantabria, Santander, Cantabria, Spain

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Diabetes treatment with insulin does no prevent the development of secondary complications. For this reason, treatments other than conventional ones are needed, which could bring about an "almost physiological" metabolic regulation. This can only be done by transplanting insulin producing tissue, such as vascularised pancreas transplantation, which is an already consolidated clinical procedure these days, or by islets transplantation, which is still a procedure in the clinical research phase. This has the same metabolic objectives as the vascularised transplant, but without the risks of major abdominal surgery, since the islets are implanted in the liver with minimal surgery or using interventionist radiology by means of a catheter. A clinical trial (Edmonton Protocol) was published in the year 2000, which improved the results after islet transplantation by obtaining normoglycaemia periods of more than 1 year in a consecutive patient series with type 1 diabetes and without using corticoids. This protocol has been endorsed in other centre in different trials. Although the initial results were good, the progress of these patients has shown that many islets transplantations do not manage to maintain insulin-independence indefinitely.

Presente y futuro del trasplante de islotes pancreáticos en el tratamiento de la diabetes mellitus

Palabras clave:
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R E S U M E N

El tratamiento de la diabetes mellitus con insulina no evita la aparición de complicaciones. Por ello son necesarios tratamientos alternativos al convencional que permitan una regulación metabólica "casi fisioloógica". Esto sólo puede realizarse mediante el trasplante de tejido productor de insulina: el trasplante de páncreas vascularizado, que hoy es un procedimiento clínico ya consolidado, o mediante el trasplante de islotes, que continúa siendo

E-mail address: danielcasanova@telefonica.net
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un procedimiento en fase de investigación clínica. Éste tiene los mismos objetivos metabólicos del trasplante vascularizado, pero sin los riesgos de una cirugía abdominal mayor, ya que los islotes son implantados en el hígado con mínima cirugía o mediante radiología intervencionista a través de un catéter. En 2000 se publicó un ensayo clínico (Protocolo de Edmonton) que mejoraba los resultados tras el trasplante insular obteniendo periodos de normoglucemia de más de 1 año en una serie consecutiva de pacientes con diabetes mellitus tipo 1 mediante un régimen sin corticoides. Ese protocolo ha sido refrendado en otros centros en diferentes ensayos. Aunque los resultados iniciales fueron buenos, la evolución de los pacientes a medio y largo plazo ha puesto de relieve que muchos de los islotes trasplantados no consiguen mantener por tiempo indefinido la independencia de la insulina

Type 1 diabetes mellitus (DM) is characterised by the selective destruction of the beta cells of the pancreas, which gives rise to an absolute deficit of insulin. Conventional treatment based on diet, insulin, exercise, and metabolic self-control is not sufficient to prevent the development of chronic complications over time, of which the most important are retinopathy, nephropathy, and neuropathy. It is known that current medical treatment of DM does not succeed in normalising the glycaemia 24 hours a day in most patients, as is demonstrated by glycaemic profiles or glycohaemoglobin values.[1]

The DCTT (Diabetes Control and Complications Trial) has shown that strict metabolic control of DM prevents microvascular complications in patients not suffering from these complications and delays the development of already-existing lesions. With a view to trying to achieve optimum metabolic control, diabetic patients make every effort to maintain their glycaemia near normal levels, but often undergo episodes of hypoglycaemia.[2] Furthermore, over time, some patients lose the initial symptoms associated with the perception of a reduction in the glycaemia, with the added risk of episodes of severe hypoglycaemia.[3,4]

All the above makes it advisable to develop alternative treatments to conventional ones, in which the homeostatic regulation of glycaemia is carried out in a continuous, effective manner.

Endocrine pancreas transplantation is the only method that fulfils these requirements since the secretion of insulin is produced in response to the metabolic demand. There are 2 ways of performing a pancreatic transplant: transplanting the whole organ or just the cells entrusted with producing insulin, that is, the islets of Langerhans. The first method is known as vascularised pancreas transplantation and the second, an islet transplant.[5]

The vascularised pancreas transplantation has long been subject to scientific discussion as to its efficacy and importance, and is now included in the range of services offered by hospitals of reference. Its results are comparable to or even better than those of other solid organ transplants. However, almost 90% of pancreas transplants are practised in combination with a kidney transplant, due to terminal kidney failure caused by the DM, meaning that it is applied at a late stage of the disease.[6]

If we assume that a transplantation of a functioning pancreas can affect secondary complications of DM through correcting the metabolism of carbohydrates, a crucial aspect would be applying this solution as early as possible. At this point, in which we would all be in agreement about the benefit of a restored metabolism of carbohydrates, there are certain controversies regarding its indication in preuremic patients. The risk/benefit balance of the transplant is not always perceived in the same way, and therefore the indications for an isolated transport are much more limited. The reason for this are the risk involved in the surgery, in addition to risks related to the immunosuppressant medication that is necessary during the patient's entire life.[7-11]

A pancreatic islet transplant has the same general objectives as a vascularised pancreas transplantation, since its mission is to eliminate the injections of insulin and dietary restrictions and, more importantly, affect the evolution of complications of the disease by normalising the carbohydrates metabolism.[12]

If we consider that endocrine cells only account for 2% of total cells in the pancreas, it is clear that the possibility of transplanting islets would resolve the problem of DM at an early stage, without the complications of a vascularised transplantation. In addition to this, the enormous disproportion between the number of organ donors and the number of potential recipients limits the solution to diabetes using the organ transplant method to a very small number of diabetic patients, and for this reason the use of insulin-producing cells in different modes could open up new possibilities for a greater number of patients.

As a result, the hypothesis of correcting DM by the transplantation of cells continues to be a reasonable objective, even though the technological and immunological difficulties involved must be considered, since:

- The lack of human cells could be offset by developing stem cells that produce insulin or beta cells from animals[13]
- It can be performed with minimally invasive techniques and even under local anaesthesia
- The fact that it is a cell transplant allows it to be applied in early stages of the disease
- The cells can be manipulated to reduce antigenicity and induce tolerance

- It is possible that in the future, there will be banks of islets from both human foetal and adult pancreatic glands and from the pancreas of animals. Therefore, theoretically, there may be an unlimited supply of tissue

Research into islet transplants is not new, since even before insulin was discovered, Williams practised a transplantation of fragments of sheep pancreas on a diabetic child.[14] In 1972, Ballinger et al[15] reported the possibility of isolating islets and reversing experimentally-induced diabetes by means of a transplantation in rodents.

In humans, the first experiments are those performed in 1974 by Sutherland et al[16] and Najarian et al[17] in Minneapolis, but the best results were obtained in 1980 on a series of patients undergoing an autologous islet transplant following a total pancreatectomy due to chronic pancreatitis.[18,19] When an attempt was made to perform this technique in the form of an allotransplant in diabetic patients, the results were very discreet, due to the technological limitation of the isolation protocol and the lack of uniformity in the way in which the resulting homogenised products are interpreted.

At the end of the eighties, Ricordi et al[20] improved a method previously developed at Washington University in St. Louis (USA). That method consisted of using a steel chamber inside which the pancreatic gland was placed. The chamber was connected to a closed circuit through which Hanks' collagenase solution was continually recirculated. The digestion of the pancreas was determined by obtaining periodic samples of the effluent and dying them with a vital colorant called ditizone.[21] The endocrinal cells were observed to have a red dye under the inverted light microscope and, therefore, the extent of digestion of the gland and the richness obtained in the endocrinal cells could be seen. Through these observations, the parameters for the quality controls during the different phases of the isolation process were established. Although there are many factors that affect the final result of an isolation operation, at present, these semi-automatic methods can be used to obtain between 600 000 and 800 000 non-purified islets from a human gland. This represents between 50% and 60% of the total insular mass.

The final calculation of the islets is evaluated in numerical terms in equivalents of islets (EI). During the second Congress on Pancreatic and Islet Transplants held in Minneapolis in 1989, this concept was standardised as a necessary vehicle of obtaining understanding in this field. We know that human pancreatic islets have an average size of 150 µm, with oscillations of between 50 and 400 µm.[22] As a result, the total number of isolated islets must be corrected so that the final result can be expressed in the terms mentioned above.

Based on these criteria and the standardisation of enzymatic digestion, thanks to the greater purification of collagenase by means of liberase, it was possible to develop protocols for isolating and transplanting in specialised centres and for obtaining data in an international register.[23-25]

Meanwhile, during the 1990s, research continued to obtain better results in human isolations, in developing other sources of tissue, in developing less toxic and better-tolerated immunosuppressant protocols and attempting to obtain immunological tolerance through cell manipulation.[26-31]

Between 1990 and 2000, 240 cases of autologous islet transplants were performed all over the world, according to the Islets Transplant Register, in 15 different institutions, of which 140 were well documented.[32] Forty-seven percent of these maintained the lack of dependence on insulin for periods of more than 1 year. When the number of islets implanted was reviewed, it was seen that in those receiving more than 300 000 EI, the percentage of insulin-dependent patients per year was 71%. The long-term feasibility of the insular function was demonstrated in some patients with more than 20 years of normoglycaemia following an autologous transplant as a result of a pancreatectomy due to chronic pancreatitis. In addition, the patients not succeeding in being insulin-independent maintained high glycaemia values but without suffering severe episodes of hypoglycaemia, taking into account that a total or almost total pancreatectomy had been performed on them.[33,34]

Encouraged by these results, more and more groups performed clinical trials with islet allotransplants, in some cases, demonstrating without doubt the survival of the total or partial insular functions for 2-3 years in centres such as Minneapolis, Miami, Milan, etc. The International Register recorded 355 cases, between 1990 and 2000, of which at least 237 had a follow-up period of more than 1 year. Among these, 41% had islets that continued to function 1 year after the transplant, but only 11% were insulin-independent. When the results were analysed 3 years after the transplant in a group of 235 patients, 94% were alive and 24% of them showed some sign of insular function, although only 4% were insulin-independent.[35]

As regards the indication, in this group of patients, corresponding to what is known as the pre-Edmonton era, the majority (55%) received a simultaneous islet-kidney transplantation (SIK), 37% received an islet transplant following islet after kidney transplantation (IAK), and only 4% received only an islet transplant (ITA). In practically all the cases, the immunosuppressant regime was based on the usual protocols used for kidney transplants.

In 2000, Shapiro et al,[36] from the University of Alberta in Edmonton, Canada, published excellent results for a consecutive series of 7 patients who were insulin-independent following an islet transplant using a protocol (known as the Edmonton protocol) based on an immunosuppressant regime without corticoids and with a combination of tacrolimus and sirolimus with daclizumab (a monoclonal antibody that fights the interleukin 2 receptors).

The advantage of this type of immunosuppression as compared to other protocols used previously is the effective combination of different drugs in small but sufficient doses for controlling rejection with minimal toxicity for the islets, thereby permitting their long-term survival. Other important factors in that study were the reduction of the cold ischaemia period following the removal of the pancreas, the isolation of islets using culture methods exempt from animal proteins and transplanting at least 9000 EI/kg, which in most cases makes it obligatory to use 2 donors for 1 transplant. The type of diabetic patient selected for that initial trial had to meet the following requirements: DM1 for more than 5 years and peptide C values after stimulation of

<0.48 ng/mL; difficult control of the DM despite strict control with insulin; episodes of severe hypoglycaemia due to labile DM demonstrated in a certified form and with an alteration in the quality of life, absence of coronary disease and not having previously undergone a transplant.

In selecting the patients, it is obvious that quality of life is a decisive factor and the risk of unstable DM was evaluated with respect to the effects of the immunosuppressant medication itself. In the initial trial, the islet transplant was performed on 7 consecutive patients and in all of these, insulin-independency was achieved for a period of more than 1 year. The same group extended the series to 15 patients with 80% of these being insulin-independent for more than 1 year. In the other patients who could not achieve insulin-independence, it was possible to interpret the residual insular function by determining peptide C and glycohaemoglobin.[37]

The success of the first experiments with the Edmonton protocol led to a renewed enthusiasm for islet transplants. In many centres from Europe and the USA, and also in Spanish centres, coordinated research projects were developed with the aim of optimising the results in this field and starting up new clinical trials.[38-40]

The immediate objective of the transplant is to reach normal glycaemia levels but in the long term, the most important objective is the influence on secondary complications. It is clear that for this purpose, the transplant must be performed before severe complications arise. For evaluating the organic changes, periods of normalisation of at least 5 and 10 years are necessary.

Although the patients in the initial series were characterised by having extremely labile DM with the risk of hypoglycaemia and without kidney failure, during the second phase some centres developed trials that combined islet and kidney transplants in uremic patients. However, the logistical difficulties in performing and coordinating this double transplant favoured the practice of an islet transplant in patients previously undergoing functioning kidney transplants. In these cases it was necessary to modify the immunosuppressant therapy to suspend the corticoids and add tacrolimus and sirolimus at low doses to prevent toxicity in the islets; furthermore, there was to be no rejection during the kidney transplant.[41]

Transplant complications may be severe in some cases, basically in relation to the technique of implanting the islets. The most frequent are bleeding and partial thrombosis of the vena porta.[42] Furthermore, the effects of the immunosupressive therapy are similar to other transplants: bucal ulcerations, anaemia, diarrhoea, kidney failure, risk of lymphoproliferative disorders, infection by cytomegalovirus, etc.

The University of Alberta, together with another 8 groups from the universities of Minnesota, Miami, Seattle, Washington, Harvard, Giessen, Milan, and Geneva, performed a multicentre clinical trial to evaluate the results at long term, the efficacy of the immunosuppression and the effects on complications.[43]

Initially, 2000 patients were evaluated for the study, but only 149 were selected (7%), of whom only 36 met the protocol requirements.

The result of this study, which included 36 patients with DM1, was published in the New England Journal of Medicine in September 2006.[44] Of these, 44% (16 cases) were insulin-independent after 1 year, 28% (10 cases) had a partial function, and 28% (10 cases) had lost the function during the first year. Of the 16 patients who were insulin-independent, only 5 (31%) continued without insulin after the second year. The conclusions of the international study were that the islet transplant is able to restore the endogenous production of insulin with a high percentage of independence, however, it is impossible to maintain that state in the long term, although important benefits are maintained due to the partial insular function, such as preventing severe episodes of hypoglycaemia and improving glycohaemoglobinaemia.

These poor long-term results have meant yet another setback in evaluating this therapeutic alternative which, from the technical point of view, has solved the problems of isolation and viability (autologous islet transplant with more than 20 years of insulin-independence), since from the immunological perspective this transplant expires within a short time. For this reason, the initial enthusiasm has given way to a new period of reflection and analysis of the results, with the aim of solving or improving some of the problems encountered. These include:

- Obtaining new sources of insulin-producing cells in order to have an unlimited number of islets: xenotrasplants, marginal donors, donors whose hearts have stopped, genetic engineering for the purpose of making some cells produce insulin, transformation of stem cells or pancreatic ductal cells in islets[45,46]
- Preserving the pancreatic gland before isolation: double-layer method with perfluoride-carbon[47-50]
- Optimising the method for isolation: liberase, recombinant collagenase, modification of the enzymatic digestion phases (the Minneapolis group performed a series of 15 transplants with islets from one donor, of whom 13 were insulin-independent)[51-53]
- Purifying the islets: optimising the density gradients method
- Preserving the islets themselves before implanting them in the adequate recipient: preserving in culture media, preserving in cold conditions, cryopreservation[54,55]
- Controlling the inflammatory reaction in the implant site: use of a factor to inhibit tumoral necrosis such as infliximab, blocking of the enzyme that induces the NO[56-58]
- Developing new immunosuppressant combinations to permit long-term viability and above all, induce immunitary tolerance: substituting tacrolimus by non-diabetogenous immunosuppressant agents, but which maintain a synergy with sirolimus, deoxyspergualine, the possibility of halting the autogenous and allogenous immuno-responses through anti-CD3 and hOKT3-gamma-1 antibodies, blocking of the co-stimulating signal of the T cells, encapsulation of the islets, etc[59-61]

In the USA, at present, the NIH (National Institute of Health) finances 10 cellular processing and islet transplant centres

that isolate cells for their own patients and for centres linked to the same protocol, to which they send the homogenised cells prepared for transplanting. In Europe the Groupe Rhin-Rhône-Alpes-Geneve pour la Transplantation d'Ilots de Langerhans (GRAGIL, Rhine-Rhône-Alpes-Geneva Group for the transplantation of islets of Langerhans) consortium is comprised of a group of several universities from France and Switzerland based in Geneva. This institution carried out important processing and transplanting activities.[62-64]

In Spain, as part of the Research Networks Programme, the Carlos III Health Institute promoted the creation of the Red Española de Trasplante de Islotes Pancreáticos (RETIP, Spanish Pancreatic Islets Transplant Network) which was initially constituted by 5 centres. This group has promoted collaborative projects for putting into practice a series of clinical trials and attempting to reproduce the Edmonton protocol in our environment.[65]

However, although the intentions are good, and this policy is aimed at maintaining our research in this field at a reasonable level, the current legislation on new medicines, which this procedure is considered to be, means that from an operative standpoint, practically none of the laboratories in Spain fulfils those legal requirements. In the United States, genetic therapy and islet transplants are considered as forming part of clinical experimentation and are regulated by the FDA. Each preparation used for a transplant must certify the origin of the samples, their content, the circuit followed, the existing distribution, the content in impurities and the different tests and quality controls of the existing manufactured product. As a result, to approve a laboratory that engages in the processing and distribution of human cells, an infrastructure must exist that goes beyond the good intentions and know-how of some researchers, since it is a difficult challenge to comply with the "normal" standards of research existing today.[66]

In 2003, through its Biological Responses Committee, the FDA described what is today considered a standard for evaluating the response of an islet transplant. It is divided into 4 sections: insulin-independence, percentage of insulin needs, hypoglycaemia episodes, and quality of life[67,68]:

- Insulin-independence: it is considered that this is achieved when the normalisation of the carbohydrates metabolism is obtained without the need to use exogenous insulin injections. The main criteria of comparison are the patients maintaining that independence every year
- The percentage of insulin usage describes the partial success of the insular transplant measured by the percentage of reduction in insulin in relation to the time previous to the transplant
- The hypoglycaemia episodes also describe the disappearance of this severe metabolic alteration due to the protective function of the functioning islets which, although they do not achieve normoglycaemia, maintain that metabolic safety margin
- Quality of life: this refers to the different studies that have succeeded in measuring the repercussion in patients of eliminating the hyperglycaemia and hypoglycaemia episodes, the effect of the transplant on eliminating dietary restrictions, the effect of continual self-monitoring of glycaemia, etc. Those studies are based on Health Utilities Index, SF-36, Inmunosuppressant Quality of Life (QQL) Survey and Hypoglycemia Fear Survey[69-71]

In the United States, other 35 centres have asked to process and transplant islets, and even the National Institute of Diabetes has been entrusted with Medicare regarding possible beneficiaries of these new therapies. Despite all this, the high cost of the current procedure, the legal difficulties and the complex administrative requirements, in addition to the difficulty in the curve for learning the technique and the existing quality controls mean that very few centres can perform the process with guarantees. However, it has become easier to detect the association of satellite transplant centres in collaboration with the processing centre, with the initial sending of pancreatic glands from donors, for the processed islets to be returned and prepared for implanting in selected diabetic recipients.[72,73]

What is the future of islet transplants? The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) set up the Collaborative Islet Transplant Registry (CITR) with the following objectives:

- To develop and implement a method for measuring all the standards and results following an islet transplant
- To develop a database containing all the information on patients undergoing transplants in the United States and Canada. European cases can be included through an addendum with the Juvenile Diabetes Research Foundation
- To increase the safety of islet transplants through the information on all the adverse effects in all the patients
- To perform a scientific analysis, emphasising that all the islet transplant aspects must be protocolised in order to increase their safety; obtain information on each and every one of the transplants and re-transplants performed; record information on the donors, the isolation characteristics and the processing of the samples, information on the recipients, and the protocol used[74]
- To publish all the results, inform the institutions involved and the scientific community about them and to stimulate prospective studies in this field

Final thoughts

It is clear that islet transplants have reached a success level that was unimaginable a few years ago, but which is still insufficient in proportion to the expectations of researchers and especially, diabetic patients.

To understand the significance of the insulin-independence percentages, we should compare them against what occurred at the beginning of the nineties when that percentage in insular transplants was practically non-existent and less than 50% after 3 years in vascularised transplants.

We believe that, as occurred with vascularised transplants at the end of the nineties, through relentless development

resulting from refining the technique and controlling rejection, it is foreseeable that something similar will occur with islet transplants – but at a different pace.

Meanwhile, the recommendations for Spain, as discussed in the conference of consensus between the NTO (National Transplant Organisation) and Scientific Societies regarding pancreatic and islet transplants, it will be necessary to set up one centre for every 10 million people, based on which it would be sufficient to have 4 or 5 specialist laboratories in Spain. All other hospitals interested could subscribe as collaborating centres for sending pancreatic glands from donors that are not used for vascularised transplants. We firmly believe that this policy is the right one and will enable us to interpret and quickly apply the new developments taking place at this time. The comings and goings of islet transplants make up the sketch of a future configuration. The development of stem cells and xenotransplants are part of a chain that is in the process of being developed. The fight against DM requires more time, more resources and more imagination.

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