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This special issue has been coordinated by V Lassmann-Vague and PJ Guillausseau

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Paris, 10 Novembre 2006.

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Editorial

J Girard (President of the ALFEDIAM)

Every year in January for more than twenty years, in a small village in the Austrian Alps, a meeting of a EASD study group is held to discuss innovative treatments of diabetes. This meeting brings together clinicians and basic research scientists interested in transplantation of the pancreas, islets, or β-cell and specialists in the mechanical artificial pancreas from all over Europe and sometimes beyond. In advance to the international congress, this group consistently offers stimulating and profitable exchanges. Tyrol is not easy to access and only the most determined attend this meeting as an intellectual pilgrimage. This sparked the idea of assembling those interested in these subjects in France during the ALFEDIAM's thematic day. French clinical and basic research on innovating treatment of diabetes is highly developed, as shown by the islet transplantation network GRAGIL, the implanted pumps network EVADIAC, and centers such as Strasbourg, Lille and Montpellier where patients have access to both types of treatment. Last December, the ALFEDIAM's Board decided that this thematic day would concentrate on β-cell. The day's program was outlined by a group of ALFEDIAM members: Hélène Hanaire-Broutin, Véronique Lassmann-Vague, Pierre-Yves Benhamou, Christian Boitard, and Michel Pinget. It balances both basic research and clinical research, following the tradition of this thematic day. The title of this meeting will be "Every aspects of β-cell". We thank these colleagues for having actively participated in elaborating this high-quality scientific program. The president of the ALFEDIAM extends particular thanks to Véronique Lassmann-Vague, who has contributed by considerable time and effort to coordinating the program.

We also wish to thank the company Novo-Nordisk which has accepted to provide the financial support necessary for this meeting. Our thanks also go to Catherine Cottenceau, the ALFEDIAM secretary, for her availability, efficiency, and her moral support to the organizers.

Véronique Lassmann-Vague and Pierre-Jean Guillausseau (Editor-in-chief) have succeeded in putting together 8 of these 12 papers in a special issue of Diabetes and Metabolism, which we are pleased to distribute during the meeting. We hope that the ALFEDIAM 2006 thematic day will meet with success and provide the most recent data on the innovative treatments for diabetes.

Artificial β-cell: clinical experience toward an implantable closed-loop insulin delivery system

E Renard[1], G Costalat[1], H Chevassus[2], J Bringer[1]

SUMMARY

Aim: Restoration of long-term normal blood glucose control in diabetic patients supports the elaboration of an artificial beta cell. The possibility of implantation of the three crucial components of such a system (insulin delivery device, glucose sensor and controller) is analyzed.
Methods: The Long-Term Sensor System® project, aiming at a fully implantable artificial beta cell, assessed the feasibility of glucose control by the combined implantation of a pump for peritoneal insulin delivery and a central intravenous glucose sensor close to the right atrium, connected via a subcutaneous lead. It was initiated in 10 Type 1 diabetic patients in our clinic from 2000. Data obtained during this experience are reviewed and confronted to reported closed-loop trials using other approaches.
Results: No significant complication related to prolonged implantation of intravenous sensors occurred and the combined implants were well tolerated. Glucose measurement by the intravenous sensors correlated well with meter values (r = 0.83-0.93, with a mean absolute deviation of 16.5%) and accuracy has been sustained for an average duration of 9 months. Uploading of pump electronics by algorithms designed for closed-loop insulin delivery allowed in-patient 48 hour-trials aiming at automated glucose control. Glucose control was similar to that reported by investigations combining subcutaneous sensors to wearable pumps for subcutaneous insulin infusion. The benefits of more physiological insulin kinetics due to intra-peritoneal delivery have been hampered by the slow response time of intravenous sensors.
Conclusion: Although the concept of a fully implantable artificial beta cell has been validated as feasible, the limited performance in achieving glucose control requests improvements in the sensor structure to increase its longevity and decrease sensor delay.

Key-words: Type 1 diabetes mellitus • Glucose control • Artificial pancreas • Glucose sensor • Algorithm • Review.

RÉSUMÉ

Cellule bêta artificielle : expérience clinique en vue d'un système implantable pour l'administration d'insuline en boucle fermée.

But : La restauration d'un contrôle glycémique normal à long terme chez les sujets diabétiques est à l'origine de l'élaboration d'une cellule bêta artificielle. La possibilité d'implantation des trois composants essentiels d'un tel système (dispositif d'administration d'insuline, capteur de glucose et module de contrôle) est analysée.
Méthodes : Le projet Long-Term Sensor System®, visant une cellule bêta artificielle complètement implantée, a évalué la faisabilité du contrôle glycémique par l'implantation combinée d'une pompe pour la perfusion intrapéritonéale d'insuline et d'un capteur de glucose intraveineux central à proximité de l'oreillette droite, reliés par un câble sous-cutané. Il a été commencé chez 10 diabétiques de type 1 dans notre clinique à partir de 2000. Les données issues de cette expérience font l'objet d'une revue et d'une confrontation avec les essais rapportés de fonctionnement en boucle fermée utilisant d'autres approches.
Résultats : Aucune complication significative liée à l'implantation prolongée des capteurs intraveineux n'est survenue et les implants combinés ont été bien tolérés. Les mesures de glucose par les capteurs intraveineux étaient bien corrélées avec les valeurs des glucomètres (r = 0,83-0,93, avec une déviation moyenne absolue de 16,5%) et l'exactitude a été maintenue sur une durée moyenne de 9 mois. La mise en place dans l'électronique des pompes d'algorithmes destinés à l'administration d'insuline en boucle fermée a permis des essais de 48 heures chez des sujets hospitalisés visant un contrôle glycémique automatisé. Le contrôle glycémique était similaire à celui rapporté par des recherches combinant des capteurs de glucose sous-cutanés à des pompes portables pour la perfusion sous-cutanée d'insuline. Les bénéfices d'une cinétique de l'insuline plus physiologique due à l'administration intrapéritonéale ont été amoindris par le temps de réponse lent des capteurs intraveineux.
Conclusion: Bien que le concept d'une cellule bêta artificielle complètement implantée ait été validé comme faisable, la réussite limitée dans l'obtention du contrôle glycémique requiert des améliorations dans la structure du capteur pour augmenter sa longévité et diminuer le délai de réponse du capteur.

Mots-clés : Diabète de type 1 • Contrôle glycémique • Pancréas artificiel • Capteur de glucose • Algorithme • Revue générale.

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A tight relationship has been established between long-term blood glucose control and the development of microangiopathic, neuropathic and cardiovascular complications in type 1 diabetes mellitus [1, 2]. Consequently, looking for sustained normoglycaemia is nowadays the ultimate goal of diabetes therapy. Although insulin analogues, insulin pumps and reinforced education reduce blood glucose variability and may improve HbA_{1c} levels, restoration of stable tight blood glucose control close to normal cannot be reached in most type 1 diabetic patients. Limited reproducibility of insulin action for a same dose, persistent insulin action while blood glucose changes and lack of modulation of insulin delivery according to the variations of glucose needs, contribute to the present failure. To face these challenges, the development of an artificial endocrine pancreas has been expected for almost 30 years [3, 4].

To achieve an artificial endocrine pancreas depends on the availability of three crucial components: 1) a safe and reliable device that delivers insulin continuously with a quick reactivity to change, 2) an accurate real-time continuous glucose monitoring system, 3) a control program to adapt insulin delivery according to blood glucose at all times [5, 6]. Historically, this combination has been made available by the development in the 1970s of the bedside external artificial pancreas, *e.g.* Biostator® [7]. In this model, an intravenous (IV) infusion of insulin from a peristaltic pump is modulated thanks to a continuous blood glucose assessment using glucose-oxidase, by following algorithms that define insulin delivery according to glucose variations. The whole system is however bulky, and requires an almost constant human assistance. Of note, improvement of the algorithms has requested a large amount of work to allow post-meal glucose control while avoiding hyperinsulinemia that induces secondary hypoglycaemia [8]. Although still used for physiological investigations, this system cannot fulfil the objective of replacement of insulin secretion as expected by the diabetic patient for daily life.

During these last years, more sophisticated insulin delivery systems that better mimic physiology and reasonably accurate glucose sensing devices have been developed that revitalized the feasibility of a closed-loop insulin delivery [9]. Short-term trials have been performed using two different approaches: 1) a subcutaneous (SC) insulin infusion combined to a continuous measurement of SC interstitial glucose, 2) an intra-peritoneal (IP) insulin infusion combined to a continuous measurement of venous glucose. Used algorithms followed two main models: 1) one that aims at reproducing the physiological characteristics of insulin secretion, including proportional, integral and derivative components, 2) a "predictive control" model based upon observed relationships between blood glucose and plasma insulin variations. The present paper reviews the data that may support the development of an implantable artificial beta cell.

Trials with SC insulin infusion and SC glucose sensing

The first historical trials of a wearable artificial endocrine pancreas in dogs and then in humans were reported with enthusiasm by Shishiri et al. from 1975 [10-12]. Regular, and later lispro, insulin was infused SC from an external pump and glucose sensing was done by using a needle-type enzymatic sensor implanted in skin. The algorithm took into account the difference between current estimated blood glucose and aimed normal target (proportional component) and the variation of estimated blood glucose upon time (derivative component), but did not consider the time needed for current blood glucose to reach target (integral component) [8]. Reported data from closed-loop trials using regular insulin in five patients taking three meals for 24 hours mentioned a control of blood glucose between 2.7 ± 0.3 and 12.5 ± 1.0 mmol/l with some hypoglycaemia, and similar trials with lispro insulin allowed near-normal control with no hypoglycaemia [11, 12]. However, these results could not be reproduced by other investigators, suggesting that recruited subjects had been specifically selected from very similar characteristics of insulin dependence and sensitivity, and that Japanese food provided at meals induced very limited glucose excursions [9].

During recent years, four projects have been reported, all using lispro insulin infusion and interstitial glucose sensing either using microdialysis or a "needle-type" sensor [13-16]. Of note, in three of these projects, only partial closed-loop was achieved since meals were preceded by a handheld programmed bolus calculated from pre-meal blood glucose level and carbohydrate component of meal [13-15]. Enzymatic sensors using microdialysis were actually used in two projects [14, 15], while in another one glucose was estimated by simulation due to the lack of availability of the initially expected glucose sensor [13]. In this latter project, called ADICOL (Advanced Insulin Infusion using a Control Loop), the investigators focused on designing specific algorithms based on a "predictive control" model [9]. The results obtained by these trials with meal announcement are presented in table I. The last reported project used a "needle-type" sensor derived from the CGMS® [16]. The physiological algorithm included proportional, integral and derivative components, whose parameters were modulated at meal-times to allow more aggressive insulin delivery. Six trials for 27.5 hours including four meals were performed during which blood glucose showed average pre-meal levels of 5.8 ± 1.2 mmol/l and average post-meal levels of 9.8 ± 1.6 mmol/l, with no hypoglycaemia.

Although promising, these trials with wearable models of artificial endocrine pancreas have shown some limitations. Basal glucose control showed the most interesting data with very few excursions, indicating that SC insulin infusion, although affected by a delay for action, was reliable and could be modulated safely by the interstitial glu-

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Table I
Reported trials of closed-loop* insulin delivery using subcutaneous glucose sensing and subcutaneous lispro insulin infusion in type 1 diabetic patients.

Number of cases	Duration (hours)	Number of meals	Sensor type	Algorithm	Glucose control	Reference
11	26.5	3	Simulation	Predictive Control	84% b/w 3.5 & 9.5 mmol/l	[13]
12	32	4	Microdialysis	Empirical	56% b/w 5.0 & 8.3 mmol/l	[15]
8	24	3	Microdialysis	Predictive Control	7.8 ± 0.7 mmol/l	[14]

* Handheld bolus for meals.

cose sensing. Mealtime glucose control was less effective since characterized by post-absorptive peaks and secondary lows. Although lispro insulin was used in the recent trials, its delay of action around 45 minutes may be involved in the early peaks, because poorly mimicking the first phase of insulin secretion. Moreover, persistent action of insulin while blood glucose decreases likely contributes to secondary trend toward hypoglycaemia. The main reason for this limited meal control seems however related to the interstitial glucose sensing when no pre-meal bolus is ordered. The well-known physiological delay between variations of blood and interstitial glucose [17] likely contributes to the post-meal peaks and valleys. This delay results in lower estimation of blood glucose when it increases and higher estimation when it decreases;hence insulin delivery is too weak to challenge early blood glucose peak but remains too aggressive when blood glucose secondarily decreases.

Beside these limitations in glucose control at meal times, the wearable models that were investigated raise the question of patient acceptance on medium- and long-term. The combination of an insulin pump and its catheter plus an implanted SC sensor or probe connected to a monitor may likely be considered as too cumbersome in daily life. The necessary catheter and sensor/probe changes every two to four days on one side, sensor initial calibration procedure and following recalibrations that need (multiple) daily SMBG on the other side, would also add to the limited system convenience.

Trials of closed-loop insulin delivery using IP insulin and IV sensing

In order to challenge the kinetic problems of SC insulin delivery and SC sensing as well as the constraints related to wearable devices, the concept of an implantable system based upon IP insulin delivery and direct IV glucose sensing has emerged. It has been materialized by the design of the Long-Term Sensor System® (LTSS) by Medical Research Group (MRG), a sister company of MiniMed Technologies (Sylmar, CA, USA), that both merged into MiniMed-Medtronic (Northridge, CA, USA) in 2002. The LTSS combines an implantable pump for IP insulin delivery and a central IV enzymatic sensor, connected via a SC lead that allows the transfer of sensor signal to the pumping unit (figure 1). The software that manages the algorithms can be uploaded in the pump electronics to allow automated insulin infusion according to measured blood glucose. This first model of implantable artificial beta cell has been investigated in diabetic dogs and then in diabetic patients from 2000, when the first of a series of ten LTSS has been implanted at Montpellier University Hospital [18].

Feasibility and performance of IP insulin delivery

The use of IP insulin delivery aims at reducing and stabilizing the time between insulin delivery and insulin action by bypassing the lag and the variability related to SC insulin

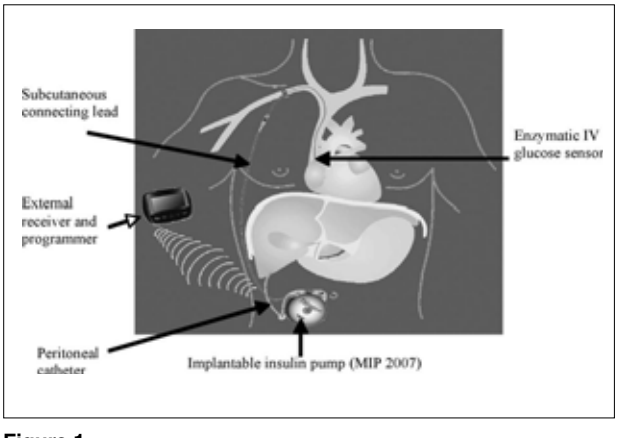

Figure 1
Scheme of human implantation of the Long-Term Sensor System® (LTSS, Medtronic-MiniMed), a prototype of implantable artificial beta-cell.

absorption. Some of our recent investigations showed that average time to peak of plasma insulin after an IP insulin bolus was 25 minutes, i.e. almost half the time measured after a SC insulin bolus [19]. Moreover pre-hepatic insulin delivery restores physiological positive porto-systemic plasma insulin gradient, with a lower peripheral insulinaemia than with SC delivery [20]. This unique insulin distribution is likely responsible for the lower incidence of hypoglycaemia associated with IP insulin infusion. The feasibility of IP insulin delivery from implanted devices has been demonstrated in clinical trials from the late 1980s [21-23]. Initial trials also investigated the feasibility of central IV insulin delivery from similar systems, but were suspended because of the occurrence of venous thromboses and frequent catheter obstructions [23]. These latter events were likely promoted by the pulsatile output of insulin from these pump models. Long-term use of implantable pumps for IP insulin infusion, mainly investigated by the French EVADIAC group, has been shown as safe and reliable [24]. Improvements of implantation procedures and of catheter components have allowed a dramatic reduction of complications at implantation site and of catheter obstructions, respectively, that were reported in early experiences [25]. However, gradual slowdowns of insulin infusion remain a current, although reversible, issue with these devices. The limited physical stability of the specific U-400 insulin preparation used in these pumps determinates these slowdowns by gradual insulin aggregation in the pumping mechanism. Periodic (mostly at 9-month intervals) rinsing by NaOH of the insulin pathway inside the pump can both prevent and fix this aggregation problem [26]. In spite of this remaining issue, IP insulin infusion from implantable pumps provides lower average HbA_{1c} levels, a significantly improved blood glucose stability and a dramatic decrease of severe hypoglycaemic events when compared to SC insulin infusion [24, 27, 28]. Implantable insulin pumps have been approved for clinical use in the European Union since 1995. Because of the more reproducible and physiological kinetics of IP insulin delivery, and of the benefits of being implantable and programmable, these devices represent a robust platform toward an artificial beta cell.

Clinical experience with IV glucose sensors

In order to develop a fully implantable closed-loop system, a specific Long Term Glucose Sensor® (LTGS) has been designed and firstly tested in diabetic dogs. This sensor is implanted by jugular or subclavian access so that the glucose sensing element is located in the central venous blood flow at the junction of vena cava superior and right atrium [18]. LTGS is an enzymatic sensor using glucose-oxidase, but its signal is generated by the oxygen consumption related to enzymatic activity in proportion to blood glucose level. Oxygen pressure at a nearby site with no glucose-oxidase is used as a reference to assess how much oxygen is consumed at the enzymatic site according to blood glucose level. The resulting signal intensity is proportional to current blood glucose level, and can be transmitted via a SC lead to the pump electronics. The initial calibration of LTGS is performed against SMBG measurements during the first days following IV implantation. Then sensor accuracy is checked once a week against a random SMBG value, and calibration may be renewed if needed. Analyses of LTGS accuracy against multiple daily SMBG values have shown an average mean absolute deviation of 16.5% and a correlation factor of 0.83 to 0.93 that can be sustained for many months with no need for recalibration [6]. Average longevity of sensor function has been found to reach around 9 months, with an extreme of 14 months. Sensor longevity appeared to be mainly depending on the mechanical resistance of sensor structure to venous blood flow. No thrombosis has been ever observed although some sensors have been implanted for almost two years. Low-dose aspirin that was taken by the patients may have prevented this eventuality. A drawback of the large glucose-oxidase pad at the sensing site to resist shearing forces created by the blood flow is an internal delay close to 3 minutes [8, 19]. Moreover, an average delay close to 20 minutes has been observed between blood glucose measurements and sensor values dispatched to the pumping unit [19]. This long delay may be explained by the difficult tuning of signal filters when using a sensor with significant transport lag [8].

Closed-loop trials using LTSS

A dozen of closed-loop trials have been performed at Montpellier University Hospital using the LTSS for periods of 48 hours including three daily meals with 40 to 70g of carbohydrates. Initial algorithm included basal, proportional and derivative components [29]. An integral component was added in the algorithm for the last four trials [19]. In some trials, insulin delivery before meals was programmed according to pre-meal blood glucose level and carbohydrate content of the meal [30]. Algorithm parameters were finally modulated during the last four trials to allow more aggressive insulin delivery at meal times [19]. Glucose control data during various trials are summarised in table II. The positive results obtained during these trials include a demonstration of the feasibility of closed-loop insulin delivery by using a fully implantable system using IP insulin delivery and IV glucose sensing, a close to normal glucose control at nighttime and between meals, and a tighter glucose control while using sensor signal to modulate insulin delivery than when adapting pump bolus and basal rates from SMBG data. Glucose control limitations were however observed at meal times that could be related to the too slow increase of plasma insulin levels when blood glucose peaks after food absorption. The sensor delays appeared as the main reason for this failure in maintaining blood glucose levels in near-normal range at meal intakes [8, 19]. These post-meal glucose peaks could however be prevented by handheld pre-meal insulin bolus or smoothened by algorithm changes to cover meal

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Table II
Trials of closed-loop insulin delivery using intravenous glucose sensing and intra-peritoneal insulin delivery [from ref. 6, 19, 30].

Number of cases	Duration (hours)	Number of meals	Algorithm	Glucose control (mmol/l) <4.4 4.4-6.6 6.6-13.3 >13.3
2	48	6	basal + proportional + derivative	6.4% 42.1% 49.6% 1.9%
1	24	3	basal + proportional + derivative + empirical meal bolus	0.0% 35.4% 64.6% 0.0%
4	48	6	proportional + integral + derivative + meal tuning	5.2% 22.5% 61.6% 10.7%

times [19, 30]. Of note, high levels of anti-insulin antibodies, which may be promoted by IP insulin delivery in some patients [31], significantly impair the feasibility of glucose control because of a "trapping effect"on insulin when plasma insulin rises and a "launching effect" of insulin when plasma insulin concentration decreases [32]. These undesired and uncontrollable variations of insulin availability make the algorithms poorly effective in glucose control with unexpected glucose highs and lows.

Prospective views about implantable systems for closed-loop insulin delivery

When analyzing data obtained with the implantable artificial beta cell approach so far, the IP route of insulin delivery from implantable devices has two advantages. The first one is the kinetics of IP insulin that allows a lower variability and a quicker insulin action than SC infusion. The second benefit is the implantable nature of the infusing system that provides a better satisfaction in terms of quality of life than wearable pumps connected to SC catheters [33]. Although initially dreaded, the IV sensor approach has resulted in no significant complication. However, the structure of IV sensors has failed in maintaining its integrity, and subsequently in allowing accurate glucose sensing, for more than 12 months in most cases. So, the invasiveness related to IV sensors would result more from the yearly replacements than from the IV implantation itself. Besides, IV sensing has shown unexpected limitations due to sensor delay that prevented timely insulin delivery at meal times. Hence closed-loop trials using IP insulin delivery and IV glucose sensing achieved almost similar glucose control as those using the SC-SC combination.

From the pilot experience with LTSS, two investigator conclusions can be drawn: 1) since a combination of IP insulin delivery and IV sensing is feasible, the concept of an implantable artificial beta cell is validated, 2) because efforts in improving sensor structure and longevity are needed, further clinical studies should wait for these improvements.

From patient point of view, until infusion and sensing systems using the SC approach will be further miniaturized and made more user-friendly (*e.g.*, calibration process), an "intelligent" implantable insulin pump would have a better long-term acceptance. However, yearly replacements of IV sensors would not be acceptable.

A straightforward strategy at present time could be to consider the feasibility of a combined model that would use the kinetic advantage of IP insulin delivery and the shorter response time of SC sensors. This intellectually-stimulating compromise looks like a feasible intermediate step toward an ultimate fully implantable artificial beta cell.

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Monitoring of the islet graft

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SUMMARY

The Edmonton trials have brought about a marked improvement in the short-term rate of success of islet transplantation with rates of insulin-independence of 80% at 1-year being reported by several institutions worldwide. Unfortunately, this rate consistently decreases to 10-15% by 5 years post-transplantation. Several mechanisms have been proposed to explain this apparent 'islet exhaustion', but are difficult to pinpoint in a given patient. Understanding the reasons for islet graft exhaustion and its kinetics is a prerequisite for the improvement of islet transplantation outcome. In this regard, efficient monitoring tools for the islet graft have been conspicuously lacking and are required to detect islet damage and diagnose its mechanisms in a timely fashion, so as to initiate salvage therapy such as antirejection treatment. Tools for the monitoring of the islet graft include follow-up of metabolic function but mostly indicate dysfunction when it is too late to take action. Progress is likely to arise in the fields of immune monitoring, molecular monitoring and islet imaging, notably thanks to magnetic resonance (MR) or positron emission tomography (PET) technologies.

Key-words: Islet of Langerhans transplantation • Monitoring • Imaging • Magnetic resonance • Positron emission tomography • Review.

RÉSUMÉ

Surveillance des greffons d'îlots de Langerhans

Les essais d'Edmonton ont amené une amélioration spectaculaire dans le taux de succès à court terme de la greffe d'îlots de Langerhans, avec des taux d'insulino-indépendance de 80% à 1 an rapportés par plusieurs institutions à travers le monde. Malheureusement, ce taux décroît de façon reproductible à 10-15% à 5 ans de la transplantation. Plusieurs mécanismes ont été proposés pour expliquer cet apparent "épuisement des îlots", mais il est difficile de déterminer avec précision lequel est responsable de la perte de fonction chez un patient donné. La compréhension des raisons de cet épuisement et de leur cinétique est une condition préalable pour l'amélioration des résultats à long terme de la greffe d'îlots. A ce propos, nous souffrons d'un manque criant de moyens de monitoring des greffons d'îlots, qui permettrait pourtant de détecter une atteinte des îlots greffés et d'en diagnostiquer la cause à temps pour pouvoir débuter une thérapie de sauvetage approprié, tel qu'un traitement anti-rejet par exemple. Parmi les outils de monitoring des greffons d'îlots figure le suivi métabolique, qui en général, indique les signes de dysfonction du greffon trop tardivement pour pouvoir réagir efficacement. De grands progrès sont attendus dans les domaines du monitoring immunologique, du monitoring moléculaire et de l'imagerie des îlots, notamment grâce aux techniques de résonance magnétique et de tomographie par émission de positrons.

Mots-clés : Transplantation d'îlots de Langerhans • Monitoring • Imagerie • Résonance magnétique • Tomographie par émission de positrons • Revue générale.

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Introduction

Islet of Langerhans transplantation has come to the forefront as one of the most promising approaches in the quest for a cure for type 1 diabetes. This is largely the result of the impact of the "Edmonton protocol", that allowed for the first time consistent achievement of insulin independence after islet transplantation, thanks to a steroid-free immunosuppressive combination, and to sequential islet infusions in order to increase the transplanted islet mass [1]. Unfortunately, figures of 80% insulin independence at 1 year have not been sustained, and the latest update of the Edmonton experience reported insulin independence rates of approximately 10-15% at 5 years, although graft function (C peptide positivity) was retained in a vast majority of patients [2]. Several phenomena are likely to be involved in late islet graft loss, such as allogeneic rejection [2, 3], recurrence of autoimmunity [3, 4], islet toxicity of the immunosuppressive (IS) drugs [5-8], lack of beta cell regeneration due to the antiproliferative properties of sirolimus, an IS drug on which the Edmonton protocol is based, or "exhaustion" of the islet graft [9].

These alleged mechanisms of islet graft loss are not mutually exclusive, and occur on a terrain of suboptimal beta cell functional reserve, as suggested by markedly decreased insulin responses to stimulation in islet transplant recipients as compared to controls [10]. The low engrafted islet mass is undoubtedly the major factor explaining the fact that reversal of diabetes with islets isolated from a single donor is very uncommon [11]. This is thought to arise as a result of: early islet loss during the isolation procedure or in the graft microenvironment within the liver, ischemia-reperfusion-like injury and non-specific inflammatory phenomena [12-17]. An acute inflammatory process that instantly destroys a large part of islets injected intraportally upon contact with blood was described recently, and is thought to be a major determinant of early islet graft loss [16, 17].

There is undoubtedly a lot of room for improvement in the long-term and short-term survivals of islet graft, but this will only be achieved when mechanisms of islet destruction have been better understood and characterized. In this regard, the whole field of islet transplantation suffers from a blatant lack of monitoring tools able to detect graft damage or decrease in graft mass or function in a timely manner. Therefore, techniques of islet graft monitoring that will be developed must address the three following aims: (i) understanding when islet grafts are damaged; (ii) understanding by which mechanism(s) islet grafts are damaged; (iii) detecting islet damage early enough to allow for appropriate intervention to salvage the graft.

Metabolic monitoring

Current clinical monitoring is based on metabolic islet function and utilises serum markers, in the basal and stimulated states [18]. These markers, recapitulated in table I, have been used for decades in the baseline assessment of the diabetic patient and need not be discussed in detail here. They are recorded at each visit and based on them, islet grafts can be classified as being fully (insulin-independence), partially (insulin required and detectable C-peptide) or not functioning (no detectable C-peptide). None of these markers are specific of the islet transplant situation and can be used in the assessment of any diabetic patient.

Table I
Metabolic monitoring of the islet graft.

Overall function	Plasma glucose
	Plasma insulin
	Plasma C-peptide
	HbA_{1c}, fructosamine
	Insulin requirement (U/kg/d)
	Secretory Unit of Islet Transplant Objects (SUITO)
	Beta-score
Glucose stability	Mean Amplitude of Glycaemic Excursions (MAGE)
	Lability Index (LI)
	Continuous Glucose Monitoring Systems (CGMS)
Stimulation tests	Arginine stimulation test
	Glucagon stimulation test
	Mixed meal tolerance test (MTT)
	Oral Glucose Tolerance Test (OGTT)
	IV Glucose Tolerance Test (IVGTT)
	Glucose-potentiated arginine stimulation test

Islet graft function

Measuring C-peptide levels is the simplest way of assessing islet function in a subject receiving exogenous insulin. Because C-peptide levels vary a lot according to blood glucose, they have hardly any quantitative value. Moreover, islet recipients have various degrees of impaired kidney function, which impacts on C-peptide excretion and prolongs its serum half-life.

The Secretory Unit of Islet Transplant Objects (SUITO) was recently developed. It computes both blood glucose and C-peptide and can be calculated with the following formula: (1500*fasting C-peptide [ng/dl]) / (fasting blood glucose [mg/dl]-63). The SUITO index allegedly represents accurately the percentage of a "normal" islet mass in a given subject but still needs validation [19]. However, none of these methods accurately quantifies islet function, because they do not take the quality of metabolic control into account.

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The Beta score was introduced to take all these parameters into account. It is based on fasting blood glucose, HbA_{1c}, daily insulin requirements, use of oral hypoglycaemic agent and stimulated C-peptide [20]. It rates islet graft function on a 0-8 scale, with 0 corresponding to total absence of function and 8 to perfect graft function (table II).

Metabolic stability

Monitoring can be refined by quantifying blood glucose instability [21]. The MAGE (Mean Amplitude of Glycaemic Excursions) index reflects blood glucose stability. It can be calculated from 14 consecutive blood glucose values taken over 48 hours at pre-defined time points, by calculating the arithmetic mean of blood glucose increases or decreases, that exceed one standard deviation of the blood glucose values measured during the study period. Control individuals have a MAGE index comprised between 1 and 3.3 mmol/l, while patients with unstable Type 1 diabetes can have values up to 15 mmol/l [22, 23]. The MAGE index is easy to calculate, can be repeated often and has been broadly used to assess metabolic stability in islet transplant recipients [1, 24, 25].

The Lability Index (LI) also reflects blood glucose stability. It was tested on a large group of islet transplant patients and was found to correlate better with the clinical assessment of lability than the MAGE index. However, it is a cumbersome index to calculate and requires several values measured over four weeks [26]. The following sum has to be computed for each one of four consecutive weeks: $\sum((Gluc_n - Gluc_{n+1})^2/(h_{n+1}-h_n))$, where "Gluc" (in mmol/l) is the n^{th} reading of the week taken at time h_n (rounded to the nearest hour). The LI can then be calculated as the mean value of this sum over the four weeks. Most patients with type 1 diabetes have a LI up to 400 $mM^2/h \cdot week$, and patients selected for islet transplantation for metabolic instability have a LI up to 700 $mM^2/h \cdot week$. To our knowledge, the LI has not been assessed in healthy volunteers.

Blood glucose stability and the occurrence of hypoglycaemia can be measured very accurately using the Continuous Glucose Monitoring System (CGMS). It requires the placement of a subcutaneous probe, which is removed at the end of the recording. The device measures capillary glucose levels continuously over a few consecutive days. Several groups have used this technology [27-29] for the monitoring of islet grafts.

Stimulation tests

While the tests described above provide a snapshot idea of islet function or glucose stability at a given time or period, they give no information on islet response to a standardized stimulus. Stimulation tests answer this question and can be classified in two groups, depending on whether they study islet response to a glucose challenge or to direct pharmacologic stimulation. All stimulation tests should be performed in the fasting state and after discontinuation of exogenous insulin for at least 12 hours, which makes them unpractical to perform in islet transplant recipients who are still on insulin.

The mixed-meal tolerance test (MTT) provides simple information about islet function. Blood glucose, C-peptide and insulin levels are measured prior to and 90 minutes after ingestion of a standardized meal, usually a commercial liquid caloric supplement. Differences in cross-border availability of these products make standardization of the meal only relative. Study subjects ingest a preparation containing 350-500 kcal and 50-65 g carbohydrates depending on product used. A normal response to the MTT in control subjects shows a stimulated C-peptide level of 1000 to 1500 pmol/l.

In the oral glucose tolerance test (OGTT), blood sugar is measured after ingestion of 75 g of oral glucose, with blood samples drawn at 30, 60, 90 and 120 min. The OGTT is the only metabolic stimulation test included in the American Diabetes Association (ADA) definition of impaired glucose tolerance and diabetes [30].

In the intravenous glucose tolerance test (IVGTT) 300 mg/kg body weight of 50% dextrose is perfused intravenously over 1 minute after two baseline samples (-10 and 0 minutes) for glucose, insulin and C-peptide were drawn. Sampling is then usually done at 3, 4, 5, 7, 10, 20, 30, 40 and 50 minutes after glucose infusion [31]. This test allows the calculation of the acute insulin response to glucose based on the mean of the insulin level at 3, 4 and 5 minutes minus the mean basal insulin level at -10 and 0 minutes. Glucose disposal rate (K_G) is calculated as the slope of the natural logarithm of

Table II
Beta score[1].

Score	2	1	0
Blood glucose [mmol/l]	≤ 5.5	5.6-6.9	≥ 7
HbA_{1c} [%]	≤ 6.1	6.2-6.9	≥ 7
Daily insulin [units/kg] or OHA[2]	none	0.01-0.24 and/or OHA	≥ 0.25
Stimulated C-peptide [nmol/l]	≥ 0.3	0.1-0.29	< 0.1

[1] Assessment of islet graft function, according to Ryan *et al* [20]
[2] OHA (oral hypoglycaemic agent)

the glucose values. It may reflect endocrine reserve as well as insulin resistance. KG values < -1.0 are considered normal. Areas under the curve (AUC) for insulin and C-peptide can also be calculated.

The arginine-stimulation test [32] is gaining high interest in the follow-up of islet transplant recipients, because it is easy to administer, devoid of significant side-effects and does not involve a carbohydrate challenge and ensuing hyperglycaemia. Serum insulin is usually measured –10, 0, 2, 3, 4, 5, 7 and 10 min from intravenous injection of 5 g arginine over 30 sec [25]. The AUC for insulin reflects islet mass. The acute insulin response (AIR) can be calculated as the mean of the three highest values between 2 and 5 minutes minus the mean of values at –10 and 0 minutes. In healthy volunteers, mean AUC was 183 ± 57 mU•min/l and mean AIR 31.5 ± 9.5 mU/l, values that are hardly achieved by recipients of successful islet transplants, indicating suboptimal engrafted islet mass [25]. We have recently reported that follow-up of the AIR in arginine stimulation tests was a good prognostic indicator of islet graft outcome and that a decline in AIR always occurred before reintroduction of exogenous insulin [33].

In many institutions, the arginine stimulation test has replaced the glucagon-stimulation test (insulin secretion after intravenous injection of 1 mg glucagon), which is linked to side-effects, such as nausea and vomiting and only provides semi-quantitative information about islet responsiveness in terms of insulin and C-peptide release in a stimulated condition [34].

The principal problem with metabolic tests (hyperglycaemia, loss of C-peptide, absence of response to stimulation, etc.) is that they are late markers of islet graft dysfunction, and generally appear when it is no longer possible to salvage a failing graft. For this reason, development of novel techniques of islet graft monitoring or identification of surrogate markers of rejection is of foremost importance.

Monitoring of rejection

Although it is far from certain that it is a significant cause of long-term islet graft loss, islets of Langerhans are prone to acute rejection. Chronic rejection of islet grafts has not been characterized and it is as yet unclear whether it could be a cause of progressive islet graft exhaustion and associated decrease of function. Therefore, it is obvious that islet of Langerhans transplants should be followed-up for rejection as closely as other organs. Unfortunately, there is currently a lack of efficient tools to monitor islet rejection, and intense research is taking place in order to develop accurate methods of diagnosis or identify surrogate markers of islet graft rejection.

Islet graft biopsy

In all solid organs, histological examination of the graft is the gold standard diagnostic test for acute rejection. However, a biopsy is an invasive procedure and complications may occur. Moreover, in the case of islet transplantation, the quantity of islets engrafted in the liver is very low. Considering that the total volume of 1 million islet equivalents is approximately 1.8 ml and that the volume of the liver corresponds to 1.5-2% of the total body weight (i.e. 1,000-1,500 ml in a 70 kg subject), a large islet graft will occupy 0.1-0.2% of the total liver volume. It is then easy to understand that percutaneous needle biopsies have low chances of sampling islets, unless multiple biopsies are performed. Therefore, liver biopsies have not entered clinical routine yet, although some centers have undergone such activity [9]. In order to have a more accessible site for biopsy, it has been proposed to transplant some of the islets in the forearm, as a sentinel graft [35]. Although the idea looks appealing, the method is limited by the fact that the implantation and the survival of islets are probably site-dependent, and islets implanted at two different sites are unlikely to behave in a similar fashion.

In the specific case of combined organ transplant, such as simultaneous islet-kidney transplantation, rejection is known to usually occur on both organs at the same time. Recipients of combined islet/kidney transplants will thus be treated whenever rejection is detected in the kidney.

Detection of humoral anti-HLA reactivity

Humoral responses against donor HLA molecules can be as deleterious to transplanted organs as cellular rejection [36]. There have been reports of the detection of circulating anti-HLA antibodies specifically directed against donor antigens and preceding the occurrence of islet graft failure [37-39]. Although these observations suggest that humoral responses might have played a role in the failure of the islet grafts, they do not provide absolute demonstration that humoral rejection indeed occurred. Nonetheless, detection of circulating anti-HLA antibodies at regular intervals during follow-up seems valuable and has entered the routine of islet transplantation programs [25]. Detection of anti-HLA antibodies can be done using several techniques with different sensitivities. The method of the panel-reactive antibodies (PRA) assesses anti-HLA reactivity by measuring the percentage of cells from a panel of blood donors against which the recipient's serum reacts using a complement-dependent cytotoxicity assay. The classic PRA method was improved with the introduction of the techniques of enzyme-linked immunosorbent assay (ELISA) and more recently of flow cytometry. Both methods utilize purified class I and class II HLA antigens as targets for the binding of anti-HLA antibodies from the patient's serum. HLA antigens are coated on the assay plate for the ELISA method or on polystyrene beads for flow cytometry. In comparison to the cytotoxicity PRA assay, these new methods are associated with higher sensitivity, especially for the detection of anti-class II antibodies, and allow determination of alloantibody specificity [40, 41].

Detection of cellular anti-HLA reactivity

The detection of cellular anti-HLA responses is more difficult and is not performed routinely, because it implies the

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realisation of labor-intensive and complex *in vitro* assays. The cytotoxic T-lymphocyte precursors (CTLp) and the helper T-lymphocyte precursors (HTLp) assays measure cytotoxicity and IL-2 or other cytokines production in limiting dilution assays, where decreasing numbers of recipient donor lymphocytes are incubated with a fixed number of irradiated donor-specific stimulator cells (or cells with any desired number of HLA matches or mismatches with respect to the donor and/or the recipient). Conflicting data have been reported on the usefulness of these assays in predicting solid organ rejection [42]. To our knowledge, the CTLp assay has been performed by one group on peripheral blood lymphocytes of islet transplant recipients, who were found to exhibit absent or low responses, except in one patient in whom strong responsiveness correlated with islet graft failure [43].

Tetramer technology has revolutionized the field of detection of antigen-specific T-cells. It consists of 4 biotinylated MHC molecules covalently linked together by streptavidin, thus increasing their affinity to the T-cell receptor during cognate interaction. Class I or class II HLA-peptide tetrameric complexes allow direct ex vivo visualization of antigen-specific CD8+ or CD4+ T-cells in straightforward, easy to perform assays [42, 44]. Tetramer technology has yet to be made available for the quantification of specific anti-HLA T-cells, but would be a welcome tool for the assessment of donor-specific cellular reactivity.

Finally, it should be briefly mentioned that immune monitoring is currently generating a lot of interest in the transplantation community. New methods for the *ex vivo* and in vitro measurement of immune events of clinical significance in a transplant recipient are being developed, with the aim to detect states not only of rejection, but also of tolerance (or rather low responsiveness) that might allow tapering of immunosuppression [42, 44]. Detection of lymphocyte gene transcripts is one method of immune monitoring currently under development and will be discussed below.

Monitoring of autoimmunity

Islet grafts are prone not only to destruction by allogeneic rejection, but also by recurrence of autoimmunity [3]. Recurrence of autoimmunity in transplanted islet tissue was clearly demonstrated by the observation of graft failure caused by insulitis in recipients of segmental pancreatic grafts from an identical twin. These patients had received no immunosuppression because there was no risk of rejection [45].

Autoantibodies

In islet transplant recipients, there is indirect evidence that autoimmunity participates to graft failure in spite of adequate conventional immunosuppression. This was first suggested when the Giessen group reported that islet graft failure occurred significantly earlier in patients testing positive for the presence of islet cell antibodies (ICA) or anti-GAD65 autoantibodies [46]. This observation was confirmed by the Milan group [47], who also reported that a rise in autoantibody titers in recipients of vascularised pancreas transplants was observed in a minority of patients (7%), but almost invariably followed by graft function failure [48]. Kinetics of autoantibody titers show great patient-to-patient variability and clear guidelines regarding action to be taken in the situation of a rise of autoantibody levels are still lacking. Nonetheless, it seems valuable to measure autoantibody titers in the follow-up of an islet transplant recipient, as a marker of autoimmunity reactivation. A marked rise in titers might prompt the investigator to perform a liver biopsy looking for insulitis or even to administer immunosuppression in the same way one would treat a bout of acute rejection.

Autoantibodies of interest comprise ICAs, anti-insulin autoantibodies (IAA), anti-glutamate decarboxylase 65 (GAD65) autoantibodies, and anti tyrosine phosphatase (IA-2) autoantibodies. Levels of ICAs are determined by indirect immunofluorescence on frozen sections of human pancreas, whereas IAAs, GAD65 and IA-2 antibodies are determined by radio-immunoassay.

Autoreactive T-cells

GAD65 and IA-2 are probably the major autoantigens in type 1 diabetes, and elicit autoantibodies that are the most specific markers of the disease. However, beta-cell destruction in type 1 diabetes is primarily, if not only, T-cell-mediated, and it is unlikely that autoantibodies are directly pathogenic [49]. Therefore it seems logical to attempt to monitor directly the presence of autoreactive T-cells in the peripheral blood of islet transplant recipients in order to detect recurrence of autoimmunity. The tetramer technology briefly described above has been used to characterize autoimmune responses in patients with Type 1 diabetes ant at-risk subjects [50]. HLA-DR tetramers containing a peptide corresponding to the immunodominant epitope GAD65(555-567) from human GAD65 were first used for that purpose, and CD4+ T-cells were detected by flow cytometry in the blood of all Type 1 diabetic patients, some at-risk subjects but no control subject [50]. Of great significance, is the recent report of the detection of CD8+ autoreactive T-cells in the peripheral blood of patients with type 1 diabetes, using HLA-A2 tetramers binding an insulin peptide (insB10-18) and the observation that their occurrence in islet transplant recipients was strongly associated with graft failure [51]. Autoreactive T-cell monitoring using the tetramer technology is undoubtedly going to be very shortly an important tool in the follow-up of islet transplant recipients.

Molecular monitoring

Insulin gene expression

We have recently reported the detection by reverse transcriptase-polymerase chain reaction (RT-PCR) of circulating insulin mRNA in the peripheral blood early after islet

transplantation, and proposed that this apparent release of beta cell material could be a reflection of early islet damage in the engraftment period, with ensuing release of beta cell material in the peripheral blood [52]. This was comforted by the observation that circulating insulin mRNA was detectable for a much longer time (up to 10 weeks) in patients on a steroid-containing immunosuppressive regimen known to be toxic to the islets than in patients on a steroid-free regimen (up to 2 weeks).

In a subsequent study, we tested the hypothesis that monitoring of circulating insulin mRNA could be a valuable tool for the prediction of injury to the islet graft, in an attempt to identify a surrogate marker of acute islet rejection or recurrence of autoimmunity. In contrast to the previous study, we used a real time quantitative RT-PCR with the aim to correlate the amplitude of the mRNA peaks with signs of islet damage, and we followed-up patients for up to 500 days [53]. In this second study, insulin mRNA was always detected immediately after islet transplantation, and the duration and amplitude of the primary insulin mRNA peak was not correlated to graft size or outcome. Subsequent peaks of insulin mRNA were sometimes detected and were associated with alterations of islet graft function (increase in the amount of injected exogenous insulin, decrease in C-peptide levels, increase in HbA_{1c}) when they were prolonged or of high amplitude. With appropriate cut-off values, positive and negative predictive values of 80% were obtained [53]. Signs of islet damage were observed on average 17 days after detection of the secondary peak, suggesting the usefulness of the assay for timely graft salvage. Interestingly, thanks to the use of quantitative RT-PCR, we were able to calculate that the average amount of insulin mRNA in the detected peaks was lower than the contents of one beta-cell, and that, in contrary to what we had previously stated, we were in all probability not detecting whole circulating beta-cells, but rather beta-cell material within phagocytes.

Our assay is in fact indicative of beta cell damage in general and is not specific for allorejection or recurrence of autoimmunity, two conditions that could be treated with a boost of immunosuppression. The fact that islets can also theoretically be damaged in the long term by non-specific inflammatory mechanisms or lose function to progressive exhaustion makes it necessary to couple it to another monitoring assay, so as not to initiate unnecessary antirejection treatment.

Cytotoxic lymphocyte gene expression

The T-cell-dependent immune activation gene products granzyme B, Fas-ligand and perforin have been involved in mechanisms of apoptotic death of target cells during the process of acute rejection. An increase of the levels of expression of these genes in the peripheral blood of kidney transplant recipients was shown to be associated with acute kidney graft rejection [54]. From this study came the idea of monitoring cytotoxic lymphocyte gene expression as a marker of rejection. In a first study on non-human primates from the Miami group, a sustained elevation of cytotoxic lymphocyte gene mRNA levels was observed 83-197 days before islet graft failure secondary to acute rejection [55]. Granzyme B was the best predictor of rejection. These findings were confirmed in a series of 13 patients, in whom a clear elevation of granzyme B mRNA levels was observed 25-203 days before onset of frequent hyperglycaemia and eventually re-introduction of insulin [56]. These markers of cytotoxic T-cell activity are likely to appear in the presence of an immune phenomenon such as rejection or recurrence of autoimmunity, but also in response to infectious or inflammatory processes, as reported in the study, accounting for a relative lack of specificity [56].

In this regard, coupling of our mRNA assay with the granzyme B assay might improve the specificity of both tests and enable to discriminate between immune and non-immune islet damage (granzyme B), and between immune islet destruction and infectious/inflammatory events (insulin). Such discriminating ability could provide an accurate trigger for the appropriate initiation of antirejection therapy.

Islet graft imaging

The field of beta cell imaging is currently generating a lot of interest, notably by the US National Institutes of Health, who have organized workshops on the theme of "Imaging of pancreatic beta cell in health and disease" and made funding available for clinical research projects on this topic. While the field encompasses the imaging of beta cells in type 1 or type 2 diabetes, emphasis was put on the need for imaging techniques of transplanted islets [57]. Imaging could be used as a tool to visualize either the islets directly to monitor graft mass, or an inflammatory process in a situation of ongoing acute rejection. Three modalities have demonstrated applicability in the near future: bioluminescence imaging (BLI), magnetic resonance imaging (MRI) and positron-emission tomography (PET).

Bioluminescence imaging

BLI uses light-generating enzymes such as luciferase, generating low-light signals easily detectable by exquisitely sensitive charged-couple device (CCD) cameras. Imaging islet transplants using this technology was reported in the mouse, with islets expressing the reporter gene luciferase, either by adenovirus-mediated gene transfer or transgene expression [58, 59]. BLI was shown to have a high sensitivity, being able to detect as few as 10 islets. Stable luminescence was obtained for as long as 18 months after transplantation [59]. Interestingly, luminescence intensity started to decrease several days before permanent recurrence of diabetes and histologically demonstrated acute rejection in an allogeneic model. Limitations for scaling up CCD technology results from the absorption of the BLI signal by mammalian tissue and ensuing penetration of only a few centimeters [59]. There

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is much to be improved in this elegant method before it can be applied to large animals, let alone the human, notably the finding of a reporter gene with a much more favorable light emission spectrum for signal detection through mammalian tissue.

Magnetic resonance imaging

In contrast to BLI, MRI can be easily used in the clinical setting. The first attempts at liver MRI after islet transplantation were aimed at studying a possible structural impact on the liver of the procedure. The Philadelphia group discovered and reported the presence of several areas of focal steatosis around portal spaces in two patients, a finding that seemed to be more prominent in patients having good islet function [60]. It was assumed that this finding was the result of the paracrine action of local insulin release around implanted islets, but was probably of no clinical significance.

Further MRI studies used superparamagnetic iron oxide (SPIO) nanoparticles, which are widely used in the clinical setting as contrast agents for liver imaging. Two groups at the University of Prague, Czech Republic, and at the Massachussets Institute of Technology have used such particles to label islets prior to transplantation in a rat model. After intraportal infusion, labeled-islets could be identified within the liver of rats and appeared as hypointense spots on T_2*-weighted MR images. The signal remained stable within the liver and could allow imaging of islets for several months after syngeneic transplantation [61, 62]. In a model of allotransplantation without immunosuppression, no more MR signal could be detected three weeks after transplantation [63, 64]. Iron labeling did not affect islet viability, nor *in vitro* or *in vivo* functions [61, 63]. A possible improvement of the method was recently reported in the rat model, in which islets were "transfected" with SPIO particles by electroporation [65]. This technique allowed detection of as few as 200 islets, but no data on its effect on islet function were provided.

Because of the availability of commercial, approved MR contrast agents made of SPIO nanoparticles routinely used for liver MR imaging, applying the method to the clinical setting is rather straightforward. We have recently included three islet transplant recipients in a pilot study, in which islets were incubated for 24 hours with ferucarbotran (Resovist®, Schering AG, Berlin, Germany) before intraportal transplantation. All three patients became insulin-independent, demonstrating the harmlessness of the iron-labelling procedure, and have shown hypointense spots on T_2*-weighted sequences, as observed in the rat model, up to six months after transplantation so far [66]. This pilot study is a proof of principle of the validity of the concept and merely a starting point for the development of the method.

Analysis of the images should be improved. Iron particles induce a disturbance of the magnetic field, and the related image is larger than the particle itself. As a consequence, two similar spots can include various numbers of iron particles. 3-D reconstruction of the liver images in order to obtain a complete representation of hypointense spots and quantification of the implanted islet mass are the next steps. Further and most important, the clinical outcome has to be better correlated to the images. The critical point will be to know whether a decrease of signal can be detected early enough in order to be able to salvage a graft failing to rejection.

Other MR compatible contrast agents have also been studied. Lipophilic Gd3+ complexes, which bind to the cell membrane and are able to label islets ex vivo have been designed by Zheng *et al* [67]. Uptake of manganese, a MR enhancing agent, by glucose-activated beta cells has been observed and proposed as a method for functional islet graft imaging [68].

Positron emission tomography imaging

The sensitivity of PET is higher than that of MRI, and it allows accurate quantification of the signal. PET-compatible tracers can be used to label islets ex vivo, prior to transplant, or if specific enough, they can be injected intravenously after transplantation.

Ex vivo labeling of islet prior to intra-portal transplantation has been successfully attempted with 2-[^{18}F]fluoro-2deoxy-D-glucose (FDG). Islets could be visualized for the first 6 hours after transplant only [69]. This study showed the feasibility of the technique in the context of islet transplantation, and was able to demonstrate that islets implanted only inside the liver. The same strategy was used with similar results in the pig [70]. Limiting factors for long-term assessment were the short half-life of the β+ -emitting radionucleotides (110 min for ^{18}F) and the high outflow of tracer from the cell. However, this technique could have some indication in studying the fate of the islets very early after transplant.

PET imaging could also be performed after transplant for sequential monitoring of the islet mass in the long-term. This option would require the identification of tracers highly specific for beta-cells, but would also allow visualization of islets within the pancreas in patients suffering from type 1 or type 2 diabetes, or conditions such as insulinoma or nesidioblastosis. Considering the very low mass of islet grafts, it is expected that the probe should be retained at least 1000 times more by islets than by the surrounding tissue [71]. This is especially challenging considering that most tracers are metabolized by the liver, inducing a high background noise [72].

Beta cell-specific antibodies have been studied as potential tracking agents. The K14D10 monoclonal antibody and its Fab fragment (similar affinity, but faster clearance) were tested for that purpose. It was estimated that its cellular specificity was in fact far too low to overcome the very low beta-cell mass in the pancreas [73]. An anti-IC2 monoclonal antibody (mAb) bound to a radioisotope chelator, showed decreased accumulation of the probe in streptozotocin-induced diabetic mice as compared to control animals. While analyses have been performed on native pancreas *ex vivo*, it is unclear whether such antibodies could be used for *in vivo*

clinical imaging [74]. A ^{125}I-labelled mAb directed against gangliosides from the beta-cell surface was also tested by radioimmunoscintigraphy in a rat model. Although in vitro staining of islets was one order of magnitude higher than that of exocrine tissue, no difference was seen in vivo [75]. The specificity issue is likely to hinder the development of such beta-cell specific radiolabelled mAbs.

While they were expected to be potential candidates, glibenclamide, tolbutamide, serotonine, L-DOPA, dopamine, nicotinamide and fluorodithizone had all low specificities for beta cells when tested in vitro [72]. [^{11}C]Dihydrotetrabenazine (DTBZ) is a radio-ligand currently used in clinical imaging of the brain. It binds specifically to VMAT2, a transporter found specifically in the brain and in beta-cells. Longitudinal PET imaging of the native pancreas of diabetic BB rats demonstrated a decline of signal, paralleling the decrease of beta-cell mass [76]. This technique appears promising, but still needs to be replicated in the islet transplant setting in the context of the generally high uptake of the liver.

Conclusion

Efficient monitoring tools of the islet graft has been conspicuously lacking but are critically needed in the current era of high rates of short-term success and long-term loss of function experienced by the procedure. A wealth of candidate methods and techniques is close to hand and should allow significant progress in the understanding of the reasons for islet graft exhaustion and its kinetics, which is a prerequisite for the improvement of islet transplantation outcome. Progress is likely to arise from the fields of immune monitoring, molecular monitoring and islet imaging.

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Alternatives routes of insulin delivery

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SUMMARY

Optimal glycaemic control is necessary to prevent diabetes-related complications. An intensive treatment, which could mimic physiological insulin secretion, would be the best one. However subcutaneous insulin treatment is not physiologic and represents a heavy burden for patients with type 1 and type 2 diabetes mellitus. Consequently, more acceptable, at least as effective, alternative routes of insulin delivery have been developed over the past years. Up to now, only pulmonary administration of insulin (inhaled insulin) has become a feasible alternative to cover mealtime insulin requirements and one of the various administration systems was recently approved for clinical use in Europe and the United States. But, due to advances in technology, other routes, such as transdermal or oral (buccal and intestinal) insulin administration, could become feasible in a near future, and they could be combined together to offer non-invasive, efficacious and more physiological way of insulin administration to patients with diabetes.

Key-words: Routes of insulin delivery • Ocular, Oral, Buccal, Intestinal, Nasal, Pulmonary, Inhaled insulin • Review.

RÉSUMÉ

Voies alternatives d'administration de l'insuline

Un contrôle glycémique optimal est indispensable pour prévenir les complications du diabète. Un traitement intensifié, reproduisant la sécrétion physiologique d'insuline, représenterait l'idéal. Mais le traitement par insuline par voie sous-cutanée n'est pas physiologique et les schémas basal-bolus représentent un lourd fardeau pour les patients atteints de diabète de type 1 et de type 2. Aussi, depuis longtemps, ont été recherchées des voies alternatives d'administration de l'insuline, plus physiologiques, plus acceptables, et au moins aussi efficaces. Jusqu'à ce jour, seule la voie pulmonaire (insuline inhalée) représente une alternative pour les besoins en insuline prandiaux, et l'un des systèmes d'inhalation d'insuline a été approuvé en 2006 par les autorités de santé européennes et par celles des Etats-Unis pour le traitement clinique des patients atteints de diabète de type 1 et de type 2. En raison des progrès technologiques, d'autres routes d'administration, comme les voies transdermique et orale (buccale et intestinale) en particulier, ont connu des progrès récents et pourraient devenir accessibles dans un futur proche. Combinées, ces voies d'administration, pourraient ainsi bientôt offrir aux patients diabétiques une alternative fiable, non invasive, efficace et plus physiologique au traitement insulinique par voie sous cutanée.

Mots-clés : Administration d'insuline • Voies alternatives • Insuline orale, nasale, pulmonaire, inhalée • Revue générale.

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Introduction

Optimal glycaemic control is the key to prevent long-term micro- and macrovascular complications of diabetes mellitus. Intensive insulin regimens that mimic physiological insulin secretion represent the best way to attain the goal of near-normoglycaemia. However in type 1 diabetes, only a few patients succeed in maintaining long-term HbA_{1c} below 7% with complex and intensive insulin treatments involving multiple daily injections or continuous insulin infusion by pumps, sophisticated glucose monitoring, and meal glucose count. In patients with type 2 diabetes, insulin therapy is often initiated late in the course of the disease and intensification of treatment by insulin meet psychological resistance both by the patient and the physician. Consequently, more acceptable alternative routes of insulin administration have been searched for many decades, with the aim to avoid the burden of multiple subcutaneous injections and to improve insulin's pharmacokinetics. Every imaginable route has been tested, but even if some of them are still promising like dermal, oral (buccal and enteral), only pulmonary inhaled insulin becomes a feasible alternative. This article will review these alternative routes for insulin administration.

Anecdotal routes

Ocular route

Insulin has been administered as eye drops to animals. When insulin is given alone at growing concentrations, no increase in systemic insulin level is observed and no toxicity is detectable [1]. Absorption is increased in animals when enhancers, like saponin, dodecylmaltoside, tretradecylmaltoside, fucidic acid or glycocholate, are added, but eye toxicity increased with the enhancer concentration [2, 3]. More recently, an acidified Gelfoam® (an absorbable gelatine sponge) -based eye device, has been tested and the results suggest an efficient systemic absorption of insulin, at least in rabbits [4].

Vaginal and rectal routes

These routes have been tested soon after the discovery of insulin but have met absorption problems through the mucosa, with very poor biodisponibility. Several classes of enhancers (bile salts, dihydrofusidate, cyclodextrins, surfactants and chelating agents) have been tested to promote the absorption, but with the induction of severe local reactions [5]. Moreover rectal route is subject to variability related to the intestinal transit. These routes do not seem to offer a real opportunity for the management of insulin-treated patients.

Transdermal route

Skin offers the advantages of an easy access and a very large surface area (1-2 m^2). However, it represents an effective barrier that limits penetration of large, hydrophilic polypeptides, like insulin [6]. The upper layer, the stratum corneum, is responsible for this impermeability via its lipid-rich matrix [7]. Various methods have been tested to overcome the skin barrier and to allow insulin absorption. They can be separated into chemical (liposome and chemical enhancers) and physical methods (mainly iontophoresis and sonophoresis).

Chemical methods

Transport of molecules across the stratum corneum is slow and the mechanism appears complex. It is controlled by thee predominant concepts which are partition, diffusion, and solubility. These parameters are to be targeted to improve the rate of absorption [8].

Chemical enhancers can increase permeability by increasing the partition coefficient of the drug, or increasing the thermodynamic action of the drug in the vehicle or modifying the nature of stratum corneum [9]. The incorporation of insulin in liposomes do not lead to systemic biological effect due to their size, which enables them to pass through the narrow pores (<30 nm) of the outer skin layer [6, 10, 11]. More recent studies have developed the concept of transfersomes which are ultraflexible, highly deformable, lipid vesicles incorporating surfactant molecules [12, 13]. Application of transfersomes including insulin (Transfusulin®) was reported to result in a transfer rate of approximately 50% of subcutaneous administration, and systemic normoglycaemia lasting at least 16 hours was achieved using a simple non-invasive epicutaneous administration of insulin in transfersomes [13]. These results, if confirmed by other studies, could imply a possible use of this route to cover basal insulin requirements. Preliminary studies using ethosomes or biphasic lipid-based vesicles have also shown possibilities of insulin transfer [14, 15].

Physical methods

Iontophoresis

Cathodal iontophoresis has been tested to increase transdermal insulin penetration. This technique uses electrically charged insulin molecules and a small electrical potential which can be manipulated to control the rate of insulin delivery [16]. It is poorly efficient, but can be improved by shaving the hairs, injurying the stratum corneum, or using monomeric insulin analogues [17, 18]. Nevertheless the amount of insulin transferred is insufficient to cover basal insulin requirements and long-term safety issues have not been assessed.

Sonophoresis

Low frequency ultrasound (20-160 kHz) (also called sonophoresis) can be used to increase transdermal insulin penetration applied as aqueous solution or mixed with a hydrogel [7, 19]. Decreases in blood glucose have been observed after application of low frequency ultrasound in animal and human studies [19-21]. This method seems feasible,

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but needs further long-term studies. Other methods have been investigated, like pressure waves and electroporation, but they are still at a preliminary stage.

Up to now, results of studies assessing insulin administration through the skin as a possible treatment for diabetic patients remain limited. Combining chemical and physical methods needs further investigations.

Per-oral (gastrointestinal) route

Oral administration of insulin is a potentially attractive route, firstly because of its easy convenience, and secondly due to the portal drainage, then a more physiological delivery of insulin to the liver. However polypeptides, like insulin, are submitted to acidic degradation in the stomach and to enzymatic attacks in the small intestine. Moreover the gastrointestinal mucosa prevents absorption of large, hydrophilic peptides.

Several strategies, alone or in combination, have been developed to increase intestinal absorption of insulin. They include use of permeation enhancers (bile salts and fatty acids), associated or not, with enzyme inhibitors like aprotinin [22, 23], the use of liposomes, emulsions, mucobioadhesives and polymer-based delivery systems [24, 25]. Previous studies performed with liposomes in animals, have shown mainly, a low absorption of insulin and variable decreases in blood glucose, depending on the physical and chemical composition of the liposomes [15]. Polymerisation, inclusion of insulin in an acrylic biodegradable polymer, encapsulation into microspheres or in biocompatible nanocubicles have been tested [27-31]. These vehicles are degraded in the liver, releasing insulin *in situ*. All these strategies have achieved partial success and need further investigations.

A more promising formulation consists to modify the insulin molecule by a covalent attachment of amphilic low molecular weight oligomers. Hexyl-insulin monoconjugate 2 (HIM 2) is made of human recombinant insulin with an amphilic oligomer covalently bound to the free amino-acid group on the lys-β29 termination. This formulation improves solubility and stability of the molecule [32]. In healthy subjects [33], oral HIM2 suppresses endogenous glucose production and increases tissue glucose disposal, in a dose dependent manner. Absorption is rapid (peak plasma insulin is reached within 60 minutes and return to baseline in 120 minutes). The effects persist up to 240 minutes after administration. In type 1 diabetic subjects [34], a phase 1/2 clinical trial suggested that oral HIM2 is safe and may prove effective in controlling postprandial hyperglycaemia. In type 2 diabetic patients [35], a randomised-dose escalation study was performed: oral HIM2 and subcutaneous insulin provided comparable control of 2h-postprandial glycaemia and comparable 0-240min area under the curve (AUC), but HIM2 resulted in lower peripheral insulin concentration. These results confirm that oral HIM2 is absorbed though the portal circulation directly to the liver and then, could reproduce the physiological route of insulin secretion.

Oral (buccal and sublingual) routes

The buccal mucosa offers the advantages of an easy accessibility and a large surface (100–200 cm^2) for absorption. Moreover, it has little proteolytic enzyme activity and is a well vascularised tissue. However the continuous, but variable, saliva flow and the robust multilayered structure of the oral epithelium constitute a barrier to penetration of drugs [36].

Several strategies, alone or in combination, have been tested to improve buccal insulin absorption: use of absorption enhancers (bile salts, surfactants, fatty acids, alcohol, chelators) protease inhibitors, bioadhesive delivery systems (gels, films, patchs), lipophilicity modifications (conjugation with polymers). All these studies, conducted in animals, have not shown a decrease of blood glucose greater than those obtained with 30% of the insulin dose administered intramuscularly. Furthermore, reproducibility seems poor [36-42].

A more promising technology has been developed by Generex Biotechnology Corporation (Toronto, Canada). It combines a liquid formulation (Oral-Lyn$^{\varepsilon}$) of recombinant human insulin and absorption enhancers and a propeller, the RapidMist® device, which sends small particles from an aqueous spray into the oral cavity. This allows rapid insulin absorption. Pharmacokinetics of insulin administered via this system has been evaluated in healthy subjects and in type 1 diabetic patients. The time to peak occurred at 25 minutes, and compared to subcutaneous regular insulin, a more rapid onset of action and a less prolonged hypoglycaemic action were observed. A dose-response relationship was noticed but pharmacokinetics were variable [45-47]. Short-term studies in patients with type 1 or type 2 diabetes, revealed that this oral insulin can be efficient in controlling postprandial glucose levels [46-48]. The oral insulin spray was generally well tolerated. This oral insulin system could represent an alternative to subcutaneous route, but needs further investigations on its reproducibility, tolerance and long-term efficacy in diabetic patients.

Nasal route

Nasal administration represents a potential route for insulin delivery due to the easy nose access, its high vascularisation and a relatively large surface (150 cm^2) of absorption. However a very active mucociliary clearance mechanism, preventing prolonged contact of the drug with the mucosa, and the presence of proteolytic enzymes, do not favour a high biodisponibility [48]. Like buccal and intestinal routes, a number of factors influence bioavailability: type, volume and concentration of insulin and enhancers, physicochemical properties of the particles, frequency of administration, and indeed, the presence of various affections at the nasal level.

For several decades, numerous enhancers have been tested to improve insulin absorption with a local toxicity as lowest as possible. They include bile salts (1 to 4% sodium glycocholate and deoxycholate), fusidic acid salt

(8% 9-laureth), phospholipids (2% didecanoyl-phosphatidylcholine) and cyclodextrins [48]. Saponin, lecithin, chitosan in gel have been tested also [49]. The kinetics profile of insulin administered by the nasal route has been evaluated in healthy subjects and in type 1 diabetic patients. These studies have shown a rapid increase of insulin concentration with a peak at 10-20 minutes, and a fast decrease of insulinemia (in about 2 hours). The bioavailability depends on the various factors already mentioned, and varies between 10 to 20% (with the exception of up to 45% in a preliminary animal study of insulin with chitosan gel). The effect is dose related, but with a huge variable inter-individual response [50, 51]. Nevertheless, this profile appeared potentially suitable for prandial insulin replacement, and then, short-term and long-term clinical studies have evaluated its efficacy in type 1 and type 2 diabetic patients [52-57]. The results were rather disappointing: in the short-term, glucose-lowering effect was too variable, and requiring high insulin doses given in one or more administrations;on the long-term, HbA_{1c} levels were not improved, and even mostly slightly deteriorated due to a too short duration of insulin action through this route of insulin delivery.

More recently, some new encouraging results have been obtained. In type 2 diabetic patients, a lyophilised formulation of insulin, using glycocholate as enhancer, was given before meals (associated to bedtime NPH insulin). Compared to twice daily subcutaneous NPH, similar glycaemic control was obtained [58]. In a 6-month study in type 1 diabetic patients, a gelified spray of insulin administered three times daily with NPH insulin twice daily, was as efficient as three subcutaneous regular insulin injections [59].

Nevertheless, using this route, side-effects were important: nasal irritation was observed very frequently, immunogenicity (insulin antibodies) was increased [53], and a potential damage of the nasal mucosa and mucociliary system is still possible, *i.e.* with a risk of an increased permeability of the mucosa to viruses or mitogens [50, 52, 55, 56, 59]. The development of a nasal insulin by the Novo-Nordisk company has been stopped, most likely due to these reasons. Up to now, the benefits/risks ratio of the nasal route does not seem favourable.

These review focuses on nasal insulin as an alternative route for insulin delivery in the treatment of diabetic patients. But recent studies have shown two different types of additional effects. First, nasal insulin alone, without enhancers, improves central nervous system function in healthy subjects and in Alzheimer-affected subjects [60, 61], without variation of peripheral insulinaemia, probably *via* a direct nasal-to-brain effect. Secondly, proinsulin administered intranasally with anti-CD3, enhances remission from recent onset autoimmune diabetes in animals [62].

Pulmonary route

Today, pulmonary inhaled insulin, seems the most promising alternative route of insulin delivery. The rationale for pulmonary administration is based on several facts: lungs provide a large, highly vascularised, potential absorption area (100–150 m^2) (composed of bronchioles, alveoles ducts, and alveoles witch represent 95% of the total absorption area). Alveoles are covered by a very thin (0.1–0.2 mm) monolayer of epithelial cells. There are few variations in mucus production, no mucociliary clearance, nor peptidases which represent barriers to absorption in other sites. The transport of molecules is not completely understood but for rather small molecules, like insulin, the predominant process is a junctional paracellular transport [63] when, for larger molecules, it is preferently transcytose.

Absorption is inversely related to molecular weight (<30 000 Da is better) and depends on MMAD (median mass aerodynamic diameter) which reflects the particle diameter and density [64]. Deposition is optimal in the deep lung for MMAD between 1.5–5µm, larger particles remaining predominantly in the upper part of the respiratory tract, and smaller are mostly exhaled. Breathing characteristics have a major influence on intrapulmonary absorption [65] and all parameters influencing breathing will have to be studied to assess their influence on insulin absorption (smoking, asthma, lung diseases, exercise and patient's ability to breath through inhaler devices).

Currently, four inhaled insulin systems have progressed to phase 3 clinical trials: AERx® Insulin Diabetes Management System (Aradigm Corp, Hayward, CA, USA and NovoNordisk, Copenhagen, Denmark) which delivers aerosol of human insulin; Exubera® system (Nektar Therapeutics/Pfizer Inc) which uses a dry powder formulation; Alkermes inhaler (Eli Lilly/Alkermes) delivers engineered human insulin powder; MedTon inhaler (Mannkind Corp., Danbury, CT, USA) delivers a powder of Technosphere®-associated human insulin. Other systems are less advanced, but will be described too. The Exubera® system was given marketing approval in 2006 by the US (FDA) and European (EMEA) health authorities for use in the treatment of type 1 and type 2 diabetic patients.

AERx® iDMS, developed by NovoNordisk

This system uses a liquid insulin formulation and expels a single dose of aerosol of fine insulin particles though a disposable nozzle on a disposable dosage strip. The AERx®iDMS emits the aerosol by extruding the solution through the holes of the nozzle. Particles have a MMAD of 2.2 µm. It is a battery powered device utilising a microprocessor to guide electronically the user to the optimal breathing pattern (flow rate and depth of breath) [66, 67]. The system allows delivering metered dose of insulin and single unit increments. It has the size of a small book. As containing a liquid formulation, it requires cold storage.

Pharmokinetics and bioavailability were studied in healthy subjects and type 1 diabetic patients [67-69]. The pulmonary delivery of insulin resulted in a rapid absorption (time to maximal concentration varying between 10 and

60 minutes) leading to a glucose-lowering effect (maximum between 60 and 255 minutes), both effects being more rapidly obtained than with subcutaneous regular insulin. A dose-response relation was observed, as well as a dose-dependent time to peak, higher doses being absorbed slightly slower than smaller doses. The duration of breath had no effect on insulin absorption. The pharmacodynamics system efficiency was 12.7% compared to subcutaneous administration.

The effect of several potentially influencing factors, have been evaluated. Smoking increases insulin absorption without affecting intra-subject variability [70]. Age does not modify pharmacokinetics of inhaled insulin in type 2 diabetic subjects, but slightly decreases lowering glucose response [71]. In asthmatic subjects, absorption is reduced compared to healthy subjects. No effect on airway reactivity was observed [72]. Acute upper respiratory infection (current and three weeks after recovery) does not modify pharmacokinetics and glucose response [73].

Intra-subject variability of insulinaemia and blood glucose profiles after inhaled insulin administration was comparable to those observed after subcutaneous injections of regular insulin (intra-patient coefficient of variation for insulin AUC during 6 hours: 14 to 27% after inhaled insulin *vs* 19% after subcutaneous administration, and for glucose AUC: 21-30% *vs* 23% respectively. The variability was similar in smokers or elderly patients but higher in subjects with asthma [70-72, 74].

Up to now, a mid-term clinical study is available in diabetic patients. A proof-of-concept, randomised trial conducted in 107 patients with type 2 diabetes compared pre-meal inhaled or subcutaneous regular human insulin, in combination with bedtime NPH insulin during 12 weeks. No difference was observed between the two treatments for HbA_{1c}, but fasting glucose was lower in the AERx®iDMS group. Frequency of adverse events was not significantly different, except a significant increase in insulin antibodies with inhaled insulin. Pulmonary assessment was normal [75]. The inhaled insulin was considered as well tolerated and provided excellent compliance. A multicentre, 24-month trial, initiated in 300 type 1 diabetic patients was designed to assess efficacy and safety of inhaled insulin, compared to subcutaneous injections of aspart insulin. To date, the results have not been published yet.

AIR (Advanced Inhalation Research), developed by Alkermes and Eli Lilly

The device is a simple, small, breath-activated system that uses capsules of dry-powder human insulin which are punctured before emission. The aerosol is made of large, porous particles, containing insulin associated with a biodegradable polymer matrix composed of phospholipids. The particles are relatively large (10-20 μm) but their MMAD is within 1 to 3 μm, and they have a reduced tendency to aggregate, thus facilitating dispersion [76]. Lung deposition of the particles (without insulin) has been studied in healthy subjects [77] with this system. Delivery was characterized by high and reproducible emitted doses, and high lung deposition (mean 51% of the total dose) with low inter- and intra-subject coefficient of variation across a various range of inspiratory flow rate. Pharmacokinetics and glucodynamics dose response of human insulin inhalation powder delivered by the AIR system were compared to subcutaneous lispro insulin in healthy subjects at various doses, using the euglycaemic clamp technique [78]. The time action profile was longer for inhaled *vs* lispro insulin (time to return to basal level: 480 *vs* 360 minutes respectively) but both treatments showed rapid initial absorption (time to maximum concentration: 45 minutes), similar overall pharmacokinetics AUC and glucose lowering effect. Inhaled insulin doses equivalences were shown to be 2.6 mg for 6 IU, 5.2 mg for 12 IU and 7.8 mg for 18 IU of lispro insulin. Tolerance was considered to be excellent. A clinical, randomised, cross-over study [79] performed in patients with type 1 diabetes has compared inhaled to subcutaneous (lispro and regular) as pre-prandial insulin associated with glargine in a *basal-bolus* regimen. However, it has to be noticed that metabolic targets were not stringent. At the end of the 12-week treatment periods, HbA_{1c} was comparable and sub-optimal in the two groups (inhaled *vs* subcutaneous: 7.95 *vs* 8.06%). Fasting blood glucose was lower with inhaled insulin. Safety profiles were comparable, except for the incidence of nocturnal hypoglycaemia which was higher with inhaled insulin. These latter facts need further explanations.

Exubera®, developed by Nektar/Pfizer

Exubera® was granted marketing approval by health authorities (EMEA in Europe and FDA in the US) in January 2006, for the treatment of type 1 (in association with basal insulin) and type 2 diabetes.

The device uses insulin powder formulation, which consists of recombinant human insulin (60%) and excipients (mannitol, glycine, sodium and nitrate). The powder is packed in blister packs, each one containing 1 or 3 mg of insulin (about 28 and 84 IU) equivalent to 3 IU and 9 IU of subcutaneous insulin respectively [80, 81] which represents a 10% relative activity. The blister is inserted into a slot at the base of the device. Activation leads to compressing trapped air, puncturing the blister and releasing air through the blister at high velocity. Insulin particles (MMAD approximately 3 μm) are aerolised into an inhalation chamber. Then, the subject inhales the respirable cloud with a full slow breath. The device is 23 cm long, but when it is folded, it has the size of devices used for asthma. Pharmacokinetics of inhaled insulin has shown a peak at about 55 minutes and a more rapid return to basal level than regular subcutaneous insulin [81]. Pharmacodynamics of Exubera® inhaled insulin was compared to regular insulin and insulin lispro in healthy subjects. Inhaled insulin has the fastest onset of action, a comparable time to maximal effect to insulin lispro, a maximal metabolic effect and duration of action comparable to regular insulin [82]. Reproducibility evaluated in type 2 diabetic

patients is similar to subcutaneous insulin [83]. Smoking influences inhaled insulin profile, the peak occurs earlier (31 *vs* 53 minutes) and its magnitude as well as the total insulin absorption (AUC 0 to 6 hours) are greater, these changes being partly reversible with smoking cessation [84]. Safety and efficacy have not yet been established in patients with asthma, chronic obstructive pulmonary disease (COPD) or acute respiratory infection.

The clinical metabolic efficacy has been evaluated in approximately 3000 patients with type 1 or type 2 diabetes. In patients with type 1 diabetes, a proof-of-concept, open-label, 3-month randomised study compared inhaled insulin given 10 minutes before meals associated with ultralente insulin at bedtime, and two or three subcutaneous injections of regular and NPH insulin at bedtime [85]. At the end of the study, glycaemic control evaluated by HbA_{1c} was similar in the two groups (7.9% and 7.7% respectively). Glycaemic profiles after a standardised meal were comparable for both treatments at the beginning and at the end of the trial, and metabolic side-effects (hypoglycaemia, weight gain) were similar. Two 6-month randomized phase 3 studies, compared inhaled with subcutaneous pre-meal regimens. The first one [86] compared pre-meal inhaled to regular subcutaneous insulin and twice daily NPH insulin injections (conventional treatment) in 335 type 1 diabetic subjects. Mean decrease in HbA_{1c} was comparable with the two treatments. A greater reduction in fasting and postprandial glucose values was observed with inhaled insulin. Hypoglycaemia was slightly lower with inhaled insulin. The second study [87] used the same design, but compared pre-meal inhaled *vs* subcutaneous regular (but not analogue) insulin, in 368 type 1 diabetic patients receiving NPH insulin twice daily in a *basal-bolus* regimen, but with conventional therapeutic objectives. HbA_{1c} and 2-h postprandial glycaemic reduction were comparable in the two groups, but fasting glycaemia was lower in the inhaled insulin group. The overall hypoglycaemia rate was slightly less (inhaled vs subcutaneous: 9.3 *vs* 9.9 episodes/patient-month) but severe hypoglycaemia frequency was comparable (inhaled vs subcutaneous: 5.5 *vs* 4.7 events/100 subject-months). No clinical study has been published yet, comparing inhaled insulin with intensified subcutaneous regimens using insulin analogues and with stringent fasting and postprandial glucose targets.

In type 2 diabetic patients, one proof-of-concept trial and several phase 3 studies assessed efficacy of Exubera® at different stages of the disease and versus different oral antidiabetic treatments. In the first one [81], metabolic efficacy (HbA_{1c}, glucose profiles after a standardised meal and frequency of hypoglycaemia) were comparable. A randomised study [88] conducted in type 2 diabetic subjects with diabetes control failing off on diet and exercise, compared pre-prandial inhaled insulin alone to rosiglitazone twice daily and showed better metabolic control with inhaled insulin. Another randomized study [89], evaluated Exubera® alone vs Exubera® and oral antidiabetic drugs and vs oral antidiabetic drugs only. Metabolic control at the end of the study was significantly improved in patients receiving Exubera®. In a 6-month study [90] with a design similar to the trial in type 1 diabetic patients previously described [82], the decrease in HbA_{1c} was comparable with inhaled or subcutaneous regimens, but more patients with inhaled therapy reached an HbA_{1c} lower than 7%.

Non metabolic side-effects (pulmonary consequences, immunogenicity), patients'satisfaction and costs, have been studied, although with various approaches, with all inhaled insulin delivery devices, but studies conducted with Exubera® are clearly at a more advanced stage. However, all these concerns are expected (or have proved) to be similar with any inhaled insulin.

In most studies, pulmonary functions were reported to remain stable, although a decrease in carbon monoxide (CO) diffusing capacity was noted [86, 90]. Mild to moderate cough was reported throughout all studies, but seems to decrease over treatment periods [86-90]. FDA recommends baseline pulmonary function testing prior to initiation of treatment and every year [80]. The use of Exubera® is contraindicated in patients with lung disease, and in patients who smoke or discontinued smoking less than six months prior to initiating it. Exubera® treatment is not recommended in patients with chronic pulmonary disease (asthma, COPD) due to its non-established efficacy and safety in these diseases.

Exubera® (like other inhaled insulin) induces a higher increase of insulin antibodies compared to subcutaneous treatment. This increase does not seem to lead to detectable metabolic consequences [91, 92].

In phase 2 and 3 clinical trial, Exubera® (like other inhaled insulin), was associated with a higher satisfaction of patients towards their treatment than subcutaneous injections [85-90]. Moreover a specific 1-year study [93] addressed specifically patients'satisfaction and demonstrated a greater satisfaction with inhaled insulin. Furthermore, this route of treatment administration has been shown to improve the acceptance of insulin treatment by type 2 diabetic patients [94].

Studies focusing on costs associated with inhaled insulin treatment compared to conventional treatments of diabetes (oral antidiabetic agents and/or subcutaneous insulin regimens) are not available yet, nor cost/effectiveness data. Inhaled insulin treatment is expected to be significantly more expensive than injectable insulin, due to the higher amount of inhaled insulin required for equivalence to subcutaneous administration (a 10% bioavailability for inhaled vs subcutaneous administration), the price of the device and its related furnitures .However this has to be balanced with a better acceptance of insulin treatment via this route, which could imply a wider use of insulin in type 2 diabetic patients (reducing the production costs) and less long-term diabetic complications through an earlier and better diabetes control and prevention. However, all these considerations await further studies [95].

Aerodose® (Aerogen Inc./Nektar Therapeutics)

Aerodose® is a system activated by breath which uses a liquid insulin formulation aerosolised in small droplets. Pharmacokinetics and pharmacodynamics studies [96, 97] have shown a time to peak insulin level shorter after insulin inhalation than after regular subcutaneous insulin (60-97 minutes vs 168-237 minutes) and an onset of action and a peak metabolic effect occurring earlier with inhaled insulin. Reproducibility was similar with inhaled or subcutaneous insulin.

Technosphere® Insulin (Mannkind Corporation)

Technosphere® insulin is a kind of lattice containing a dry-powder formulation of crystallized insulin in gelatine capsules. The insulin delivery mechanism uses a high-impedance inhaler with a powder deagglomeration system. Pharmacokinetics and pharmacodynamics studies have shown a very fast absorption (time to peak insulin level: 12-14 minutes, time to maximum metabolic effect: 20-40 minutes) and a short duration of action (2 to 3 hours). Bioavailability was proportional to the administered dose and the biopotency was around 15% [98]. This formulation seemed to be well tolerated and is currently entering phase 3 studies.

Spiro System (Dina Pharmacy Inc/Elan Corporation)

Spiro System provides a dry-powder insulin formulation encapsulated in blister-disks via a breath-activated inhaler. After inhalation, peak insulin level was observed at 70 minutes and a dose-response relationship was observed [99].

Conclusion

More acceptable, painless routes of insulin delivery have been searched for many years to avoid the burden of insulin injections to diabetic patients and thus, alternative routes of insulin delivery is already a long time story [review in 100-102]. Interest in these alternative routes has grown up over the last few years, in parallel to progress in insulin formulations and advanced technology of delivery systems, as illustrated by the large number of excellent reviews on these topics [103-109].

At the time being, the most promising alternative to subcutaneous insulin injections is represented by insulin inhalation via the pulmonary route. Among the number of various inhaled insulin systems in development, the first one, Exubera®, was given approval for use in the treatment of type 1 and type 2 diabetic patients, by the FDA and the EMEA in January 2006; most of the other systems being currently in phase 2/3 studies. Pre-prandial inhaled insulin has proved to be as efficient as conventional treatments/regimens using subcutaneous insulin injections in type 1 and type 2 diabetic patients. Inhaled insulin seems to be well tolerated and to improve patients'acceptance of insulin treatment, which could lead to an improved diabetes control and prevention of long-term diabetic complications. However, comparisons with intensified insulin regimens using stringent metabolic control goal (HbA_{1c}), are still lacking, as well as long-term studies on cost/effectiveness and pulmonary safety.

Development of other alternative, painless, routes of insulin delivery are also in progress, and oral routes (intestinal and buccal) have recently shown very interesting advancements. Furthermore the intestinal route (via hepatoportal drainage) has the potential advantage of a more physiological administration.

Today, complete (basal and prandial) replacement of subcutaneous insulin treatment by alternative routes is not available yet, and likely, would require a combination of different approaches. But non subcutaneous pre-prandial insulin treatment has become available with the health authorities approval of pulmonary inhaled insulin for treatment of type 1 and type 2 diabetes, and this may be the beginning of a lighter burden for diabetic patients. The exact place of these new routes of insulin administration in the broad range of currently approved diabetes treatments deserves further research.

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Immunology of pancreatic islet transplantation

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SUMMARY

Clinical protocols in type 1 diabetic patients to optimize islet survival and function post-transplantation improved dramatically in the last decade, but it is clear that this approach still has potential limitations to provide long term insulin independency. Islet allografts administered in the liver via the portal vein are exposed to several factors contributing to a rapid loss of function that may reach 50% of the initial beta cell mass. Allo- and auto-immune reactions – an unique situation in clinical transplantation - are partially overcome with immunosuppressive regimen. Serological markers and T cell reactivities may correlate with graft failure. Most of the drugs that are used, including rapamycin (sirolimus) or the calcineurin inhibitor tacrolimus (FK506), have deleterious effects on beta function and/or insulin sensitivity. Immediate factors that limit initial islet engraftment have been elucidated, including instant blood mediated inflammatory reaction and angiogenesis. Newer interventions designed to promote islet survival, to prevent apoptosis, to promote islet growth and to protect islets in the long run from immunological injury are rapidly approaching clinical trials.

Key-words: Islets of Langerhans • Transplantation • Immunosuppression • Immunosuppressive treatment • Sirolimus • Calcineurine • Tacrolimus • Review.

RÉSUMÉ

Immunologie de la greffe d'îlots de Langerhans

Les récents programmes de greffes d'îlots chez les diabétiques de type 1 ont démontré des progrès considérables, mais il reste des limites pour assurer une fonction sur le long terme et une insulino-indépendance. Les allogreffes d'îlots injectés par voie portale dans le foie sont exposés à de nombreux facteurs d'agressions, expliquant une perte de fonction estimée à 50 % dans les premiers jours de la greffe. Les réactions allogéniques et autoimmunes, une situation unique en transplantation, sont partiellement contrôlées par l'immunosuppression chronique. La plupart des immunosuppresseurs utilisés, comme la rapamycine (sirolimus) ou les inhibiteurs de la calcineurine tel le tacrolimus (FK506), ont des effets délétères sur la fonction des cellules bêta et/ou sur la sensibilité à l'insuline. Des facteurs immédiats peuvent limiter la viabilité des îlots, comme une réaction immédiate de thrombose et la réduction de l'angiogenèse. De nouveaux traitements qui ont comme objectifs la survie des îlots et/ou la réduction de l'apoptose sont attendus.

Mots-clés : Greffes d'îlots de Langerhans • Immunosuppression • Traitement immunosuppresseur • Sirolimus • Calcineurine • Tacrolimus • Revue générale.

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Introduction

Optimised insulin injections remain the mainstay life-sustaining therapy for patients with type 1 diabetes (T1D) in 2006. However, a small subset of patients with T1D are very sensitive to insulin and lack counter-regulatory measures, putting them at higher risk of neuroglycopenia. Patients have been enrolled in islet cell transplantation programs with significant improvements during the last few years. In a landmark study published in 2000, Shapiro et al. [1] reported seven consecutive patients treated with islet transplants, all of whom maintained insulin independence for one year. The approach included: (i) selection of T1D subjects for islet alone who suffered from severe hypoglycaemia unawareness or labile diabetes; (ii) the immunosuppressive protocol was steroid-free, consisting of dacluzimab (an anti-CD25 monoclonal antibody) induction, the anti-rejection drugs sirolimus and calcineurin inhibitor tacrolimus; (iii) islets were prepared for transplant in the absence of xenogeneic proteins using human albumin rather than bovine albumin; and (iv) 10,000 IEs (islet equivalents)/kg of body weight was the minimum islet transplant administered to each patient, often administered as two or sometimes three infusions, from sequential donors. Since the original report of the Edmonton protocol in 2000, an estimated 500 islet transplants have been conducted worldwide using variants and further advances [2], including 50 in France. Clinical results of these grafts were encouraging although less efficient than the original Edmonton study with 30 to 60% insulin-independent patients at one year. These half tone results reinforced the need for better understanding of the underlying cellular mechanisms associated with the reintroduction of functional beta cells in long-standing T1D patients and on the possible immune factors implicated in the loss of function or destruction of islet grafts. Approximately 80% of grafts continue to function and secrete C-peptide however, and patients benefit considerably from near-normal HbA_{1c} and avoidance of hypoglycaemic reactions. The exact cause for progressive islet dysfunction is incompletely defined to date, but most likely reflects multifactorial aetiologies, including not only acute and chronic rejection, but the recurrence of autoimmunity and the fact that islets are placed in a non-physiological environment and are exposed further to chronic drugs that have diabetogenic and antiproliferative side effects. Furthermore, islet exhaustion may set in when a subtherapeutic islet engraftment mass is forced to continually secrete insulin at maximum capacity. This review will expose the respective contribution of the immunosuppression regimen, immediate pro-apoptotic factors during islet engraftment, allogeneic immune response and recurrence of autoimmunity in beta cell loss.

Immunosuppression

Early strategies relied on protocols that had proven success in solid organ transplantation which consisted of azathioprine, cyclosporine and corticosteroids. Under these protocols, fewer than 10% of patients were able to achieve insulin-independence [2]. The Edmonton protocol (and more recent variants) use a glucocorticoid-free combination of immunosuppressive agents, typically including Dacluzimab (anti-CD25 mAb) administered in the peri-transplant period, sirolimus and tacrolimus which are administered to prevent chronic rejection. Regular monitoring of sirolimus and tacrolimus levels ensures adequate and effective immunosuppression and avoids unnecessary overdosing, which could result in rapid and severe toxicities. Although the current immunosuppressive therapies have improved outcome in transplant recipients at 1- and 3-year time points post-transplantation, it is now emerging that insulin-independence is not sustainable in most subjects once they reach 5 years post-transplantation [3]. Rapamycin (sirolimus) is a macrolide fungicide with immunosuppressant properties, which may cause post-transplant diabetes mellitus. Rapamycin had a dose-dependent, time-dependent, and glucose-independent deleterious effect on MIN-6 cell viability [4]. A supra-therapeutic rapamycin concentration of 100 nmol/l had a deleterious effect on the viability of rat and human islets, causing apoptosis of both alpha- and beta-cells. Last, studies in Sprague-Dawley rats have demonstrated that rapamycin, with its known antiproliferative properties, is also associated with insulin resistance [5] and when combined with FK506 induces diabetes. Tacrolimus (FK506) time-dependently suppressed glucose-stimulated insulin secretion from rat islets, and at a therapeutic concentration of 0.01 micromol/liter, it suppressed glucose-stimulated insulin secretion to 32 ± 5% of the control value after 7-day incubation [6]. Most of these in vitro effects were reversible after drug withdrawal. Therefore, chronic immunosuppression with calcineurin inhibitors has proven its efficacy to limit T cell activation, but may seriously affect long term function of islet transplants [7, 8]. The need for calcineurin inhibitor-free immunosuppressive regimen appears a high clinical priority as well as strategies for long-term tolerance induction. New immunosuppression protocols are planned. T cell depleting agents such as alentuzumab (Campath-H, anti-CD52mAb) which as shown its efficacy in the management of autoimmune diseases [9], as well as several compounds that bind to CD80 and CD86, blocking the interactions with the T cell co-stimulatory receptor CD28, such as the analogue LEA29Y (Belatacept) [10] are promising. An alternative approach to traditional immunosuppression which has targeted lymphocyte activation is to inhibit lymphocyte migration to their site of activation. Emerging compounds of interest include FTY720 a potent inhibitor of lymphocyte egress from the thymus and lymph nodes which as shown promising results in islet transplantation in non human primates [11] and in autoimmune diabetes prevention in NOD mice.

Immediate islet engraftment

An important and rapid tissue loss is associated with islet transplantation, which explains the need to graft large numbers

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of islets from different donors. Human islets exposed to human blood trigger an "instant blood mediated inflammatory reaction", IBMIR, characterized by platelet consumption and activation of the coagulation and complement systems [12]. Interestingly, human islets express tissue factor (TF), an integral component in the coagulation cascade [13]. Its role in this adverse clotting reaction is suspected. The islets become surrounded by clots and infiltrated with leukocytes with evidence of islet damage. Addition of heparin reduces IBMIR and islet damage. TF and MCP-1 (macrophage chemoattractant protein) expression in human islets can also be decreased by adding nicotinamide to the culture medium [14]. These encouraging results explained why nicotinamide is used in a non randomized fashion in most islet transplantation programs. Intraportally transplanted islets are avascular at the time of transplantation and take up to 14 days to fully revascularize [15], during which time, up to 60% of islet mass may be lost. This complex glomerular-like network of blood vessels which coalesce at the periphery or traverse the central core of the islets is destroyed after enzymatic digestion. The antiproliferative effects of sirolimus may theoretically be disadvantageous both for angiogenesis in newly transplanted islets [16, 17] and for islet neogenesis from ductal stem cells [18]. Ischemia– reperfusion and coagulation-thrombosis lead to inflammation, and islets are very susceptible to injurious effects of activated macrophages and proinflammatory cytokines.

Recurrence of autoimmunity

Type 1 diabetes results from a selective destruction of the beta cells by an autoimmune mechanism. Results from twin to twin pancreas transplantation have underlined the importance of the recurrence of autoimmunity with the presence of memory T cells with CD8+ T cells that rapidly infiltrate the islets and destroy the beta cells [19]. We have previously reported in 68 C-peptide-negative diabetic patients receiving pancreatic allografts, that chronic graft failure was associated with positivity of both antibodies to GAD65 and IA-2 [20]. When immunosuppression was not adapted, rapid destruction of islet grafts were observed with a sudden rise in GAD65 and IA-2 autoantibody titers [21]. Recent pathologic observations in a patient with long-standing diabetes have shown the persistence of T cells and macrophages at the vicinity of insulin positive cells in the exocrine tissue, which limit beta cell regeneration [22]. These observations, clearly illustrate, the necessity of an efficient immunosuppression to block recurrence of autoimmunity. The predictive value of islet cell antibodies is a matter of controversy, since immunosuppressive regimen are unable to control perfectly anti-pancreas antibody production without immediate metabolic consequence. Direct analysis of islet cell infiltrates is needed to assess autoimmune recurrence through access to islet tissue or specific imaging procedures such as injection of magnetic labelled T cells and MRI [23].

Allograft rejection

The role of pretransplant sensitization to human leukocyte antigen (HLA) in islet transplantation is crucial, in theory due to the multiple sources of tissue donors. A recent study addressed this question and concluded that humoral and cellular sensitization to histocompatibility antigens, prior to and after islet transplantation, are associated with the failure of transplanted islets [24]. Rapid failure (< 3 weeks) in three cases was accompanied by increases in precursor frequencies of graft-specific alloreactive T-cells [25]. T-cell reactivities in peripheral blood can therefore be used to monitor immune mechanisms, which influence survival of beta-cell allografts in diabetic patients. Improvements in purity, yield and viability of islet preparations are rendering single donor islet transplants sufficient for insulin independence [26]. Living donor islet transplantation is another strategy to use more strict criteria of HLA matching without the haemodynamic instability and pro-inflammatory cytokines that are common in non-heart-beating and brain-dead donors and to reduce warm and cold ischaemia time [27].

Protecting islets from immunological injury through beta cell growth

Expanding stem/progenitor cells and then to convert them into beta cells by treatment with GLP-1 [28], reducing beta cell apoptosis, are additional strategies to prevent or limit the initial beta cell loss. Access to GLP-1 receptor agonists (exenatide) in clinics, have led to promising results in open trials which necessitate confirmation in randomised trials. Recent observations in NOD mice with gastrin + EGF therapies [29] are very encouraging, with the increase in islet cell mass and prevention of autoimmune diabetes. These approaches, if confirmed in humans, may play a central role in the future of islet transplantation.

Conclusion

Re-exposure of type 1 diabetic patients to living allogeneic beta cells is a complex but fascinating model of experimental immunology. Controlling both allo- and autoimmune responses is challenging. Novel immunosuppressive and inflammatory blockade agents in the field of islet transplantation have made significant improvements. Those agents should be non-diabetogenic or reduce the need for more diabetogenic immunosuppressive agents, reduce initial damage of islet cells and promote engraftment, induce a functional tolerance, and aim to manage autoimmunity, in addition to stopping allograft rejection processes. In the future, special emphasis will be placed on new immunotherapeutic strategies, as a means to produce tolerance to islet allografts without the spectrum of islet toxicity, and on additional pharmaceutical interventions to promote islet cell growth.

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Innovative therapies: some ethical considerations

G Reach

SUMMARY

The purpose of this text is to try to understand why certain innovations are not permitted, although they are possible. Our guiding thread will be the four principles of biomedical ethics defined by Beauchamp and Childress: beneficence, non-maleficence, justice and autonomy. We shall show how they can guide the ethical inquiry in the field of pancreas or islet transplantation, leading to an analysis of the risks and benefits of the innovations epistemologically taking into account their historical context.

Key-words: Innovative therapies • Four principles of ethics • Pancreas transplantation • Islet transplantation • Beta cell engineering • Review.

RÉSUMÉ

Thérapeutiques innovantes : considérations éthiques.

Le but de ce texte est d'essayer de comprendre pourquoi certaines innovations, qui sont possibles, ne sont pas permises. Nous prendrons comme fil directeur les quatre principes de l'éthique médicale, définis par Beauchamp et Childres : les principes de bienfaisance, de non-malfaisance, de justice et d'autonomie. Nous montrerons comment ils peuvent guider la réflexion éthique dans le domaine de la transplantation de pancréas ou de cellules insulinosécrétrices, conduisant à une analyse des bénéfices et des risques qui doit prendre en compte de manière épistémologique le contexte historique.

Mots-clés : Thérapeutiques innovantes • Quatre principes de l'éthique • Transplantation de pancréas • Transplantation d'îlots • Ingénierie des cellules β • Revue générale.

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Caute: On Spinoza's seal

Medical progress proceeds by steps that are sometimes innovations where we observe a break from the previous practices. The purpose of this text is to try to show how these innovations must be described in the framework of a historical context and how the very meaning of the word possible must be considered: the aim is to understand what makes it that certain things, while they are possible, are not permitted. This question of the "possible" and the "permitted" represents an essential part of the ethical inquiry. We shall take as our guide the four principles of the biomedical ethics defined by Beauchamp and Childress [1]: be beneficent (principle of *beneficence*), do not harm the patient (principle of *non-maleficence*), guarantee equity in the allocation of health care resources (principle of *justice*), and respect the autonomous choices of the patient (principle of *autonomy*). We shall take as an example the history of the treatment of diabetes (figure 1), a disease that today is treated by several daily injections of insulin, by trying to describe, from an epistemological point of view, the place of the transplantation of pancreatic tissue in the treatment of this disorder.

In 1894, i.e. 5 years after the discovery of the diabetogenic effect of total pancreatectomy performed on a dog by Minkowski, Williams in England tried to transplant fragments of sheep pancreas onto a diabetic patient [2]. At that time insulin had not yet been discovered and diabetes was a rapidly fatal disease: the question of the *possible* and the *permitted* was irrelevant. In 1966, insulin therapy was available. However, diabetes remained a disease with frightening complications, due to the lack of understanding of the role of glycaemic control in the occurrence of these complications, since it would be necessary to wait for the work of Pirart and Tchobroutsky at the end of the 1970s. That year, the first pancreas transplant was performed by Najarian in Minneapolis. Since that time, approximately 25,000 pancreatic transplantations have been performed, with success in more than 80% of the cases, patients being able to stop insulin, but at the price of a heavy immunosuppressive treatment. The justification of this practice was challenged in the late 1980s [3], at a moment marked by significant progress in the conventional treatment of diabetes. Only recently, studies provided compelling evidence of the beneficent character of the procedure, i.e. the improvement in the life expectancy and the quality of life of the transplanted patients [4, 5]. By the way, a potential "innovative therapy", the transplant of half a pancreas in the absence of immunosuppression between twins led to a double failure:

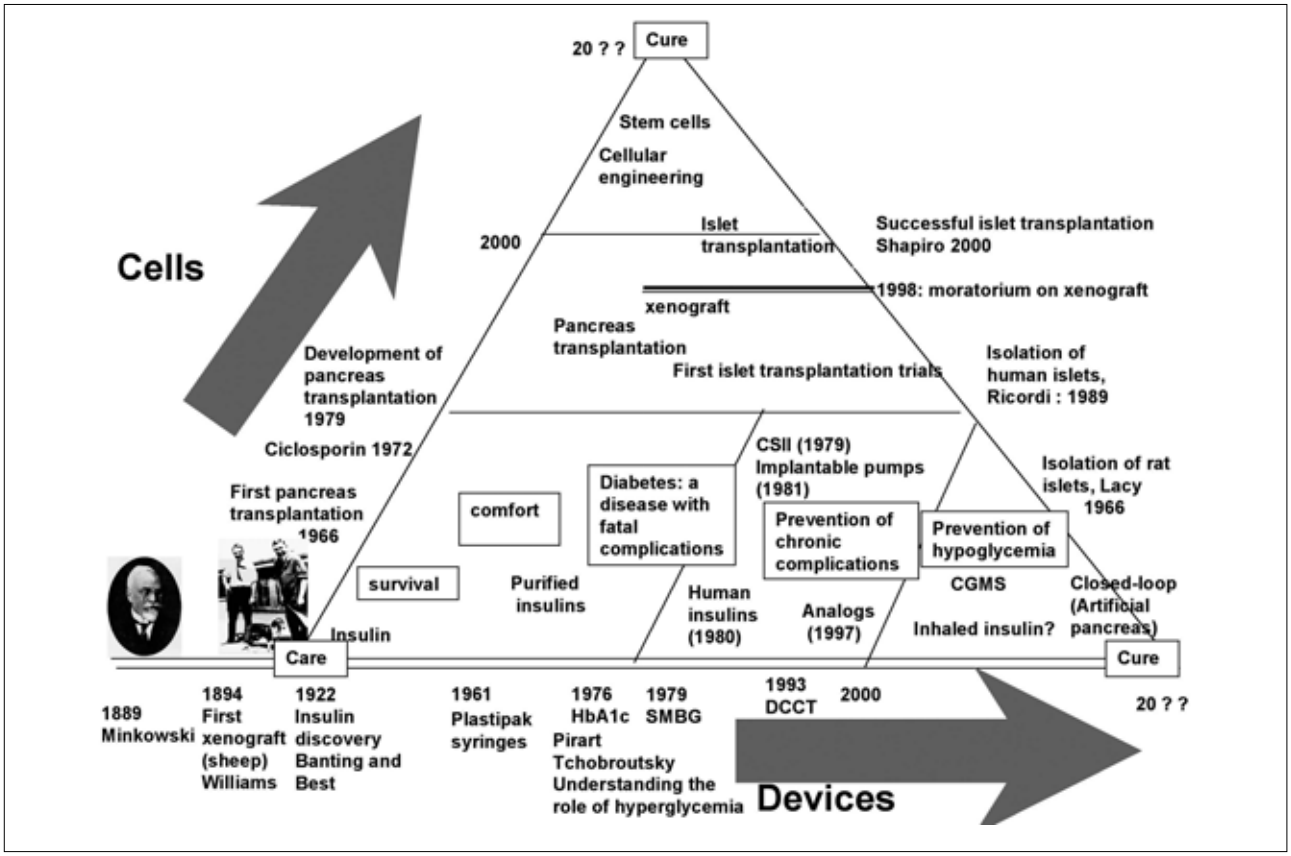

Figure 1

The pyramid of diabetes therapy: an epistemological perspective, showing landmarks of two competitive pathways, using either cells (biological) or devices (artificial). Both approaches aim to transform diabetes care into cure.

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it was not *beneficial* to the recipient: the grafted pancreas was rejected by the diabetogenic autoimmunity; it was *harmful* to the donor: their glycaemia was altered by the hemipancreatectomy [6, 7].

The transplantation of islets of Langerhans was made possible by the development of methods of isolation of islets of Langerhans from the human pancreas by Ricordi et al in 1989 [8]. Between 1990 and 2000, approximately 400 islet transplantations were performed. Nevertheless, it was not *beneficial*: their rate of success (i.e. patients off insulin) was around 5%. The analysis of the causes of this failure – diabetogenic effect of corticoids and ciclosporin, role of microangiopathy (the patients had end stage renal failure), role of an insufficient number of transplanted islets -, led Shapiro and his colleagues in Edmonton to propose a triple innovation: immunosuppressive therapy containing neither corticoids nor ciclosporin, change in the indications (patients not presenting an end stage renal failure, but brittle diabetes and recurrent severe hypoglycaemic episodes), increase in the number of transplanted islets requiring their isolation from two to three pancreas per patient. This led to the spectacular first publication of a rate of 100% success in seven consecutive patients [9]. Thus, it seems now that islet transplantation is both technically *possible* and *beneficial*. However, considering the criterion of brittle diabetes and recurrent severe hypoglycaemia, further studies are clearly needed to provide the evidence that the prescription of a long term immunosuppressive therapy, which may be *harmful* (risk of cancer and lymphomas), is permitted. It is necessary to consider the context of the treatment of the diabetes, the comfort and the efficiency of which continue to improve (figure 1). Furthermore, the fact of having to use three pancreas by recipient brings up the question of *justice* in the allocation of organs which could be used for a whole organ pancreas transplantation in several patients.

Let us suppose now that we find an effective and safe means to avoid immune rejection of the transplanted cells, for example by encapsulating them within an artificial protective membrane: this dream of the bio-artificial pancreas has been pursued by numerous teams for more than twenty years. The problem of the source of the transplantable tissue would then arise, because we would want to treat a large number of patients, which would exceed the resources of human pancreas. These considerations led to the concept of xenograft - transplantation of animal tissues -, for example from a pig. This appears to be technically possible: the method for isolating porcine islets of Langerhans is established, the physiology of the porcine islet is close to that of the human, porcine insulin has been used in humans since 1923, the breeding of pigs exempt from specific pathogens is feasible. It is even possible to create transgenic pigs, in order to avoid acute immune rejection. In fact, porcine cell transplants in humans were already performed by a Swedish team at the beginning of the 1990s: for several weeks it was possible to observe evidence of small amounts of porcine C-peptide in the urine of the patients, proving the survival of transplanted porcine cells [10]. In other words, xenograft is *possible*.

However, it is no longer *permitted*, ever since the demonstration of a possible in vitro infection, by porcine endogenous retrovirus, of human kidney cells, fibroblasts and human B and T lymphocytes under culture conditions [11]. Some raised the question of the possibility of a nightmare scenario involving the creation of a new viral disease in humans. It is true that it was possible to show in 160 recipients having had a porcine xenograft (extracorporeal bio-artificial liver and kidney, islets, skin), that there was no argument in favour of any infection by a porcine retrovirus [12]. However, these negative results do not completely reassure concerning the complete absence of risk: the extension of the technique to a large number of subjects might allow the possibility of the appearance of this rare occurrence. Therefore, currently there is a moratorium forbidding xenograft, a moratorium which was requested by some of the pioneers in this domain [13]. Thus, the case of xenograft seems ethically complex. Without even evoking the question of ethics considered from the point of view of the animal, it remains to be proven from the point of view of the human beings, first that it will be *beneficial* to the patients: to this day, there is not a single case where the real benefit of a xenograft was demonstrated;second, that it does not present the risk of being *harmful*, neither to the recipient, nor to the society in general. Furthermore, its practice brings us to consider the principle of *autonomy*: The recommendations to limit the risk of infection require an intensive follow-up of the recipients, and the list of the prohibitions with which they will have to comply with may be not compatible with this principle [14]. Thus, in the case of a disease like diabetes, which already has a treatment - insulin - , the legitimacy of this strategy is really difficult to defend.

We saw that therapeutic innovation often arises from a necessity, or from a new possibility, opened by technical progress. Now, as the legitimacy of the xenograft became questionable, other ways of obtaining insulin secreting tissue appeared. The progress of molecular biology makes it possible to create insulin secreting cells [15], by introducing, for example in pituitary cell lines, the genes which contain the code for the proteins of the insulin secretion machinery: the genes of insulin, of the glucose transporter, of the glucokinase. Or we introduce, for example, the insulin gene into hepatocytes under the control of a promoter sensitive to glucose. But will these cells, capable of producing some insulin in answer to glucose, have the fine-tuning of the genuine β-cell, where the control of insulin is regulated at the level of the secretion, not at the level of the synthesis? It will be necessary to make sure that these cells are capable of controlling diabetes, at least as well as the conventional treatment by insulin. Certainly, they represent what is made possible by the exploits of the molecular biology, but will their use be really beneficial to the patients?

Other procedures are emerging: it is possible in the case of a mouse to transform hepatic cells into insulin secreting

cells by intravenous injections of a virus carrying the gene of PDX-1, a factor of transcription which intervenes in the differentiation of the islets of Langerhans [16]. Will this be applied one day to humans? Finally, this domain, as the entire field of medicine, risks to be made obsolete by the recent discovery of the potential of cell plasticity. It is possible to obtain insulin secreting cells from stem cells either from adult tissues [17], or from embryos [18, 19]. We know that the use of human embryonic cells represents itself an ethical issue which goes far beyond the mere domain of its application, the innovative therapies, but once it will have been accepted that it is ethical under some conditions to use this kind of material, we will have to justify its use in term of beneficence/maleficence balance, just as for the other innovations which have been described previously. And it will be always necessary to consider the context: for instance, in the same period of time when we will be waiting for the successful development of novel insulin secreting cells, the possibility of closing the glucose-insulin loop may have become a reality (figure 1). Incidentally, the same ethical discussion concerning the development of these technologies is needed. For instance, a closed-loop insulin delivery system will represent as well an exploit of technology, but its performance should be critically examined in terms of its true benefits: real normalization of blood glucose profile without hypoglycaemia, improvement in glycated hemoglobin, and, last but not least, prevention of diabetic complications and improvement in the quality of life.

Thus, the preceding arguments show that therapeutic innovations, prompted by necessity when we are faced with a rapidly fatal disease (Williams and his sheep pancreas fragments), or by the analysis of a failure (Shapiro's success in 2000), or on the contrary arisen from the consequences of success (the need to develop mass production of islets), appear generally when they become possible. We saw also that they can be stopped by the occurrence of unexpected events, even to be made obsolete by the advent of competitive innovative therapies. And still, they are necessary, and from there, it is advisable to wonder what makes it so that certain things, while they are possible, are not permitted. This question should be asked by the researchers, if they do not want to expose themselves to the criticism beautifully expressed by R. Weiss at the time of the demand for a moratorium on xenografts: "I'm not telling you that it would be better not to realize clinical trials , but I ask you the question: did you stop thinking?"

Fundamental research tries to develop pure knowledge, *épistémè*. The object of knowledge, nature, is eternal: only our understanding of nature changes, not nature. Thus, the method of fundamental research is to interrogate nature without modifying it. Therapeutic innovation does not belong to the field of fundamental research, but to applied research. Applied research aims to develop téchnè. Its aim is to dominate, to force nature. Its method is to create tools. Thus, it tries to modify nature, its domain of investigation. Therefore, it has to integrate the notion of future, which is by definition contingent. This is why therapeutic innovation is at the same time necessary for progress and presents intrinsic risks. Let us remind the readers that the word "risk" comes from the Latin *risicare*, to double a cape. What is behind the cape, or rather after the cape? The unknown, the danger!

We are thus driven to justify risk-taking. As pointed out by Evandro Agazzi [20], risk represents an essentially anthropological category: nature does not know the categories of choice and decision; God does not take risks; only we, humans, are capable of taking risks, of deciding to realize a project. It is this possibility of risk-taking that made possible all exploration, all investigation, all progress. But it is not justifiable to defend any risk. Agazzi distinguishes between the "sectorial", individual risk, at the level of the patient, requiring a decision on a case by case basis, depending on the context, risk which can be possibly taken, and the total risk, the collective risk which puts in danger the future of humanity. The latter kind of risk cannot be taken. It is this last type of risk that Hans Jonas evokes in his *Responsibility Principle* [21], with his concept of fear heuristics. We understand that the most real anxieties concern the risks of modification of the nature of humanity: infectious risk of the xenograft, which imposes a moratorium as long as it is not proved that it exists, problem of the embryonic cells, where some people see a risk of drift towards reproductive cloning.

If we come back to the more common sectorial risk, involving only the individual patient to whom the innovative therapy is proposed, it is important to recognize that risk is a relative concept. 1) The respective risks of action and of inaction must be taken into account in the deliberation: let's remember that diabetes remains nowadays a severe and distressing disease and that any efficient and safe method to improve the quality of metabolic control and the quality of life will be welcome. 2) The ethical inquiry has to consider the severity of the disease and the availability of conventional therapies. The fact that type 1 diabetes has already a treatment, relatively efficient, is obviously a brake to the implementation of novel techniques, which will have to prove that they are at least as efficient (similarly in clinical trials aimed to prove the interest of a novel drug, it will not be compared to a placebo, but to a competitor, if available). 3) Therefore, as shown in this paper, the evaluation of the risk/benefit of a novel therapy cannot be definitive, and has to take into account the appearance of new "competitors". 4) It is also important to consider the risks linked to rare events, which may not be detected in small scale clinical trials. This, points out the importance of registries reporting for each individual patient the outcome of novel therapies.

An ethics of caution is thus necessary. Indeed, according to Aristotle in the Nichomachean Ethics [22], what characterizes the cautious man, it is the "good consideration", that allows him to avoid immoderation, to appreciate the obstacles, to take into account particular cases, to choose the convenient

moment, to foresee even the unpredictable. To take into account particular cases: the ethical answers can be only casuistic. To choose the convenient moment is to take into account the context, which, as we saw in the example of the history of the treatment of the diabetes, is evolving. To appreciate the obstacles is to be capable of being able to by-pass them, thus to take the risk of going beyond the cape. But also to avoid immoderation, the *hybris* of the Greek philosophers, and this will protect us against the temptation of unjustified risks. The Latin word *caute* ("be cautious") was written on Spinoza's seal.

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Development of the endocrine pancreas

G Gradwohl

SUMMARY

This short review presents the recent breakthroughs in our understanding of the important steps controlling pancreas morphogenesis and differentiation, and on the transcription factors regulating pancreas organogenesis and islet cell differentiation and involved in the specification of the beta and alpha cell lineages. All these studies should permit a comprehensive view of the full genetic program necessary to produce mature and functional beta cells and thus, should be instrumental to guide future strategies for cell replacement therapies in type 1 diabetes.

Key-words: Development • Endocrine pancreas • Transcription factors • Research • Review.

RÉSUMÉ

Développement du pancréas endocrine

Cette brève revue présente les développements récents qui nous permettent de mieux comprendre la morphogenèse et la différenciation pancréatique, et les facteurs de transcription impliqués dans la détermination des lignées cellulaires alpha et bêta pancréatiques. L'ensemble de ces données devrait permettre une meilleure identification du programme génétique nécessaire à la production de cellules bêta pancréatiques matures et fonctionnelles, et ainsi guider les futures stratégies de recherche pour les thérapies cellulaires dans le diabète de type 1.

Mots-clés : Développement • Pancréas endocrine • Facteurs de transcription • Recherche • Revue générale.

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Understanding the mechanisms controlling multipotent stem cells differentiation into specialized cells during the embryonic life is not only one of the current challenge in stem cell biology but will also have a crucial impact on future cell replacement therapies designed to treat diseases such as type 1 diabetes. Indeed, *de novo* generation of beta cells from pancreatic stem/progenitors occurs essentially during embryogenesis. Understanding the underlying molecular mechanisms is thus essential if we want to recapitulate the beta cell differentiation program and generate functional insulin-secreting cells for therapy starting from plastic cells such as embryonic or somatic stem cells.

In the last years, major breakthroughs in our understanding of the important steps controlling pancreas morphogenesis and differentiation have been obtained [review in 1, 2]. In rodents, the first signs of pancreas organogenesis are the formation of two pancreatic buds (ventral and dorsal) emanating from the foregut endoderm at mid-gestation at the level of the future duodenum. Pancreatic endocrine, exocrine and ductal cells have an endodermal origin. The specification and growth of the two pancreatic buds is controlled by different signals originating from the adjacent mesodermal tissues. Dorsally the pancreatic bud is sequentially exposed to signals from the notochord, dorsal aorta and pancreatic mesenchyme. Ventrally both the cardiac mesoderm and vitellin veins control pancreas development. The two buds later fuse and the pancreatic epithelium branches within the surrounding mesenchyme. Concomitantly the different pancreatic cell types differentiate from immature pancreatic progenitor cells. The future endocrine cells delaminate from the epithelium, migrate and aggregate in clusters called islets of Langerhans.

Through the analysis of genetically modified mice, a hierarchy of transcription factors regulating pancreas organogenesis and islet cell differentiation was established recently [review in 3, 4]. Two transcription factors, the genes Pdx1 and Ptf1a/p48 regulate the very early steps of pancreatic endoderm specification. Research performed in our laboratory focuses on the transcriptional program implemented subsequently in these early pancreatic progenitor cells to determine their endocrine fate as well as endocrine subtype specification. In this line we identified a master gene, the bHLH (basic helix-loop-helix) transcription factor Neurogenin3 (Ngn3) as a specific marker of islet progenitor cells in the mouse and essential regulator of the endocrine lineage determination [5]. Insulin-glucagon-somatostatin-PP- and the recently discovered Ghrelin-producing cells all derive from Ngn3-expressing immature cells [6, 7]. We showed that Ngn3-deficient mice die from diabetes because islet cells are lacking demonstrating that Ngn3 is required for the development of the five pancreatic endocrine cell types including insulin-producing beta cells. These results together with gain of function studies [8, 9] demonstrated that, during development, Ngn3 acts as a genetic switch controlling endocrine fate decisions in multipotent pancreatic progenitor cells. The transcription factors Pax4 and Arx have been shown to be important, downstream of Ngn3, for the specification of the beta and alpha cell lineages respectively [10, 11]. To further understand the molecular and biological characteristics of islet progenitor cells we have generated mice where this cell population can be purified [12]. To find additional regulators of islet sub-type specification and endocrine differentiation we have performed a series of DNA microarray hybridization and determined the complete transcriptome of the purified islet progenitor cells as well as identified the panel of Ngn3-target genes (unpublished). These studies led to the identification of the zinc finger transcription factor IA1/Insm1 a direct target of Ngn3, essential for the maturation of islet cells [13, 14]. Additional known and unknown genes enriched in islet progenitor cells induced by Ngn3 an dlost in Ngn3-dficient mice are currently being characterized.

Taken together, these and other studies should generate a comprehensive view of the full genetic program necessary to produce mature and functional beta cells and should thus be instrumental to guide future strategies for cell replacement therapies in type 1 diabetes.

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Continuous glucose monitoring and external insulin pump: towards a subcutaneous closed loop

H Hanaire

SUMMARY

The development of an artificial pancreas for the treatment of type 1 diabetes is a highly desired endeavour for type 1 diabetic patients, physicians, scientists and engineers. The development of the first miniaturized external pumps in the 70s and the pharmacokinetic properties of short acting insulin analogues, closer to physiology, have raised hopes for the elaboration of such a device. Recent technological progress in the development of continuous glucose sensors, have improved the reliability and accuracy of these devices. This has led to the development of prototypes of closed-loop system based on the combination of a continuous monitor, a control algorithm, and an insulin pump. This review focuses on the SC-SC approach, employing subcutaneous glucose monitoring and subcutaneous insulin delivery. The feasibility of this solution has been proven at a small scale, but remains to be confirmed in the home setting. Intermediate solutions, such as semi-automatic systems, might be immediately valuable.

Key-words: Type 1 diabetes • Continuous glucose monitoring • Continuous subcutaneous insulin infusion • Artificial pancreas • Automated insulin delivery • Review.

RÉSUMÉ

Surveillance glycémique continue et pompe à insuline externe : vers une boucle fermée sous-cutanée

Le développement d'un pancréas artificiel est un objectif hautement désirable pour les patients diabétiques de type 1, les médecins, les scientifiques et les ingénieurs. La mise au point des premières pompes externes miniaturisées dans les années 70, et les propriétés pharmacocinétiques des analogues de l'insuline de durée d'action brève, plus proche de la physiologie, ont suscité des espoirs pour l'élaboration d'un tel outil. Les progrès technologiques récents dans le développement des capteurs de glucose ont amélioré leur sûreté et leur précision. Ceci a permis l'élaboration de prototypes de systèmes en boucle fermée fondés sur la combinaison d'un moniteur continu de glucose, d'un algorithme de contrôle et d'une pompe à insuline. Cette revue est centrée sur l'approche SC-SC, qui utilise la mesure sous-cutanée du glucose et l'administration sous-cutanée de l'insuline. La faisabilité de cette démarche a été prouvée à petite échelle, mais reste à démontrer en ambulatoire. Des solutions intermédiaires, telles que les systèmes semi-automatiques, pourraient présenter un intérêt immédiat.

Mots-clés : Diabète de type 1 • Mesure continue du glucose • Perfusion sous-cutanée continue d'insuline • Pancréas artificiel • Administration automatisée d'insuline • Revue générale.

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The goal of type 1 diabetes treatment is to achieve tight glucose control, in order to avoid chronic complications, while limiting the frequency of hypoglycaemic episodes in the day-to-day life. Although considerable efforts have been made to improve the pharmacokinetics of insulin and to develop user-friendly monitoring and treatment tools, this goal remains difficult to achieve, and the desire and demand for an automated artificial pancreas is still up to date. Such a system includes an insulin pump, capable of delivering insulin continuously, a monitoring device, capable of sensing continuously glucose, and algorithms for calculating the insulin delivery rate, in order to achieve the normalisation of glucose concentrations [1].

Several control algorithms have been proposed for the automated regulation of glucose by insulin. The pioneering device, the Biostator, was developed in the 1970's [2]. However, the use of this bulky device remains limited to hospitals and research centres.

In the last decades, considerable technological progress has been made. Since the introduction of continuous subcutaneous insulin infusion, the insulin pumps have been miniaturised and their reliability improved. The accuracy and reliability of glucose sensing devices has also been improved. It is then tempting to develop a closed-loop system based on these two devices, despite the drawbacks of the subcutaneous tissue, i.e. the delays in glucose sensing and in insulin delivery.

The present review focuses on the development of subcutaneous – subcutaneous (SC-SC) closed-loop systems, which adopt the subcutaneous route for both glucose monitoring and insulin delivery. The intravenous– intraperitoneal approach is developed in this issue by E. Renard *et al* [3].

Continuous glucose monitoring

The information obtained with the glucose monitoring system should be accurate, real-time, and continuous (or frequent if intermittent). The glucose sensors that measure glucose concentrations in the interstitial fluid can be non-invasive or minimally invasive. Most non-invasive approaches are carried out using optical glucose sensors. The basic premise of optical glucose sensors is to direct a light beam through the skin and to measure the alterations of the properties of the reflected light. Although this approach seems attractive, the specificity of glucose measurement is poor, because of numerous interferences [4]. The reverse iontophoresis approach (Glucowatch®, Cygnus) allows the measurement of glucose concentration in the interstitial fluid after its transdermal extraction [5]. After a 3-h warm-up period, the device is capable of providing up to three glucose readings per hour for 12 h after a single point calibration with a self blood glucose measurement. However, the measurement result is not immediately available (the time required for sample extraction and analysis is 20 minutes), and the current applied to the skin causes some degree of irritation. The device cannot be used in case of increased sweating, and this is a concern for the detection of hypoglycaemia. For all these reasons, these two approaches are not suitable for the closed-loop.

Minimal-invasive methods are based on the microdialysis or on the use of amperometric enzyme electrodes.

The microdialysis system developed by Menarini (Glucoday®) can be used for three days [6]. The semi-permeable dialysis fibre is inserted into the subcutaneous tissue and perfused with glucose-free isotonic fluid. Due to the concentration gradient, glucose diffuses from the interstitial fluid through the dialysis membrane into the perfusate. The dialysate is pumped to a glucose sensor outside the body where the glucose concentration is measured continuously. A calibration is required once daily. As the glucose sensor is outside the body, no significant signal drift has to be feared. However, the time lag inherent to the technique is a disadvantage. It is related to the length of the tubing and the perfusion flow rate.

The other minimal invasive approach is based on the use of electrodes covered with glucose oxidase and submitted to the application of a potential. The electrode is inserted in the subcutaneous tissue and measures the change in current flow caused by the enzyme-catalyzed production of hydrogene peroxide, which is proportional to the amount of glucose at the site of insertion. The main drawback of this technique is the signal drift induced by the reaction of the subcutaneous tissue to the electrode and the changes in glucose and oxygen diffusion near the electrode. This can be compensated for by frequent recalibrations.

The continuous glucose monitoring system (CGMS®, Medtronic MiniMed) is the first currently available monitoring system based on this technique. It uses a subcutaneously inserted flexible needle sensor containing glucose oxidase, which converts interstitial glucose into a measured electrical current [7]. It requires four calibrations per day, does not display the current glucose concentration measured, but allows retrospective analysis of interstitial glucose readings every 5 minutes for 72 hours. The next generation of the system (Guardian RT®) functions in real time, with hypoglycaemic and hyperglycaemic alarms, and allows extended use by the patient himself [8].

Two other devices based on the glucose electrode technique and capable of displaying glucose measurements in real-time are currently submitted to the FDA approval: the Navigator® system (Abbott) [9] and the Dexcom® system [10].

All these devices have been studied for their reliability, and most of them for their ability to improve glycaemic control (HbA_{1c} and/or frequency of hypoglycaemic episodes) when used in the real life. At present, the non invasive techniques based on spectroscopy and the reverse iontophoresis technique do not fulfil the desirable features of a glucose monitoring system included in a closed-loop. Minimal invasive techniques, microdialysis and glucose electrodes, allow frequent, durable and reliable glucose monitoring, and can

therefore be used for an automated insulin delivery system. Their weaknesses are the delay of glucose measurement and the disparity of interstitial and venous glucose measurement [11], and the need for frequent recalibration.

Continuous insulin delivery

Continuous subcutaneous insulin infusion (CSII) with external insulin pumps was introduced in the 1970s as a way of achieving and maintaining strict control of blood glucose concentrations in type 1 diabetic patients [12], by means of more physiological insulinisation than that with multiple daily injections (MDI). The exclusive use of soluble, short acting insulin, infused subcutaneously at the same site for 2 or 3 days, reduces the variability of insulin absorption when compared to long acting insulins. CSII allows a wide flexibility of insulin infusion, thanks to the ability of programming several basal rates and adjusting meal boluses when required. Several studies have concluded to the superiority of CSII over MDI in terms of HbA_{1c} [13-16]. In the DCCT (Diabetes Control and Complication Trial), HbA_{1c} levels in the intensive group were significantly lower with CSII than with MDI (-0.2 to -0.4%) [17]. Recent meta-analyses report an overall benefit of CSII over MDI, with a reduction of HbA_{1c} in the range of 0.4 – 0.5% [18, 19].

Several randomised controlled trials have shown that the use of short-acting insulin analogues is more efficient on HbA_{1c} levels than human insulin [20-22], this has been confirmed by a meta-analysis [23]. The pharmacokinetic properties of the analogues are certainly responsible for the improvement in postprandial glucose levels and stability. However, the efficacy of CSII versus MDI therapy has been evaluated only in a limited number of randomised controlled trials in which rapid-acting analogues were used for both regimens, two out of three concluding to a superiority of CSII [24-26]. The pooled analysis of these three studies suggests that CSII is associated with better glycaemic control, particularly in those patients with suboptimal initial control [27].

The subcutaneous route of insulin delivery is easy to use, and recent improvements in terms of reliability of the devices and pharmacokinetics of the insulin analogues allow to consider its use for an automated insulin delivery, although the subcutaneous site introduces additional delays in insulin kinetics not seen with intravenous delivery.

Subcutaneous – subcutaneous closed loop – system

Two major practical solutions of a closed-loop system based on the body interface exist. The IV– IP approach relies on intravenous glucose monitoring and intraperitoneal insulin delivery and is described elsewhere. The developments of the SC– SC approach, which adopts the subcutaneous route for both glucose monitoring and insulin delivery, is discussed here.

Clinical considerations for the use of a SC-SC closed-loop

The SC– SC approach has the advantage of a minimally invasive solution, with the greatest potential to achieve widespread application. On the other hand, the use of the subcutaneous site is responsible for delays in glucose reading and in insulin action that may be difficult to overcome, especially when rapid changes in insulin delivery are needed to compensate for rapid and large glucose levels changes, especially during the meals.

There are several causes explaining these delays. When glucose levels change, there is a lag in the equilibration between the interstitial and plasma glucose that will vary depending on physiologic conditions. Following a glucose load, the interstitial glucose concentration lags behind the blood glucose. On the contrary, following insulin administration, the decline in glucose concentration in the interstitium precedes that in the blood [28]. With microdialysis based systems, there is an additional lag required to transfer the interstitial fluid sample to the glucose sensor [29]. The delayed absorption kinetics of subcutaneously delivered insulin is an additional factor to be taken into account. It may lessen the efficacy of insulin when glucose levels change rapidly after a meal, but also result in an extended postprandial glucose lowering effect, compromising the efficacy of the system. Therefore, the first control systems based on the SC– SC approach will probably be semiclosed-loop systems or hybrid systems, requiring at least a partial manual assistance to the delivery of insulin for meals [30].

System variability

Not only the difficulty to overcome wide glucose fluctuations after the meals can disturb the efficacy of a control system. Even in healthy individuals, insulin sensitivity varies both day-to-day and throughout the day. Diurnal variance can result either from a change in insulin sensitivity per se, or a change in endogenous glucose production. In a type 1 diabetic patient, these changes result in varying basal insulin requirements throughout the day [31]. Insulin requirements for meals of identical carbohydrate content can also vary, depending on the type of carbohydrate and the presence of dietary fat and alcohol. Insulin sensitivity is also modified by physical exercise, in an individual-specific manner. A large number of parameters can influence insulin requirements, and might interfere with the efficacy of a closed-loop insulin delivery algorithm.

Model Predictive Control (MPC)

In this control approach, a mathematical model of the subject's glucose response is derived from one of the many models of the glucoregulatory system [32]. Measured glucose values enter a "parameter optimiser" which estimates individual parameters of the glucoregulatory model. These parameters are used to make individualised predictions of

glucose excursions, and take into account the estimation of insulin sensitivity. The model predicts how insulin would affect the future glucose profile, and calculates the first insulin delivery value. On the next sample interval, the difference between the measured glucose and the model-predicted values is reassessed, and the optimal insulin delivery profile is calculated. These steps are repeated, improving the predictive accuracy of the model.

The MPC-based system has been studied by the European consortium of partners on the Advanced Insulin Infusion using a Control Loop (ADICOL) since 2000 [33]. The first clinical studies have been performed with an intravenous (IV) sensor with a 15 minutes sample time and subcutaneous delivery of insulin lispro. The following studies were performed with the same IV sensor, but the measurements were delayed by 30 minutes in order to mimic the time lag associated with a subcutaneous sensor. In these two sets of experiments, the MPC system was able to achieve normoglycaemia during fasting conditions while avoiding hypoglycaemia. A progressive and pronounced reduction in the standard deviation of plasma glucose was observed, demonstrating the ability of the algorithm to bring all subjects close to the target. Another experiment was conducted in the fasting and postprandial condition, the prandial bolus being individually determined according to the carbohydrate content of the meal, and the MPC being run for three additional hours after the meal.

All these experiments were conducted with IV glucose sensing. Only 5 subjects could benefit from the MPC system with a subcutaneous sensor, at the end of the program. Nevertheless, the ADICOL project gives an interesting approach to a semi-closed-loop control with subcutaneous insulin delivery.

Physiologic Insulin Delivery system (PID)

The PID system, aims at mimicking the mechanisms by which the beta-cell maintains tight glucose control. The main points are that the beta-cell adapts its secretory response to the individual's underlying insulin sensitivity, and that it adjusts the ratio of first- to second-phase insulin to compensate for a delay in insulin action. The Medtronic MiniMed external PID system [34] includes three terms: proportional, integral and derivative (figure 1). Basal insulin delivery is determined by the slow component (integral). Once the meal begins, the rate of change component (derivative) results in a rapid rise in insulin delivery, and is accompanied by a proportional component (proportional), as glucose rises above a set-point [35]. This third component is equal to zero when glucose is at target concentration. The derivative component counteracts rapid changes and can be considered to reproduce the first phase of insulin secretion. The integral component adapts to changes in insulin sensitivity and links insulin administration to the difference between the ambient and the target glucose.

The closed-loop insulin delivery system developed by Medtronic-MiniMed is composed of a Guardian-type subcutaneous sensor equipped with a transmitter, and transmitting

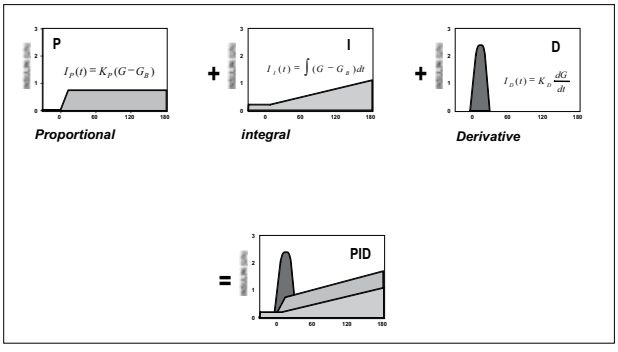

Figure 1
Components of the Physiologic Insulin Delivery (PID) system [adapted from ref. 34].

glucose values to a computer which calculates insulin delivery rates every minute and transmits the rate to an external pump. The first studies with a fully closed-loop were carried out in dogs. The algorithm allowed to reach the glucose target under fasting conditions, but failed to achieve normoglycaemia in the postprandial state [35]. A recent evaluation of the PID system was carried out in 10 type 1 diabetic subjects over 24 hours with meals. Satisfactory glucose control was obtained overnight, but postprandial glycaemic excursions remained excessive. Therefore, a hybrid, semi-automatic control with "priming" conventional pre-meal bolus is currently under investigation in the Yale group.

Conclusion

Glucose monitoring has been the main limiting factor to the development of a viable closed-loop solution, and the perspective of a closed-loop system has been one of the main driving forces for glucose sensor development. The currently available sensors display satisfactory properties in terms of reliability and accuracy. If the subcutaneous route of insulin infusion remains a barrier, the reliability of insulin pumps and the pharmacokinetics of insulin analogues have given adequate answers enough to encourage the hopes for an automated artificial pancreas. Several algorithms are under evaluation, and if the results obtained in the fasting state are more than encouraging, the postprandial state remains difficult to handle with. Before a fully automated device is available, intermediate steps may already be valuable. Continuous glucose monitoring with real-time access to the glucose values, facilitating self-adjustments of diabetes management by the patient, has already proven efficient in pilot studies. Semi-automatic systems, with a partial control of the postprandial state by a manual bolus, are also of interest. The development of closed-loop solutions in controlled environments such as in the intensive care units, is an interesting area, as the benefits of tight control have been proven in this field. From this application area, spin-off to other areas should be possible.

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Immunosuppressive drug-induced diabetes

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SUMMARY

Post-transplant diabetes mellitus (PTDM) has emerged as a major adverse effect of immunosuppressive drugs (ISD). As recipients of organ transplants survive longer, the complications of diabetes mellitus have assumed greater importance. The predominant factor for causing PTDM by corticosteroids seems to be the aggravation of insulin resistance, however several studies have displayed deleterious effects on insulin secretion and β-cells. Calcineurin inhibitors induce PTDM by a number of mechanisms, including decreased insulin secretion and a direct toxic effect on the pancreatic β-cells. Recent *in vitro* studies stress on the increased apoptosis of β-cells when exposed to these drugs. Studies involving other immunosuppressive agents (mycophenolate mofetil [MMF], sirolimus) are scarcer and lead to conflicting results, while daclizumab seems to have a neutral effect. Clinical studies have consistently shown a greater potential of tacrolimus to induce PTDM compared with cyclosporine. Reducing PTDM incidence is a feasible goal while using corticosteroid-sparing regimens and/or lower tacrolimus trough levels. In patients developing PTDM, conversion from tacrolimus to cyclosporine could improve or reverse glucose tolerance abnormalities. In the absence of well-designed studies in this specific indication, treatment of PTDM is based on the same principles as type 2 diabetes mellitus. Thiazolidinediones do not display any pharmacological interaction with calcineurin inhibitors, but their safety and efficacy in PTDM need to be confirmed in large-scale randomized trials. Use of sulfonylureas has to be cautious regarding the suspected interaction of some of them with calcineurin inhibitors. If needed, insulin regimens have to be adapted in patients who display the particular glycaemic profile of corticosteroid-induced diabetes. Incretin-based therapies, due to their specific action on β-cell apoptosis and proliferation, raise promises that have to be confirmed in clinical studies.

Until methods for inducing specific graft tolerance become available, immunosuppressive regimens should be tailored to the individual patient on the basis of predictive criteria for the development of PTDM.

Key-words: Post-transplant diabetes mellitus • Immunosuppressive drugs • Tacrolimus • Cyclosporin • Corticosteroids • Review.

RÉSUMÉ

Diabète induit par les immunosuppresseurs

Le diabète post-transplantation (DPT) est un des principaux effets secondaires des immunosuppresseurs. Avec l'augmentation de la survie des receveurs d'organes, les complications du diabète sont devenues un problème majeur. L'action diabétogène principale des corticoïdes passe par l'aggravation de l'insulinorésistance, mais plusieurs études ont rapporté des effets délétères sur la sécrétion d'insuline et sur la cellule β pancréatique. Les inhibiteurs de la calcineurine peuvent induire un DPT par plusieurs mécanismes, principalement en diminuant la sécrétion d'insuline et par un effet toxique direct sur la cellule β. Des études *in vitro* récentes ont montré une augmentation de l'apoptose des cellules , soumises aux inhibiteurs de la calcineurine. Dans de rares études qui impliquent d'autres immunosuppresseurs [mycophénolate mofétil (MMF), sirolimus] les résultats apparaissent contradictoires, le daclizumab semble, quant à lui, ne présenter aucun effet délétère.

Les études cliniques s'accordent, cependant, à démontrer le fort potentiel du tacrolimus à induire un DPT en comparaison à la ciclosporine. La réduction de l'incidence du DPT est possible et repose sur l'utilisation de schémas d'immunosuppression sans glucocorticoïdes et/ou avec de faibles doses de tacrolimus. Chez les patients développant un DPT, la substitution du tacrolimus par la ciclosporine est susceptible d'améliorer ou de corriger les anomalies du métabolisme glucidique. En l'absence d'études spécifiques réalisées dans ce domaine, la prise en charge du DPT est semblable à celle du diabète de type 2. Les thiazolidinediones ne présentent aucune interaction pharmacologique avec les inhibiteurs de la calcineurine, mais leur efficacité et leur tolérance nécessitent d'être confirmées dans des études randomisées à grande échelle. Le recours aux sulfamides hypoglycémiants doit rester prudent en raison, pour certains d'entre eux, d'interactions avec les inhibiteurs de la calcineurine. Si nécessaire, les schémas d'insulinothérapie doivent être adaptés chez les patients présentant le profil glycémique particulier du diabète cortico-induit. Les incrétino-mimétiques, de par leur action spécifique sur l'apoptose et sur la prolifération des cellules β, représentent une thérapeutique d'avenir dans cette indication du DPT, mais ces promesses doivent être confrontées aux résultats d'études cliniques. Dans l'attente de méthodes susceptibles d'induire une tolérance spécifique en greffe d'organe, le recours aux immunosuppresseurs doit être adapté à chaque patient en fonction de son risque potentiel de développer un DPT.

Mots-clés : Diabète post-transplantation • Traitement immunosuppresseur • Tacrolimus • Cyclosporine • Glucocorticoïdes • Revue générale.

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New-onset diabetes mellitus after solid organ transplantation is frequent [1-3] and associated with increased morbidity and mortality [4]. Among several risk factors predisposing to post-transplantation diabetes mellitus (PTDM) [4], the type of immunosuppression plays a major role, accounting for 74% of the variability of 12-month cumulative incidence of PTDM cases in a systematic review of the literature up to 2000 [5], with inclusion of corticosteroids and/or high-dose cyclosporine (CsA) or tacrolimus being the main risk factors. Even if insulin resistance is a major contributor to the physiopathology of PTDM, we will focus on the effects of immunosuppressive drugs (ISD) on the pancreatic β-cell function. The challenge for the transplant teams is to reduce the diabetogenicity of the immunosuppressive regimens and decrease the cardiovascular risk of the recipients, while containing a low rate of acute and chronic rejections.

ISD implicated in diabetes mellitus

Corticosteroids

In vitro and animal studies

If the role of corticosteroids in increasing hepatic glucose production, through gluconeogenesis stimulation, and peripheral insulin resistance has been established for a long time [6], and is considered to be the main contributor of their diabetogenicity, more recent studies have underlined their deleterious effects on insulin secretion [7, 8]. Several mechanisms, displayed in vitro studies on murine β-cells or human cell lines incubated with dexamethasone, have been proposed: insulin secretion inhibition by increased expression of α_2-adrenergic receptors [9, 10], decreased cAMP levels [11, 12], GLUT2 protein decrease at the β-cell plasma membrane [13, 14], downregulation of glucokinase mARN [15], increased voltage-gated K^+ channel activity [16], β-cell apoptosis through the activation of the calcineurin phosphatase and the corticosteroid receptor [17]. In this last study, dexamethasone-induced β-cell apoptosis was inhibited by the GLP-1 analogue, exendin-4 [17]. Whether a treatment with GLP-1 analogue could prevent or decrease the severity or reverse corticosteroids-induced diabetes would deserve to be studied.

Calcineurin inhibitors

The diabetogenicity of calcineurin inhibitors has been demonstrated, in both animals and humans, to be mediated through suppression of pancreatic insulin secretion [7, 18-21].

In vitro and animal studies

Morphologic abnormalities, including nuclear inclusions, cisternal dilatation of both the rough endoplasmic reticulum and the Golgi apparatus, vacuolisation [22], severe cytoplasmic degranulation and degeneration of islet β-cells [23], and cell death are observed with both calcineurins inhibitors in rodent β-cells.

Furthermore, both calcineurin inhibitors impair insulin gene transcription regulation [24] through inhibition of calcineurin signalisation [25-29]. Other mechanisms have been proposed: closing of the ATP-sensitive potassium channel [30], interference with mitochondrial function of pancreatic β-cells (CsA) [31], impairement of glucose-stimulated insulin secretion downstream of the rise in intracellular Ca^{++} at insulin exocytosis [32], reduced ATP production and glycolysis derived from reduced glucokinase activity [33], decreased islet cell viability by a downregulation of anti-apoptotic factors and an accumulation of pro-apoptotic mediators [34] in cultures of freshly isolated human islets. A very recent work by Heit *et al*. demonstrated the crucial role of the calcineurin phosphatase regulatory subunit, calcineurin b1, in regulating multiple factors that control growth and hallmark β-cell functions in mice [35]. β-cell-specific deletion of this subunit led to age-dependent diabetes characterized by decreased β-cell proliferation and mass, reduced pancreatic insulin content and hypoinsulinaemia. Moreover, β-cells lacking Cnb1 have a reduced expression of established regulators of β-cell proliferation.

Very interestingly, a report of D'Amico E *et al*. demonstrates that GLP-1 is capable of preserving β-cell function and protecting cells from apoptotic cell death in mouse insulinoma cells exposed to a cocktail of ISD [36].

In summary, if both calcineurin inhibitors alter insulin secretion by several mechanisms, the effects of tacrolimus seem to be more profound and intense compared with the CsA-induced ones. One possible explanation could come from that the tacrolimus specific binding protein, *i.e.* FKBP-12, is preferentially located in β-cells, leading to a strong concentration of the drug in these cells. In contrast, the CsA specific binding site (ciclophiline) is preferentially located in the heart, the liver and the kidneys.

Clinical studies

In spite of some conflicting results [37], clinical studies have confirmed the deleterious effects of tacrolimus on insulin secretion. Boots et al. have examined the respective effects of steroids and tacrolimus in 15 non diabetic kidney transplant recipients, using IVGTT [38]. After withdrawal of 10 mg of prednisolone, insulin resistance significantly decreased. After tacrolimus trough level reduction from 9.5 to 6.4 ng/ml, pancreatic β-cell secretion capacity significantly improved, along with a HbA_{1c} improvement, from 5.9 to 5.3% (p=0.002). Strumph et al. studied seven non-diabetic, non-transplanted subjects who were to receive FK506 for autoimmune diseases. All subjects underwent two standard oral glucose tolerance tests and two 180-min hyperglycaemic clamps immediately before and 10 weeks after starting FK506. FK506 decreased insulin secretion, regardless of initial glucose tolerance, while insulin sensitivity did not change.

It has also been reported a correlation between tacrolimus blood levels and PTDM incidence in kidney transplant recipients [39] as well as a statistically significant negative correlation between CsA concentration and insulin, proinsulin, C-peptide blood levels and a statistically significant positive correlation between CsA and glucose blood level in heart transplant recipients who developed hyperglycaemia after transplantation [40].

Striking features observed in most of the above-mentioned studies when they have been examined, are the dose-dependence and the reversibility of the deleterious effects on calcineurin inhibitors on the β-cells [41]. These findings are, of course, of paramount importance from a clinical point of view.

Other ISD: mycophenolate mofetil (MMF), sirolimus, daclizumab

The effects of ISD other than calcineurin inhibitors or corticosteroids are fairly less documented. Furthermore, the results of the studies are quite discordant.

In vitro and animal studies

Insulin secretory parameters and insulin gene expression of cultured human islets have been studied in the presence of ISD [19]. In opposite to FK506 and CsA, MMF had no deleterious effects. Other experimental in vitro and in vivo studies conclude to the neutral or beneficial role of sirolimus [42]. By contrast, Paty et al. exposing HIT-T15 cells and Wistar rat islets to various concentrations of five immunosuppressive agents found that glucose-stimulated insulin secretion was significantly inhibited after exposure to MMF and sirolimus, even more that after exposure to CsA or tacrolimus [7]. No reduction in insulin secretion was observed after exposure to daclizumab. In another study [34], MMF and sirolimus were able to decrease islet cell viability by downregulate anti-apoptotic factors in cultures of freshly isolated human islets. Other studies have displayed deleterious effects of sirolimus on MIN-6 cells and rat islets, but at supra-therapeutic concentrations [43].

Clinical studies

In a recent work [44], Italian authors have investigated the effect of the withdrawal of calcineurin inhibitors and the switch to sirolimus on peripheral insulin résistance and pancreatic β-cell response in 41 kidney transplanted patients: 26 in whom CsA was converted to sirolimus and 15 who were treated with sirolimus and tacrolimus for the first three months after grafting and then with sirolimus alone. Based on the results of OGTT and IVGTT before and six months after conversion to sirolimus-alone therapy, the withdrawal of anti-calcineurins was associated with a significant fall of insulin sensitivity (P=0.01) and with a significant defect in the compensatory β-cell response, as measured by the disposition index. These deleterious effects significantly correlated with the change of serum triglyceride concentration after the conversion to sirolimus-based therapy. Clinically, the switch to sirolimus was associated with a 30% increase of impaired glucose tolerance incidence and with the occurrence of four *de novo* diabetes.

By contrast, in the randomized PROGRAF Study, there was no difference of PTDM incidence in steroid and tacrolimus-treated kidney recipients whether they received sirolimus or MMF (about 7% at six months) [45].

Altogether, and from a pancreatic β-cell point of view, these data would point to daclizumab for the induction of immunosuppression and for the use of MMF instead of sirolimus as the ideal immunosuppressive regimen. Whether the use of MMF could allow to decrease the diabetogenic tacrolimus trough level as the same extent as sirolimus remains to be established. Furthermore, no clinical data has validated this theoretical assessment yet.

Clinical trials comparing the diabetogenic effects of CsA and tacrolimus after transplantation

Comparison of the respective effects of the two calcineurin inhibitors in inducing PTDM has been hampered for a long time in the absence of prospective randomised clinical trials. Several recently published works have however confirmed the former suspicion raised by retrospective analysis [46] on the higher incidence of PTDM in tacrolimus-treated.patients. In the 6-month, open-label, randomized, prospective multicenter DIRECT study, tacrolimus and CsA were compared in 567 non-diabetic kidney graft recipients [47]. PTDM or new impaired fasting glucose occurred in 26.0% of CsA-treated patients and 33.6% of tacrolimus-treated patients (P=0.046). This increased risk of PTDM of solid organs (kidney, liver, heart, lungs) with tacrolimus has also been documented in a meta-analysis of 56 prospective and randomised clinical trials [48] The PTDM incidence was 16.6% with tacrolimus *vs* 9.8% with CsA, without any difference according to the transplanted organ.

However, in protocol-driven studies, steroid doses are comparable in both treatment arms, while in clinical practice, steroid dose used in conjunction with tacrolimus or CsA may differ. A retrospective study analysed renal transplant recipients without pre-existing diabetes receiving tacrolimus (n=100) or CsA (n=100) for whom one-year follow-up data were available [49]. Although tacrolimus-treated patients received a significantly lower cumulative dose of corticosteroids over the first three months post-transplant, significantly more tacrolimus-treated patients had new-onset diabetes than CsA- treated patients at 3, 6, 9 and 12 months. At 12 months, 18 patients receiving tacrolimus and two receiving CsA had diabetes (P<0.0001). After stratifying patients by age group, the frequency of diabetes was significantly higher with tacrolimus than with cyclosporine

in any age group. These results confirm that new-onset diabetes is strongly and significantly associated with tacrolimus vs CsA in renal transplant recipients, even when steroid dosing is lower with tacrolimus.

This higher risk of PTDM with tacrolimus is amplified in HCV+ recipients (2.5 to 7 fold higher risk) and may be restricted to these patients [50]. Although the mechanism underlying the strong association between tacrolimus-induced PTDM and HCV+ remains quite elusive, the hypothesis stands on the increased insulin resistance in HCV patients [51-54]. The maximally compensated pre-existing insulin levels, allowing to maintain euglycaemia in face of HCV-induced insulin resistance, are suppressed after immunosuppression with tacrolimus, resulting in the development of PTDM.

However, in the randomized LIS2T study comparing CsA (n=250) and tacrolimus (n=245) in liver transplant recipients according to the VHC status, 14% of tacrolimus-treated patients vs 6% of CsA-treated ones developed PTDM at month-9 post-transplant [55].

Effect of the dose of tacrolimus

There was a progressive decline in the incidence of PTDM induced by tacrolimus-based regimens, from 20% in the early 1990s to 0-5% most recently [56]. The low incidences of PTDM were achieved with those protocols employing lower blood levels of tacrolimus and/or corticosteroid elimination. In these studies, the risk of developing PTDM was not increased in comparison with CsA-based therapy. These results emphasize the importance of reducing the immunosuppressive medication load and the role of corticosteroids in the development of PTDM.

ISD and islet transplantation

The deleterious effects of ISD on β-cells have also to be taken in consideration in islets transplantation. Assuming that neogenesis contributes to the long-term function of islet grafts, Gao *et al*. have studied the effects of ISD on precursor cell proliferation and differentiation [57]. Examining the effects of clinically used doses of ISD on freshly isolated human pancreatic cells, they showed that MMF has a potent inhibitory effect on human islet neogenesis primarily through an antiproliferative effect on the precursors, whereas tacrolimus mainly affects β-cell differentiation. Sirolimus and daclizumab have no adverse effects on these parameters. These data are consistent with the dramatic improvement of islet grafts with the immunosuppressive Edmonton protocol [58].

However, in spite of reaching long-term insulin-independence and HbA_{1c} normalisation, successful islet cell transplantations are characterized by an altered insulin secretion profile with a decrease or absent first phase insulin response after a glucose load [59]. Although the causes of these insulin secretion abnormalities could be multifactorial, with suboptimal islet number, low engraftment, chronic rejection, loss of islet-acinar integrity, heterotopic site, denervation, or insulin resistance, avoidance of diabetogenic immunosuppression is pivotal to enhance outcomes of clinical islet transplantation [60].

Reduction of PTDM

Effect of corticosteroid-sparing regimen on PTDM

In the pre-CsA era, chronic high-dose steroid therapy was a major contributing factor to the development of PTDM [61-63]. However, even if steroid contribution to PTDM has since decreased, a still valid statement is that the rate of occurrence of PTDM fells significantly when using corticosteroid sparing protocols. Compared with CsA-based regimen, immunosuppression using tacrolimus appears to decrease the rate of acute rejection episodes [64, 65], allowing to withdraw steroids 3 to 6 months after renal transplantation [66-68]. Boots et al. have investigated the hypothesis of the safety of even earlier steroid withdrawal after transplantation [69]. Sixty-two patients treated with tacrolimus were prospectively randomized to stop 10 mg prednisolone after day 7 posttransplantation (STOP) or to gradually taper steroids in three to six months (TAP). While there was no difference between the two groups after a median follow-up of 2.7 years concerning patient and graft survival, incidence and severity of acute rejections and of renal function, the incidence of PTDM (defined as the use of antidiabetic medication) was 8.0% in the STOP group and 30.3% in the TAP group (P=0.04). Futhermore, all cases occurred after one year in the STOP group, raising the question of the relationship with immunosuppressive regimen, while all cases of the TAP group occurred in the first four months (P<0.001). Recently, Rostaing et al. showed that by employing induction therapy with daclizumab plus concomitant therapy (MMF + tacrolimus) in 538 kidney transplant recipients, it is possible to taper tacrolimus blood concentrations rapidly and completely avoid the use of maintenance corticosteroids [70]. At 6 months post-transplant, this regimen resulted in a significant reduction in the incidence of PTDM compared with the standard arm, which employed tacrolimus, MMF and concomitant corticosteroid (P=0.001) (table I). A quite similar study in liver transplant recipients compared the effects of daclizumab induction + tacrolimus, without corticosteroids, with progressively tapered prednisone + tacrolimus [71]. At 3 months post-transplantation, the incidence of PTDM (defined by insulin therapy > 30 days) was 3-fold higher in the steroid group (17.8% vs 5.1%; P<0.001). A single-center study conducted in Minneapolis, involved 349 kidney transplant recipients [72]. The induction immonosuppression, including thymoglobulins and steroids, was stopped at day 5. For the maintenance immunosuppressive regimen, patients were randomised for the cacineurin inhibitor (tacrolimus vs

Table I
Incidence of acute rejection and PTDM with tacrolimus-based regimens [68].

Six-month incidence	Tacrolimus + MMF + corticosteroid (n=278)	Tacrolimus + MMF + daclizumab (n=260)
Biopsy-proven acute rejection (%)	16.5	16.5
Corticosteroid-resistant acute rejection (%)	4.3	5.0
PTDM (%)	5.4	0.4*

MMF = mycophenolate mofetil;PTDM= post-transplantation diabetes mellitus.
* p=0.001 for the difference between the two treatment groups.

CsA) and between sirolimus and MMF. At 3 years post-transplantation, without any steroid treatment in 84% of the patients, only four (1.8%) had developed a PTDM, all in the tacrolimus-sirolimus arm and within the first 6 months. The results of a subanalysis of all 12 European kidney studies [56] whereby the tacrolimus-based, corticosteroid-free treatment arms were compared with the reference tacrolimus-based, corticosteroid-containing treatment groups were consistent with those reported by Rostaing et al. [70].

Altogether, and since the use of low doses of tacrolimus, these data underline the major role of corticosteroid in inducing PTDM.

Recent clinical studies in liver transplantation have reported safety advantages and similar acute rejection rates with early steroid withdrawal. The aim of this French study was to evaluate the efficacy and safety of an immunosuppressive regimen with steroid withdrawal at day 14 in a multicenter, 1-year, comparative, double blind, placebo-controlled design. All patients received basiliximab + CsA + intravenous methylprednisolone, and they were randomized at day 7 to receive a maintenance regimen with CsA + prednisolone (group 1; n=90) or without steroids (CsA + placebo; group 2; n=84). While fewer patients received an antidiabetic treatment in the placebo group (2 *vs* 10), the incidence of acute rejection at 6 months was 38.1% in group 2 *vs* 24.4% in group 1 (P=0.03) [73]. If this study confirms the beneficial role of early steroid withdrawal on the glucose tolerance in liver transplant recipients, CsA-based maintenance immunosuppression is obviously not safe enough and would need to be reinforced by non- or less diabetogenic ISD, such as MMF, to avoid a higher incidence of acute rejection.

Effect of conversion from tacrolimus to CsA

Even if several PTDM risk factors have been determined, the individual risk assessment remains elusive and most of the transplantation teams do not base their immunosuppression choice on that single risk in pre-transplantation non-diabetic patients. Instead, some authors have studied the conversion of tacrolimus to the other calcineurin inhibitor, CsA. Bouchta *et al*. retrospectively analysed the outcome of the glucose metabolism after conversion to CsA in 34 renal transplant patients who developed PTDM under tacrolimus treatment [74]. HbA_{1c} levels decreased from 6.8 ± 0.8% at conversion to 6.0 ± 0.6% at 12 months. From 11 patients receiving insulin before conversion, three could stop it, and the insulin dose was reduced in seven. The average daily insulin dose among these patients was reduced from 31 ± 17 units at conversion to 13 ± 12 units at 12 months (P<0.05). Diabetes reversed (fasting plasma glucose ≤ 126 mg/dL without therapy) in 44% of patients during the first year after conversion (P<0.001).

This study, like others [75-78], does not provide the definitive proof of a causal association between conversion and improved glucose metabolism. Indeed, spontaneous reversals of PTDM in tacrolimus-treated patients have been previously reported [79]. These "spontaneous" reversals can, in particular, be explained by the ongoing tapering of steroid doses along with the conversion. However, several arguments suggest that conversion contributes to the observed benefits. The maximum reduction in glycaemia and insulin requirements occurs rapidly after conversion. In addition, in patients who already have prednisolone reduced to maintenance doses before conversion, the improvement of glycaemia and HbA_{1c} levels is of similar timing and magnitude as in patients who have a slight reduction of their prednisolone dose after the conversion [74, 75].

Although this review focuses on glucose metabolic effects of immunosuppressive agents, it is important to notice that calcineurin inhibitors have distinct consequences on other cardiovascular risk factors. While tacrolimus has hardly any effects on lipid levels and blood pressure, CsA is known to cause hyperlipidaemia and arterial hypertension [80]. However, these risk factors can usually be efficiently controlled more easily than diabetes [74].

Treatment of ISD-induced diabetes

As for the physiopathology and risk factors which are very similar to those of type 2 diabetes, objectives and modalities of the treatment of PTDM are not different from the usual management of type 2 diabetes. We will not detail the

guidelines in this review [81], in the absence of specific randomized studies in this population. We will focus on some studies that have evaluated the safety and drug interaction of some oral anti-diabetic agents in transplanted patients.

The safety of thiazolidinediones have been studied in PTDM in 10 patients treated with pioglitazone for a mean of 242 days [82] and in 18 patients receiving rosiglitazone for a mean duration of 381 days [83]. The addition of pioglitazone caused no significant changes in mean tacrolimus (82, 83] or CsA doses [83]. This absence of drug interaction has been confirmed in 22 renal transplant patients with PTDM who received rosiglitazone therapy [84]. In this study, fifteen patients were treated with tacrolimus and seven patients with CsA. There were no changes in CsA and tacrolimus blood levels. In this study, one patient had to stop rosiglitazone because of edema after 5 days [83]. In another study, 40 consecutive patients with PTDM after liver or kidney transplantation received 4 mg of rosiglitazone, in addition to insulin in 33 of them [85]. After a mean follow-up of 26 weeks: insulin was able to be discontinued in 30/33 (91%) patients; 25/40 (63%) continued on 4 mg/d of rosiglitazone, and 15/40 (37%) required an increase to 8 mg/d. Mild edema developed in 13% of patients; significant weight gain did not occur.

These preliminary results suggest that thiazolidinediones are safe oral agents for the management of PTDM, but further large-scale evaluations are required for both evaluate the efficacy in randomised studies and confirm the safety concerning the risk of heart failure [86] in these at risk population [87].

With regard to the sulfonylureas, co-administration of CsA and glibenclamide in six post-transplant diabetic patients resulted in a 57% increase in the steady-state plasma CsA levels, despite normal hepatic and renal functions in the patients [88]. This elevation in CsA level is possibly due to an interaction between the two drugs resulting from an inhibition of CYP3A4-mediated metabolism of CsA by glibenclamide. In contrast, glipizide does not interfere with CsA pharmacokinetics in renal allograft recipients [89].

At last, insulin regimen in transplant patients with a corticosteroid-induced glycaemic profile (hyperglycaemia during the day and in the evening with fasting normo- or hypoglycaemia) has to be adapted in order to avoid nocturnal hypoglycaemic episodes [90].

Conclusions and prospects

PTDM has emerged as a major adverse effect of ISD. As recipients of organ transplants survive longer, the long-term complications of diabetes mellitus have assumed greater importance.

While the cellular and molecular mechanisms involved in ISD-induced diabetes are better explained, the reduction of PTDM and of its well-documented impact on survival and functional outcomes warrant efforts to develop immunosuppressive regimens and drugs that eliminate or reduce the need for corticosteroids and calcineurin inhibitors without jeopardising graft function. Until methods for inducing specific graft tolerance become available, immunosuppressive regimens should be tailored to the individual patient on the basis of predictive criteria for the development of PTDM. Finally, the tools for reaching a tight glycaemic control exist and should be use more aggressively. In the near future, the specific properties of incretin-based therapies, including the decrease of β-cell apoptosis and the stimulation of their proliferative capacities, have to be assessed in the treatment of PTDM. In addition, comprehensive care of transplant recipients must include attempts to reduce other cardiovascular risk factors such as hypertension, smoking, dyslipidaemia, and obesity through a multidisciplinary team approach.

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