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REVIEW

Rapamycin in islet transplantation: friend or foe?

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Summary

The Edmonton protocol was undoubtedly a major step forward in the history of islet transplantation. Its immunosuppression regimen was largely based on the mTOR inhibitor rapamycin (sirolimus), which remains the most frequently used immunosuppressive drug in clinical islet transplant protocols. As time reveals the somewhat disappointing long-term results achieved with the Edmonton protocol, a number of publications have appeared addressing the potential beneficial or deleterious role of rapamycin on islet cell engraftment, function survival and regeneration, as well as on its side-effects in human subjects. This paper reviews the sometimes contradictory evidence on the impact of rapamycin in islet transplantation.

Introduction

Immunosuppression (IS) in what is commonly known as the 'Edmonton protocol' is largely based on rapamycin [1]. Rapamycin was considered at first as a rather gentle immunosuppressive agent in this setting, devoid of toxicities directed against islet or kidney, and indeed a key determinant for the success of the protocol. As time reveals the somewhat disappointing long-term results of islet transplantation with this protocol [2], a number of publications have appeared, addressing a possible deleterious role of rapamycin on islet or β-cell engraftment, function, survival and regeneration. This paper reviews the sometimes contradictory evidence on the favorable or negative impact of rapamycin in islet of Langerhans transplantation.

Rapamycin: mechanisms of action and synergism with calcineurin inhibitors

Rapamycin or sirolimus is a macrolide immunosuppressor that bears similarities to the calcineurin inhibitor (CNI) tacrolimus in its molecular structure. Sirolimus and tacrolimus bind to the same intracellular receptor, the immunophilin FKBP-12, but the sirolimus-FKBP-12 complex binds to and inhibits the mammalian target of rapamycin (mTOR). mTOR inhibition leads to arrest of the cell cycle at the G1 to S phase, and thus to blockade of growth-factor-driven proliferation not only of activated T cells, which is the basis of its immunosuppressive action, but also of other hematopoietic and nonhematopoietic cells [3]. Both IL-2 receptor- (signal III) and CD28-dependent (signal II) pathways of T-cell activation are affected by mTOR inhibition [4].

Perhaps one of the most interesting features of sirolimus is its pharmacologic synergism with cyclosporine, both *in vitro* and *in vivo*, which has long been described, and allows combination of both drugs at reduced doses with lower side-effects and robust IS efficacy [5,6]. This synergism was also demonstrated in a dog model of islet transplantation, in which diabetic animals receiving low doses of either rapamycin or cyclosporine as monotherapy did not reverse diabetes after islet transplantation, whereas animals treated with a low dose of both drugs as combination therapy showed a significant prolongation of islet graft function [7]. In spite of *in vitro* evidence that

sirolimus and tacrolimus bind to the same cytosolic receptor (FKBP-12), suggesting that competitive inhibition for this protein would prevent synergism [8], *in vivo* observations in animal models have shown strong potentiation of the efficacy of both drugs [9,10]. In fact, the tacrolimus–sirolimus association was shown by the Halifax group, in Canada, to be extremely potent in terms of prevention of acute rejection in a mixed series of recipients of liver, pancreas and kidney transplants [11], and the tacrolimus–sirolimus combination has become a common IS regimen for most types of organ transplants [3].

Rapamycin in autoimmune diabetes

Besides allogeneic rejection, islet grafts are prone to destruction by recurrence of autoimmunity. Recurrence of islet-directed autoimmunity in transplanted patients has been clearly demonstrated by the observation of insulitis in recipients of segmental pancreatic grafts from an identical twin [12]. However, the process is rarely observed in recipients of pancreatic allografts receiving full-dose IS [12]. The effects of rapamycin on the prevention of autoimmunity relapse versus rejection are impossible to distinguish in the clinical situation of human recipients of islet grafts. However, this effect can be selectively studied in animal models. The NOD mouse is arguably the animal model that bears the closest resemblance to human type 1 diabetes, and is characterized by spontaneous occurrence of T-cell-mediated insulitis and selective immune destruction of β cells, leading to diabetes in a majority of females between 10 and 20 weeks of age [13]. IS mimicking the Edmonton regimen (sirolimus, tacrolimus, anti-IL2 mAb) in NOD recipients of allogeneic islet grafts was able to prevent both rejection and autoimmunity and achieve prolonged insulin independence [14]. Looking specifically at recurrent autoimmunity, sirolimus treatment was able to prevent or delay the development of diabetes in a majority of mice. This effect was synergized by concurrent administration of tacrolimus, but not observed with tacrolimus alone [15,16]. With regard to diabetes recurrence on islet grafts, spontaneously diabetic NOD mice transplanted with syngeneic islets, in which allogeneic rejection does not occur, lose insulin independence within 2 weeks. Again, treatment with sirolimus prevented recurrence of diabetes in this model, an effect that was synergized by tacrolimus co-treatment, but not observed with tacrolimus monotherapy [16,17].

Rapamycin and immune tolerance

The achievement of immune tolerance to maintain graft survival in the absence of lifelong IS is seen as the Holy Grail of clinical transplantation. Several strategies are being explored, including through the establishment of mixed hematopoietic chimerism, co-stimulatory blockade or T-cell depletion [18]. Robust tolerance was achieved in a small number of kidney or liver transplant recipients using some of these strategies. In the case of islet transplantation, it is likely that restoring tolerance to autoantigens might be more complex than inducing tolerance to alloantigens [19]. Therefore, it may be reasonable to combine these strategies with initial treatment with or long-term low doses of 'conventional' immunosuppressants. Rapamycin is an interesting compound for this aim, because of its compatibility with tolerance-induction protocols. Simultaneous blockade of signals 1 and 2 of T-cell activation prevents apoptosis of alloreactive T cells, an effect that is abolished by rapamycin treatment, but not by CNIs. This effect was shown to lead to peripheral heart and skin allograft tolerance [20]. More recently, it was shown that mTOR, the intracellular receptor of the rapamycin-FKBP-12 complex, plays a central role in determining the outcome of antigen recognition by T cells with regard to activation versus anergy [21]. Importantly, T cells engineered to express a rapamycin-resistant mTOR were resistant to anergy induction caused by rapamycin [21]. A large number of studies have appeared on the role of rapamycin in the induction of $CD4^+CD25^+FoxP3^+$ regulatory T cells (Treg). Rapamycin is able to selectively induce and expand $CD4^+CD25^+FoxP3^+$ Treg in murine and human *in vivo* or *in vitro* allogeneic systems [22–28]. This effect is mediated by mTOR [26,29] and not observed with CNIs [25,27]. Interestingly, functional Treg expansion was also observed when stimulating and treating with rapamycin T cells isolated from patients with type 1 diabetes [23]. In the clinical setting, kidney transplant recipients immunosuppressed with rapamycin have higher circulating levels of Tregs than patients on CNIs [30]. $CD103^+CD8^+$ T cells are another recently described subset of Tregs. Rapamycin was shown to be able to increase *in vitro* the numbers of $CD103^+CD8^+$ Tregs in mixed lymphocyte reaction experiments, an effect that was not observed with CNIs [31].

Overall, there is a growing body of evidence to suggest that rapamycin has the unique ability to elicit and expand antigen-specific Tregs. Whether this effect is beneficial for long-term islet graft survival or may be a component of future tolerance induction protocols remains to be determined.

Rapamycin and the 'Edmonton protocol'

The seminal publication of the 'Edmonton protocol' [1] was undoubtedly a major step forward in the history of islet of Langerhans transplantation, showing that the achievement of lasting insulin-independence, which had

been anecdotal so far [32], was a realistic goal. This success was achieved thanks to a comprehensive protocol that addressed several issues such as donor selection and pancreas procurement, technicalities of the islet isolation procedure, recipient selection, transplantation technique and IS regimen, and has since represented a new baseline for all clinical islet transplantation programs. One major point was the use of multiple donors to increase transplanted, and thus engrafted, islet mass [1]. The diabetogenicity of conventional IS agents [33] was tentatively avoided by designing a steroid-free, low-CNI regimen, associating high doses of sirolimus (trough levels 12–15 ng/ml), low doses of tacrolimus (trough levels 3–6 ng/ml) and induction with daclizumab. Since then, the use of rapamycin has been considered by and large as a key determinant for the success of the Edmonton protocol, and sirolimus has been described as the single most important contribution to the IS armamentarium in islet transplantation [34]. Indeed, rapamycin analogues are currently used in the vast majority of IS regimens for clinical islet transplantation, in as many as 98% of islet transplant recipients (Table 1; 35) Conversion from the cyclosporine-mycophenolate mofetil combination to the Edmonton IS regimen was even reported to be beneficial in islet grafts with borderline function [36].

The sirolimus–tacrolimus combination has been used by other groups with equal or even higher success, in association with depleting anti-T-cell antibodies as induction agents. The only reports of consistent single-donor insulin-independence using this maintenance regimen have come from the Minneapolis group, in which thymoglobulin or the humanized anti-CD3 monoclonal antibody (mAb) hOKT3 γ1 (ala-ala) were used for induction [37,38]. The Edmonton group has used alemtuzumab (humanized mAb Campath-1H) as induction therapy in a group of islet transplant recipients. Results equivalent to those obtained with the original Edmonton protocol have been reported [2].

Interestingly, as much as sirolimus appears as the key component of maintenance IS in this combination, low doses of tacrolimus seem to be equally critical, in view of the poor results of sirolimus maintenance therapy recently reported in a protocol using antithymocyte globulin for induction. None of the patients on sirolimus monotherapy achieved insulin independence, as compared to 60% of those on the sirolimus–tacrolimus association [39].

Table 1. Immunosuppressive regimens for islet transplantation.

	Total	Sirolimus	Everolimus	mTOR inhibitors*	No mTOR inhibitors
Protocols	31	23 (74)	2 (7)	25 (81)	6 (19)
Active protocols†	23	18 (78)	2 (9)	20 (87)	3 (13)
Patients	244	232 (95)	6 (2)	238 (98)	6 (2)

Values in parenthesis are percentages.
Based on data reported to the CITR for the 2007 Annual Report [35].
*Protocols based on sirolimus or everolimus.
†Protocols in which at least one patient was transplanted.

Side-effects and toxicity in sirolimus-based protocols of islet transplantation

Perhaps no other type of clinical transplantation has been the subject of as much scrutiny and reporting of complications and side-effects as islet transplantation. Because of this, the morbidity of islet transplantation may have been overestimated in comparison to other organ transplants. Nonetheless, the morbidity rate of islet transplantation on the Edmonton regimen appears to be very high, and mostly related to sirolimus side-effects and toxicity. A recent, very comprehensive paper from the University of Miami has reported a 100% rate of side-effects, most of them of low severity, including a high incidence of hematologic (leukopenia, anemia or thrombocytopenia), metabolic (hypercholesterolemia, hypertriglyceridemia), gastrointestinal (mouth ulcers, diarrhea, vomiting), and dermatologic (skin rash, edema) adverse events [40]. The rate and nature of these events was similar in the report of the first, and so far only, multicenter trial of islet transplantation using the Edmonton protocol [41]. Mouth ulcers are a common side-effect of sirolimus after islet transplantation, and have a higher incidence than what is reported for other organ transplants. This may be a result of the high doses administered and of the steroid-free regimens designed for this indication. Sirolimus-induced small bowel ulcerations have even been reported in two islet transplant recipients on the Edmonton regimen, an adverse event that had never been reported before in transplantation [42].

Other side-effects attributed to sirolimus and reported only after islet transplantation pertain to female gonadal function. In a cohort of 13 female islet transplant recipients, alteration of the menstrual cycle was observed in all women with a regular cycle pre transplant, and benign ovarian cysts developed in eight women (61.5%). Most women had evidence of impaired gonadal function as assessed by undetectable blood levels of progesterone [43].

An issue of significant concern has been that of sirolimus nephrotoxicity, especially in subjects with type 1 diabetes, in whom kidney lesions secondary to diabetic nephropathy may be exacerbated. Although initially considered as a kidney-sparing immunosuppressive drug, sirolimus has been associated with renal toxicity and delayed graft function in animal models and in clinical trials [44–47]. It has become obvious that sirolimus can

in fact potentiate the nephrotoxicity of CNIs in the clinical setting [48–50]. Accordingly, a decrease in kidney function and/or the development of overt proteinuria, sometimes leading to end-stage renal disease, was reported after islet transplantation alone and islet-after-kidney transplant recipients on the sirolimus–tacrolimus combination [51–54]. Although alterations in kidney function were difficult to predict, patients with lower creatinine clearance, presence of albuminuria or longer duration of diabetes were at higher risk [52,53]. A change in the immunosuppressive therapy, notably sirolimus withdrawal, has been noted to reverse proteinuria or correct glomerular filtration rate decreases [51,54].

Overall, the frequency and significance of these side-effects have necessitated a switch from sirolimus to mycophenolate mofetil in a sizeable proportion of human islet recipients worldwide [35,40].

Islet toxicity and metabolic effects of rapamycin

A major challenge in the design of immunosuppressive regimens pertains to the diabetogenicity of a number of classical immunosuppressants, notably steroids and CNIs [33]. In contrast, rapamycin is thought to be a rather harmless agent in terms of islet toxicity.

Early studies in the dog showed that there was no impairment of *in vivo* canine islet function in a model of autologous transplantation [55]. In experiments done with murine or rat islets or β-cell lines, *in vitro* impairment of islet function was seen only at extremely high rapamycin concentrations, and hyperglycemia was only observed in transplanted animals when they were treated with 10–50 times the effective antirejection dosage [55–58].

Other animal studies, performed in murine or rat models, have challenged this view. Dose-dependent reduced β-cell viability [59] or impaired glucose-stimulated insulin release, coinciding with decreased PDX-1 and GLUT2 gene expression, were observed *in vitro* after sirolimus exposure [60], as well as decreased *in vivo* function and intra-graft insulin content in transplanted animals [60,61]. These observations should be related to the findings that one of the β-cell signaling pathways that ultimately lead to insulin exocytosis is regulated by mTOR activation, and consequently blocked by rapamycin [62]. Other studies have shown that sirolimus or sirolimus–tacrolimus treatment induced hyperglycemia in normal or islet-transplanted animals, with normal or even increased insulinemia, suggesting the induction of insulin resistance [63,64].

While it seems difficult to reconcile contradictory observations on the impact of rapamycin on β-cell function, it should be emphasized that all these studies were performed on cells of various animal origins, and that β-cells from different animal species may behave differently [65]. In this regard, very few studies have explored the effect of rapamycin on isolated human islets. Two studies reported impaired glucose-stimulated insulin secretion *in vitro* in human islets cultured in the presence of sirolimus, as compared to control conditions [55,66]. Sirolimus concentrations required for this effect were in the therapeutic (10 ng/ml) or largely supratherapeutic (50–100 ng/ml) ranges. In sharp contrast, another paper that has studied the effect of sirolimus *in vitro* on human islets, has reported a dose-dependent increase of glucose-stimulated insulin release, islet insulin content and islet viability, assessed by apoptosis staining and ATP content, at sirolimus concentrations up to 32 ng/ml [67].

Islet toxicity might be seen under a different light, considering the intriguing data recently reported about portal versus systemic blood levels of IS drugs in general, and of sirolimus in particular. In a large mammal experimental model, peak levels of sirolimus in the portal blood were approximately twice as high as in the systemic circulation. Exposure to sirolimus, calculated by the area under the curve was also approximately twice as high [68]. This effect was dramatically more pronounced for tacrolimus. This observation was confirmed in human subjects by simultaneously measuring portal and systemic sirolimus and tacrolimus blood levels in islet transplant recipients. IS levels were up to three times higher in the portal blood [69]. Orally administered IS drugs reach the liver, where they are metabolized, via the portal circulation. The intrahepatic engraftment site of the islets means that they are exposed to higher IS concentrations than those measured in the peripheral blood. Whether this confers stronger IS, and thus protection against rejection or autoimmunity, or exposes to higher risk of drug toxicity remains to be demonstrated, but results of *in vitro* experiments performed in conditions regarded as 'supra therapeutic doses' might be more relevant than sometimes considered.

Rapamycin has also shown 'extra-islet' metabolic effects. A cohort of islet-transplanted patients treated with sirolimus studied by insulin clamp and infusion of [6,6-$^2H_2$]glucose to quantify glucose turnover, showed an increase of the metabolic glucose clearance rate. This effect was observed both at the peripheral and hepatic sites, and was not observed in patients treated with tacrolimus. Interestingly, the same effect was obtained in type 1 diabetic patients on rapamycin before islet transplantation [70]. On the other hand, rapamycin did not affect the development of intrahepatic fatty deposition [71], a feature frequently observed in islet transplant recipients [72]. In fact, patients with type 1 diabetes submitted to islet transplantation, and studied using in- and

out-of-phase magnetic resonance imaging and ^{1}H MR-Spectroscopy to assess intrahepatic tryglyceride content in a noninvasive fashion did not show any impact of rapamycin on this parameter [71].

Effects of rapamycin on islet engraftment

The near-consistent need for more than one donor for the success of islet transplantation under the Edmonton regimen, along with a wealth of data from experimental models of islet transplantation, strongly suggest the involvement of innate immunity in the partial destruction of the islet graft by inflammatory mechanisms. Cells involved include monocytes/macrophages, granulocytes, platelets, and endothelial cells, with cytokines and chemokines, free radicals, adhesion molecules, and the complement and coagulation cascades as inflammatory mediators [73]. The need for islets freshly implanted into the liver to revascularize before full engraftment is achieved, and the ensuing ischemia they sustain during the process is another cause of immediate partial islet graft loss [74].

Some experimental data suggest that rapamycin may play a beneficial role in the early post-transplant inflammatory phenomena described above. At concentrations consistent with usual whole blood therapeutic levels, rapamycin is able to induce caspase-independent apoptosis in cultured human monocytes or monocyte-derived dendritic cells [75,76]. In patients who are candidates for islet transplantation, pretreatment with rapamycin led to a significant and prolonged decrease in peripheral blood monocyte counts, as well as in cells from the myeloid lineage in general, assessed by the CD14 and CD33 surface markers. Lymphocyte counts were unaffected by rapamycin treatment [75]. Reversely, in islet transplant recipients who were switched from rapamycin to mycophenolate mofetil, a significant increase in peripheral blood monocyte count was observed [75]. Because of the expected anti-inflammatory effect, the group at the San Raffaele University in Milan pre treated with sirolimus a cohort of patients 1–6 months prior to islet transplantation alone on the Edmonton IS regimen and compared their outcome with historical controls on the original Edmonton protocol [77]. In a preliminary report, significantly better results in terms of islet function (C-peptide >0.5 ng/ml at 3 years in 75% of patients, vs. 17% in the control group) and maintenance of insulin independence (44% at 3 years vs. 25% in the control group) [77].

On the other hand, rapamycin was shown to decrease expression and release of vascular endothelium growth factor (VEGF) by islet cells, in particular islet endothelial cells [59,66,78]. The ensuing blockade of VEGF-mediated pathways may be detrimental through two sets of mechanisms: first, VEGF acts as a antiapoptotic factor for islet cells [59,66], and second, the angiogenic effect of VEGF facilitates islet revascularization [78]. Accordingly, *in vitro* angiogenesis was markedly inhibited by therapeutic concentrations of rapamycin [78], and vascular density in islet grafts was significantly lower 30 days post-transplant in sirolimus-treated animals [60].

In summary, rapamycin may exert dual effects on the noxious inflammatory stimuli able to impede early islet engraftment. Although not randomized, the only clinical trial to have addressed this issue so far, by administering prolonged pre transplant sirolimus treatment, suggests a beneficial overall effect [77].

Antiproliferative effect of rapamycin on β-cell regeneration

The recent updates of the Edmonton protocol have shown that graft function decreases over time, the approximate rates of insulin-independence dropping from 80% at 1 year to 15% at 5 years [2]. It is established that β-cells have a limited lifespan and undergo a constant turnover within the native pancreas [79]. The most likely sources of new β-cells are β-cells themselves that may have the ability to self-duplicate [80] or progenitor pancreatic ductal cells [81]. Recent evidence suggests that an impairment of β-cell regeneration, from either of these sources, caused by the antiproliferative action of rapamycin could explain the observed graft function loss.

Human ductal cells cultured with growth factors have the ability to expand *in vitro* almost twofold over a 6-day period. This expansion was prevented, and even reversed, by the addition of sirolimus at therapeutic concentrations [61]. This effect was not observed in another study in which sirolimus prevented human ductal cell proliferation *in vitro* only when combined with mycophenolate mofetil [82]. These conflicting results can be explained by differences in culture conditions, in particular with respect to growth factors used. It must also be stressed that, while an effect on proliferation of potential pancreatic β-cell progenitors is clearly demonstrated, no direct evidence on β-cells themselves is provided in these papers.

A recent study by Nir *et al.* [83] used a transgenic murine model of conditional β-cell destruction and generation. This study convincingly demonstrated, at least in the mouse, the powerful capacity of β-cells to regenerate. Interestingly, β-cell regeneration was completely inhibited in animals treated with a sirolimus–tacrolimus combination [83]. Although the authors did not study the effect of sirolimus administered alone as a monotherapy, this paper strongly suggests that this inhibitory effect of the Edmonton IS regimen may play a role in long-term loss of islet function by inducing a decrease in β-cell mass.

Table 2. Summary of benefits and harms of rapamycin in islet transplantation.

Action	Rapamycin beneficial or potentially beneficial*	Rapamycin harmful or potentially harmful*
Synergy with CNIs	5–7,9–11	
Control of autoimmunity	14–17	
Activation-induced T-cell apoptosis	20,21	
Induces the generation of regulatory T cells	22–31	
Causes high incidence of side-effects		35,40–43
Causes nephrotoxicity		44–54
Promotes/impairs β-cell function	55–58,67	59–62,66
Induces insulin resistance		63,64
Increases glucose clearance rate	70	
Causes apoptosis of inflammatory cells from monocyte/macrophage lineage	72–74	
Decreases VEGF expression and release		55,66,75
Impairs β-cell regeneration		61,79–81

*List of references from this review addressing the specific issue.

Reduction of the rate of β-cell proliferation *in vivo* by sirolimus treatment was also demonstrated in the murine pregnancy model. Sirolimus treatment almost completely inhibited the proliferation of β-cells induced by pregnancy, assessed by *in vivo* BrdU incorporation [84]. This was corroborated by the significantly smaller size of islets recovered from pancreata of sirolimus-treated pregnant mice as compared to controls, and by the lower insulin content per weight unit of pancreas.

Overall, although a direct effect on proliferation of transplanted β-cells still has to be shown, there is convincing evidence that sirolimus treatment impairs β-cell regeneration *in vivo*, and that this may be a critical determinant for the long-term fate of islet grafts.

Rapamycin: friend or foe to the islet graft?

The literature covered by this review suggests that rapamycin can exert both beneficial and detrimental effects on the islet graft and also on the transplanted patient at several levels. It can impact on β-cell survival and function, on islet engraftment, on islet graft recipient health and on islet graft regeneration. Comprehensive consideration of published data suggests that the effect of rapamycin may vary from helpful to harmful depending on timing and circumstances. It should also be kept in mind that observations made *in vitro* may not apply *in vivo*, and that observations made in rodent models may not apply to human islets. However, while there seems to be a balance between the benefits and hazards of rapamycin in the early engraftment period, evidence points to the benefits of avoiding long-term utilization of the drug for the sake of preserving the regenerative ability of β-cells (Table 2).

Finally, one cannot rule out that there may be individual differences in the response to rapamycin from islet graft to islet graft or from patient to patient. Thus, the concept of 'individually tailored IS' [85] might well apply to the field of islet transplantation with regard to the use or avoidance of rapamycin.

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