Evidence for Rapamycin Toxicity in Pancreatic β-Cells and a Review of the Underlying Molecular Mechanisms

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Rapamycin is used frequently in both transplantation and oncology. Although historically thought to have little diabetogenic effect, there is growing evidence of β-cell toxicity. This Review draws evidence for rapamycin toxicity from clinical studies of islet and renal transplantation, and of rapamycin as an anticancer agent, as well as from experimental studies. Together, these studies provide evidence that rapamycin has significant detrimental effects on β-cell function and survival and peripheral insulin resistance. The mechanism of action of rapamycin is via inhibition of mammalian target of rapamycin (mTOR). This Review describes the complex mTOR signaling pathways, which control vital cellular functions including mRNA translation, cell proliferation, cell growth, differentiation, angiogenesis, and apoptosis, and examines molecular mechanisms for rapamycin toxicity in β-cells. These mechanisms include reductions in β-cell size, mass, proliferation and insulin secretion alongside increases in apoptosis, autophagy, and peripheral insulin resistance. These data bring into question the use of rapamycin as an immunosuppressant in islet transplantation and as a second-line agent in other transplant recipients developing new-onset diabetes after transplantation with calcineurin inhibitors. It also highlights the importance of close monitoring of blood glucose levels in patients taking rapamycin as an anticancer treatment, particularly those with preexisting glucose intolerance. ***Diabetes* 62:2674–2682, 2013**

The macrolide rapamycin is both an antiproliferative and potent immunosuppressant. It is produced commercially as sirolimus and its derivative, everolimus. Sirolimus is predominantly used as an immunosuppressant in transplantation, while everolimus is used mainly as an anticancer agent. Early data suggested little or no diabetogenic effects of rapamycin, particularly in comparison with other immunosuppressants, and it was this that prompted its use in islet cell transplantation. It was also for this reason that rapamycin has been promoted as a second-line therapy for recipients of solid organ transplants who have developed new-onset diabetes after transplantation (NODAT) while taking calcineurin inhibitors (CNIs). However, there is an increasing view that rapamycin has profound effects on pancreatic β-cells, as well as altering insulin sensitivity. Evidence for this arises from in vitro and in vivo experiments and clinical studies. This article will review this evidence and also explore the potential mechanisms of rapamycin toxicity, drawn from experiments of β-cell physiology.

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Received 21 January 2013 and accepted 22 April 2013.

DOI: 10.2337/db13-0106

Clinical evidence of rapamycin β-cell toxicity

Islet cell transplantation. Rapamycin has been one of the primary immunosuppressants for islet cell transplantation since the publication of the landmark Edmonton study in 2000 (1). The Edmonton immunosuppression protocol was designed to avoid the diabetogenic effects of corticosteroids and to minimize the effects of tacrolimus. Rapamycin was the main immunosuppressant used, as it was thought to have little or no detrimental effects on islet survival or function. Initial results were promising, with seven consecutive islet transplant recipients attaining insulin independence over a median follow-up of 11.9 months. After the initial success of the Edmonton protocol, a number of other centers adopted a similar regimen for their islet transplant programs. However, enthusiasm was tempered when the 5-year results of the initial cohort of patients from Edmonton was reported, with only ~10% maintaining insulin independence (2). Long-term results from other centers using the Edmonton protocol were equally disappointing, with insulin independence at 2 years ranging from 14 to 20% (3–5).

Although the cause of this decline in islet graft function is multifactorial, there is evidence that it is partly related to the toxicity of the immunosuppressive agents used. For example, pathological examination of one patient who died with a failed islet transplant performed under the Edmonton protocol and one who died with a functioning islet transplant showed no evidence of allo- or autoimmune damage to the transplanted islets (6,7). This provides evidence for predominantly nonimmunological causes for the chronic loss of intrahepatic islets, such as toxicity from immunosuppressive agents. Interestingly, in the context of this Review, arguably the most promising long-term survival data reported for islet transplantation to date was achieved with a regimen that avoided rapamycin after the first year posttransplant (8). This resulted in 80% insulin independence at over 3 years posttransplant.

Solid organ transplantation. Rapamycin is used predominantly for immunosuppression after renal transplantation, although it is also given after pancreas, liver, and cardiac transplants. Evidence for rapamycin toxicity in β-cells can be obtained from studies of NODAT in patients receiving rapamycin. One study showed that tacrolimus to rapamycin conversion in renal transplant recipients was associated with a 30% increase in impaired glucose tolerance (9). Furthermore, a study of renal transplant recipients from the U.S. Renal Data System showed that patients treated with rapamycin in combination with either tacrolimus or cyclosporine had the highest incidence of NODAT (10). Other studies have found sirolimus, on multivariate analysis, to be a risk factor for NODAT in kidney transplant recipients (11–15). Furthermore, in a large-scale randomized control trial of immunosuppressive regimens in renal transplantation, sirolimus was associated with the highest incidence of hyperglycemia (5 vs. 4.7% low-dose tacrolimus

vs. 4.4% high-dose cyclosporine vs. 2.9% low-dose cyclosporine), although the incidence of NODAT was higher in the tacrolimus group (16). However, as patients in these studies also received other immunosuppressants, including corticosteroids, it is not possible to determine the exact influence of rapamycin on the development of NODAT. However, as part of their U.S. Renal Data System study, Johnston et al. (10) analyzed the risk of NODAT in renal transplant recipients receiving tacrolimus in combination with mycophenolate mofetil (MMF) or azathioprine versus those receiving tacrolimus and sirolimus. This demonstrated a hazard ratio of 1.25 (95% CI 1.03–1.52), suggesting an increased risk for NODAT from sirolimus independent of any effect of tacrolimus.

The data on the risk of NODAT with sirolimus use after liver transplantation are more sparse. However, in one study the incidence of NODAT in liver transplant recipients receiving sirolimus without CNIs was 10.5% compared with 29.4% in a historical control group receiving only CNIs (17).

Further insights into the effects of rapamycin on glucose homeostasis can be drawn from studies of its use in whole-organ pancreas transplantation. Reports of sirolimus use in combination with CNIs as part of steroid-free (18) or rapid steroid elimination (19) regimens after pancreas transplantation have shown equivalent endocrine graft function compared with patients receiving mycophenolate, CNIs, and steroids. The greater exposure to corticosteroids in the comparator groups does, however, bias the results in favor of sirolimus. More robust data come from a randomized trial of rapamycin versus mycophenalate after simultaneous kidney pancreas transplantation, with otherwise identical immunosuppressive regimens. This demonstrated significantly lower HbA_{1c} levels in the mycophenalate group, while 6% of patients on rapamycin developed NODAT compared with 3% in the mycophenalate group (20). A further nonrandomized comparison of rapamycin with mycophenalate in pancreas transplant recipients also receiving tacrolimus demonstrated significantly lower plasma insulin levels during an intravenous glucose tolerance test with rapamycin (21).

Anticancer agent. The rapamycin derivative everolimus is licensed for the treatment of advanced renal cell, neuroendocrine, and breast cancers. Evidence for pancreatic β-cell toxicity can be gleaned from rates of hyperglycemia reported in studies of everolimus monotherapy given for various advanced cancers. Reported rates of severe hyperglycemia are shown in Table 1. There are difficulties in interpreting such data, such as lack of information on the number of patients with preexisting diabetes and the potential tumor effects contributing to the hyperglycemia. However, the overall inference is that everolimus does have a detrimental effect on pancreatic β-cells. The randomized control trial by Baselga et al. (22) provides more persuasive evidence. Patients with advanced breast cancer received an aromatase inhibitor alone or in combination with everolimus. The incidence of hyperglycemia was 13% in the everolimus group against 2% in the noneverolimus group. As a randomized control group, this study has fewer of the confounding factors seen in the studies above and provides more robust evidence of everolimus toxicity in β-cells. It is important to note that the dose of everolimus (10 mg/day) used in the above oncological studies is higher than that used after transplantation (1.5 mg/day) and, as such, the relevance of these findings to a transplant setting is reduced.

TABLE 1
Reported rates of severe hyperglycemia in oncological studies of everolimus monotherapy

Authors (ref.)	Cancer type	Dose (mg/day)	Hyperglycemia rate (%)
Baselga et al. (22)	Breast	10	13
Doi et al. (77)	Gastric	10	4
O'Donnell et al. (78)	Solid organ	10	3
Oh et al. (79)	Nonfunctioning neuroendocrine	10	6
Tabernero et al. (80)	Solid organ	10	2
Wolpin et al. (81)	Pancreatic	10	18
Yee et al. (82)	Hematological	10	22
Yoon et al. (83)	Gastric	10	20

Everolimus has also been used successfully as a treatment for refractory hypoglycemia in unresectable or metastatic insulinoma (23–25). Although some of this benefit may result from the antitumor effect of the drug, improvement in hypoglycemia has also been seen in patients with no radiological tumor regression. This suggests a direct effect of everolimus on glycemic control, which appears to be due to both a reduction in serum insulin levels and reduced peripheral uptake of glucose in muscles (23).

A further clinical study of interest is a phase I clinical trial of a combination of rapamycin and interleukin-2 treatment in type 1 diabetic patients (26). The hypothesis was that this treatment would reduce autoimmune damage to β-cells and improve function. Interestingly, the combination therapy resulted in transient impairment of β-cell function, as measured by C-peptide levels. However, what is not clear is whether this impairment was due to rapamycin, interleukin-2, or a combination of both. Furthermore, it is not possible to determine whether the impairment was due to direct effects on β-cells or due to indirect effects on immune regulatory cells.

Taken as the whole, the evidence from studies of rapamycin use for different clinical indications does point toward rapamycin having a detrimental effect on glucose homeostasis. However, the majority of these studies have significant confounding factors, precluding any definitive conclusion. Also, none of these studies are able to address whether the hyperglycemia seen is predominantly due to a result of direct effects on β-cells or due to peripheral effects on glucose metabolism, such as increases in insulin resistance.

Direct effects of rapamycin on pancreatic β-cells. There have been a number of studies investigating the direct effects of rapamycin on pancreatic β-cell function, survival, and proliferation, which are summarized in Tables 2–4. This research is heterogeneous, using a variety of cell types and rapamycin concentrations. There is some disparity in the results, which likely reflects differing rapamycin doses and treatment periods. It may also reflect species-specific differences in rapamycin sensitivity. A further explanation for the disparities is that it is a reflection of islet purity. A study comparing the effects of rapamycin on pure (>90%) and impure (40–60%) islets found glucose-stimulated insulin secretion (GSIS) to be significantly higher in impure islets treated with rapamycin but saw no difference in pure islets (27). Overall, the majority of these studies demonstrate significant

TABLE 2
Summary of studies investigating the effects of rapamycin on pancreatic β-cell function and insulin sensitivity

Authors (ref.)	Experimental model	Rapamycin dose	Treatment duration	Significant findings
In vitro				
Barlow et al. (36)	MIN6 cell line	200 nmol/L	24–72 h	↓ GSIS
D'Amico et al. (84)	MIN6 cell line	25 ng/mL	48 h	↓ GSIS, ↓ insulin content
Fuhrer et al. (85)	RIN-5F cell line	100 nmol/L	30 min	↓ GSIS
Fuhrer et al. (85)	HIT-T15 cell line	100 nmol/L	30 min	↓ GSIS
Paty et al. (86)	HIT-T15 cell line	1–100 ng/mL	48 h	↓ GSIS
Ex vivo				
Barlow et al. (36)	Rat islets	200 nmol/L	72 h	↓ GSIS
Bell et al. (87)	Rat islets	100 nmol/L	4 days	↓ GSIS
Bussiere et al. (88)	Human islets	20 ng/mL	6 days	⇔ GSIS
Bussiere et al. (88)	Neonatal porcine islets	20 ng/ml	6 days	⇔ GSIS
Fabian et al. (89)	Murine islets	100 ng/mL	72 h	↓ Insulin secretion
Fraenkel et al. (65)	*P.obesus* rat islets	50 nmol/L	4 days	↓ GSIS, ↓ proinsulin synthesis
Hyder et al. (90)	Postnatal rat islets	15 ng/mL	3 weeks	↓ GSIS 1 and 3 weeks
Hyder et al. (90)	Adult rat islets	15 ng/mL	3 weeks	↓ GSIS >3 weeks
Hyder et al. (90)	Adult porcine islets	15 ng/mL	3 weeks	↓ GSIS 1 and 3 weeks
Marcelli-Tourvieille et al. (91)	Human islets	16 ng/mL	120 h	↓ Basal insulin secretion
Zhang et al. (92)	Mouse islets	30 ng/mL	24 h	↓ GSIS
In vivo				
Bussiere et al. (88)	Murine syngeneic graft	0.2 mg/kg	26 days	Glucose intolerance
Fabian et al. (89)	Murine allograft	5 mg/kg/day	7 days	↑ Blood glucose
Fraenkel et al. (65)	*P.obesus* rat	0.2 mg/kg/day	16 days	↑ Serum insulin, ↑ insulin resistance
Fraenkel et al. (65)	*P.obesus* diabetic rat	0.2 mg/kg/day	16 days	↑ Blood glucose, ↓ insulin content, ↑ insulin resistance
Kneteman et al. (93)	Canine autotransplant	1 mg/kg	30 days	↑ Glucose clearance, ↑ insulin release
Song et al. (94)	Rat	0.3–0.6 mg/kg	4 weeks	↑ Blood glucose, ⇔ HOMA-R
Whiting et al. (95)	Rat	1.5 mg/kg/day	13 days	↑ Plasma and urinary glucose
Yang et al. (66)	Rat	0.5–5 mg/kg	3 weeks	Glucose intolerance, ↓ GSIS, ↑ HOMA-IR
Zhang et al. (92)	Murine syngeneic graft	0.5–1.5 mg/kg	14 days	↓ GSIS

HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-R, homeostasis model assessment index ratio; *P.obesus*, *Psammomys obesus*.

effects of rapamycin on glucose homeostasis, and the combined evidence strongly suggests that rapamycin adversely affects GSIS from β-cells.

Viability studies in murine and human β-cells treated with rapamycin conclusively demonstrate significant detrimental effects on cell survival, with increased levels of apoptosis (Table 3). Importantly, this includes studies in human islets at clinically relevant rapamycin doses similar to those measured in the portal vein of islet transplant recipients (28).

One downside of in vitro studies is that only the acute effects of rapamycin over a few days can be studied because of the difficulties in maintaining islets in culture for extended time periods. Of greater clinical importance is the effect over the months and years patients will be taking rapamycin. Yet, even after a relatively short period of

TABLE 3
Summary of studies investigating the effects of rapamycin on pancreatic β-cell survival

Authors (ref.)	Experimental model	Rapamycin dose	Treatment duration	Significant findings
In vitro				
Barlow et al. (36)	MIN6 cell line	200 nmol/L	24–72 h	↓ Viability, ↑ apoptosis
Bell et al. (87)	MIN6 cell line	10–100 nmol/L	1–4 days	↑ Apoptosis
Ex vivo				
Barlow et al. (36)	Rat islets	200 nmol/L	48 h	↑ Apoptosis
Bell et al. (87)	Human islets	1–10 nmol/L	4 days	↓ Viability (but not significant)
Bell et al. (87)	Human islets	100 nmol/L	4 days	↓ Viability
Bell et al. (87)	Rat islets	100 nmol/L	4 days	↓ Viability
Bussiere et al. (88)	Neonatal porcine islets	10 ng/mL	6 days	↑ Apoptosis
Johnson et al. (61)	Human islets	10–30 μg/L	24 h	↑ Cleaved caspase-3
Marcelli-Tourvieille et al. (91)	Human islets	8–32 ng/mL	120 h	↓ Apoptosis
In vivo				
Fraenkel et al. (65)	*P.obesus* rats	0.2 mg/kg/day	16 days	⇔ Apoptosis
Fraenkel et al. (65)	*P.obesus* diabetic rats	0.2 mg/kg/day	16 days	↑ Apoptosis

P.obesus, *Psammomys obesus*.

incubation with rapamycin cultured islets show signs of decreased function and viability (Tables 2 and 3).

Mechanism of action of rapamycin. In order to appreciate the possible mechanisms of rapamycin toxicity, it is important to understand its mechanism of action. Rapamycin binds to the immunophilin FK506-binding protein 12 (FKBP12) to form a complex that binds to and inhibits the serine/threonine kinase mammalian target of rapamycin (mTOR) (29). This kinase is a key regulator of cell metabolism, growth, and proliferation. Importantly, the inhibition of mTOR by rapamycin results in cell cycle arrest in mid- to late G1 phase and thus has the potential to repress tumor cell growth and, importantly with respect to its immunosuppressive function, inhibit T- and B-cell proliferation. However, FKBP12 and mTOR are ubiquitously expressed. Thus, there is the potential for possible "off target" effects on cells other than tumor and immunoregulatory cells.

mTOR. The mTOR kinase exists in two distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). These are differentially regulated and have distinct substrates (Fig. 1). Although they share some core

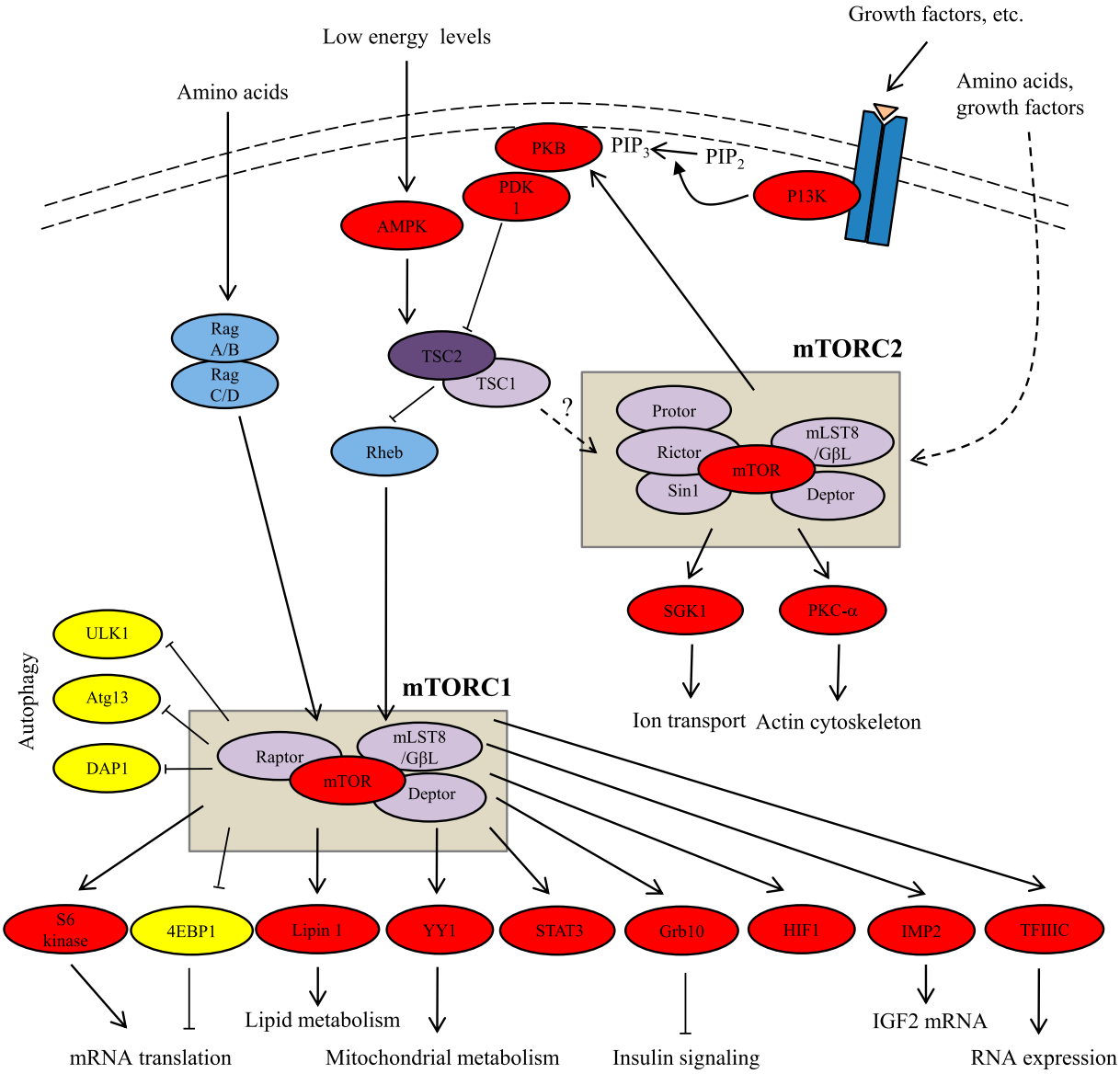

FIG. 1. mTOR signaling pathways. After stimulation by insulin and other growth factors, phosphatidylinositol 3-kinase (PI3K) converts phosphatidylinositol 4,5-bisphosphate (PIP_2) into phosphatidylinositol 3,4,5-triphosphate (PIP_3), which localizes PKB to the membrane where it is activated by PDK1 and mTORC2. Activated PKB phosphorylates and inhibits tuberous sclerosis complex (TSC1/2). Rheb, a small GTPase that is inhibited by TSC2, positively modulates mTORC1 activity. mTORC1 phosphorylates S6 kinase 1/2 and 4EBP1, resulting in increased mRNA translation. Amino acid sufficiency activates mTORC1 via Rag A/B and C/D. Under low-energy conditions, the AMP-to-ATP ratio rises and activates AMP kinase (AMPK), which phosphorylates and activates the TSC1/2 complex, resulting in mTORC1 inhibition. mTORC2 activity is mediated via predominantly unknown pathways. mTORC2 phosphorylates and activates PKB, serum- and glucocorticoid-induced protein kinase 1 (SGK1), and PKC. Arrows, stimulatory effects; block ends, inhibitory effects; solid lines, direct effects, dashed lines, indirect effects. Atg13, autophagy-related protein 13; DAP1, death-associated protein 1; Deptor, DEP domain–containing mTOR-interacting protein; 4EBP, eIF4E-binding protein; GβL- G, protein β-subunit-like protein; HIF1, hypoxia-induced factor 1; IMP2, insulin-like growth factor 2 mRNA-binding protein; mLST8, mammalian lethal with Sec13 protein 8; PDK1, phosphoinositide-dependent protein kinase 1; Protor, protein observed with Rictor; Raptor, regulatory associated protein of mTOR; Rictor, rapamycin-insensitive companion of mTOR; Sin1, stress-activated protein kinase–interacting protein 1; TFIIIC, transcription factor 3C; ULK1, Unc-51–like kinase 1.

components such as mTOR, mLST8, and DEPTOR, they also contain additional proteins that are distinct and define them. For example, a unique component of mTORC1 is RAPTOR (regulatory associated protein of mTOR), which serves as a scaffold binding both mTOR and its downstream effectors (30,31). An essential component of mTORC2 is the protein Rictor (rapamycin-insensitive companion of mTOR), which is required for both mTORC2 complex formation and its kinase activity (32,33). Importantly, mTORC1 is acutely sensitive to inhibition by rapamycin, whereas mTORC2, although originally thought to be resistant to rapamycin (32,33), is in fact sensitive to prolonged rapamycin treatment in certain cell types (34–36). Thus, both complexes could potentially play a role in both the immunosuppressive and toxic effects of rapamycin.

mTORC1. Consistent with its role as a key regulator of cell metabolism, proliferation, and growth, mTORC1 activity is regulated by nutrients, growth factors, and cellular energy levels (Fig. 1). The best characterized targets of mTORC1 are the eIF4E-binding proteins (4E-BPs) and the S6 kinase proteins (S6K), both of which play important roles in the regulation of protein synthesis. However, a number of other downstream targets have been identified (Table 5).

Role of mTORC1 in β-cell function. A vital aspect in the preservation of glucose homeostasis is the maintenance of pancreatic β-cell mass and also the ability for β-cell mass to increase in response to insulin resistant states such as obesity. This increase in β-cell mass results from increases in neogenesis (generation from progenitor cells) and proliferation (hyperplasia), hypertrophy, and reductions in apoptosis.

There is evidence from a number of studies that rapamycin significantly reduces proliferation of both β-cells and progenitor cells (Table 4), with consequences for the maintenance of β-cell mass.

Although in vitro studies provide important insights into the role of mTORC1 in the regulation of β-cell mass, more compelling evidence comes from in vivo transgenic mouse models (rev. in 37). Hyperactivation of mTORC1 by overexpression of Rheb (38) or deletion of TSC1 (39) or TSC2 (40,41), selectively in β-cells, leads to increases in β-cell size and mass, coincident with improvements in insulin secretion and glucose tolerance. These effects may be mediated, at least in part, through S6K, as mice deficient in S6K1 or rpS6 are also hypoinsulinemic and glucose intolerant with diminished β-cell size (42,43). Furthermore, transgenic mice overexpressing constitutively active S6K exhibit improved glucose tolerance and enhanced insulin secretion, with increased β-cell size (44). Although these studies are strongly suggestive of a key role of mTORC1, it is possible that manipulation of upstream regulators of mTOR such as Rheb may affect pathways other than mTORC1, and therefore a causative effect cannot be categorically proven.

There is an immense body of work investigating the role of mTOR in the regulation of cell proliferation for certain cell types but much less knowledge on the exact mechanisms by which mTORC1 signaling regulates β-cell cycle progression. However, mTORC1 is known to modulate the synthesis and stability of cyclin D2 and D3 in β-cells (45). These cyclins form a complex with cyclin-dependent kinase 4, which controls cell cycle progression. Interestingly, reduced cyclin D1 and D2 levels have been observed in rat islets treated with rapamycin, with associated reduction in β-cell proliferation (46).

mTORC1 also appears to play a role in insulin secretion in pancreatic β-cells. Knockdown of TSC1 in mice results in significant increases in insulin production, independent of β-cell number (39). In addition, chronic treatment with rapamycin inhibits GSIS in clonal β-cell lines as well as rodent and human islets (Table 1). However, whether this effect is via mTORC1 or mTORC2 is unclear. The control of insulin secretion in β-cells involves a number of complex signaling pathways, and as such the mechanisms by which rapamycin may regulate insulin secretion remain unknown. One proposed mechanism is that inhibition of mTORC1 decreases mitochondrial function, specifically, the activity of α-ketoglutarate dehydrogenase. This results in reduced carbohydrate metabolism and therefore reduced mitochondrial ATP production (47), which is known to regulate insulin secretion in β-cells (48). Other explanations are that rapamycin promoted autophagy, a process primarily controlled by mTORC1 rather than mTORC2, or that the intracellular degradation of cytoplasmic proteins involved in insulin production results in the inhibition of insulin secretion (49).

mTORC2. It is not entirely clear how mTORC2 activity is regulated, but there is evidence that it can be stimulated by amino acids and growth factors (50,51). Downstream

TABLE 4
Summary of studies investigating the effects of rapamycin on pancreatic β-cell proliferation

Authors (ref.)	Experimental model	Rapamycin dose	Treatment duration	Significant findings
Ex vivo				
Bussiere et al. (88)	Human pancreatic ductal cells	20 ng/mL	6 days	↓ Proliferation
Bussiere et al. (88)	Neonatal porcine islets	20 ng/mL	6 days	↓ Proliferation
Niclauss et al. (46)	Rat islets	0.1–10 ng/mL	1–5 days	↓ Proliferation
Sun et al. (96)	Porcine neonatal pancreas cell clusters	10–30 ng/mL	8 days	↓ Proliferation
In vivo				
Balcazar et al. (45)	RIP-rtTA/tetOAkt1 transgenic mice	5 mg/kg	14 days	↑ Proliferation without rapamycin, ↓ proliferation with rapamycin
Fraenkel et al. (65)	*P.obesus* rats	0.2 mg/kg/day	16 days	↓ Proliferation (not significant)
Niclauss et al. (46)	Murine syngeneic graft	0.3 mg/kg/day	8 days	↓ Proliferation
Rachdi et al. (40)	βTsc2($^{-/-}$) transgenic mice	5 mg/kg	14 days	↑ Proliferation without rapamycin, ↓ proliferation with rapamycin
Zahr et al. (97)	Murine pregnancy	0.2 mg/kg/day	5–7 days	↓ Proliferation

P.obesus, *Psammomys obesus*.

TABLE 5
Downstream targets of mTORC1

Target (ref.)	Acronym	Function
eIF4E-binding proteins (98)	4EBPs	Translation repressor proteins
S6 kinase proteins (99)	S6K1, S6K2	Promote mRNA translation
Lipin-1 nuclear import (100)	—	Mediates lipid metabolism
Ying Yang 1–peroxisome proliferator–activated receptor γ coactivator 1-α (101)	YY1-PGC1α	Transcription factor complex regulating mitochondrial metabolism
Growth factor receptor–bound protein 10 (102)	Grb10	Inhibits insulin signaling via IRS inhibition
Transcription factor 3C (103)	TFIIIC	Transcription factor
Insulin-like growth factor 2 mRNA-binding protein (103)	IMP2	Controls IGF-2 biosynthesis
Signal transducer and activator 3 (104)	STAT3	Transcription activator
Hypoxia-induced factor 2 (105)	HIF2	Transcription factor that responds to hypoxia
Unc-51–like kinase 1 (106)	ULK1	Regulates autophagy
Autophagy-related protein 13 (107,108)	Atg13	Regulates autophagy
Death-associated protein 1 (109)	DAP1	Regulates autophagy

targets of mTORC2 include protein-kinase C (PKC)-α (44–46) and protein kinase B (PKB) (52)—both serine/threonine kinases that play roles in the regulation of a number of key cellular processes including apoptosis, proliferation, motility, and differentiation—and serum- and glucocorticoid-induced protein kinase 1 (53), which has a role in control of ion transport (54).

Role of mTORC2 in pancreatic β-cell homeostasis. β-cell–specific deletion of Rictor, an essential component of mTORC2, in mice is associated with reduced plasma insulin levels due to reduced insulin secretion from islets with resultant hyperglycemia (55). This was associated with reductions in β-cell mass and proliferation but no increase in β-cell apoptosis. Our group has demonstrated that knockdown of Rictor by small interfering RNA in rat islets results in increases in β-cell apoptosis and reduced GSIS (36). These studies specifically demonstrate that mTORC2 activity plays a leading role in both β-cell survival and function. Importantly, prolonged rapamycin treatment (24 h) of MIN6 cells, rat islets, or human islets (36) results in the inhibition of mTORC2 through the dissociation of mTORC2. This precedes the toxic effects of rapamycin on both function and viability and is coincident with a decrease in PKB phosphorylation and downstream signaling. Interestingly, the expression of constitutive active PKB in MIN6 cells and rat islets can ameliorate the detrimental effects of rapamycin on GSIS and cell viability (36). Taken together, this suggests that rapamycin β-cell toxicity may be predominantly mediated via inhibition of mTORC2 and its subsequent effect on PKB signaling. However, this is based on in vitro experiments in β-cells and requires further confirmation in vivo.

A large number of studies have demonstrated that PKB, a key downstream effector of mTORC2, plays an important role in both survival and function of β-cells. These studies provide further insight into the possible role of mTORC2 in β-cell homeostasis. For example, transgenic mice expressing a constitutively active PKB in β-cells exhibit significant increases in β-cell mass resulting from an increase in both β-cell number and size (56,57). This manifested in significantly higher plasma insulin levels, improved glucose tolerance, and resistance to streptozotocin-induced diabetes. Expression of constitutively active PKB has also been shown to protect against fatty acid–mediated (58), cytokine-mediated (59), and AMP kinase–mediated (60) cytotoxicity in INS-1 cells, a rat β-cell line, and primary rat β-cells, respectively. Conversely, studies in transgenic mice lacking PKB show significantly higher blood glucose levels, lower insulin levels, and impaired glucose tolerance.

These studies raise the question of whether in vivo activation of PKB might improve the outcome of islet transplantation by improving the function and survival of transplanted β-cells or, indeed, provide some protection against rapamycin-induced NODAT. There are a number of potential pharmacological mechanisms by which PKB can be activated in β-cells in vivo. Experimental data highlight GLP-1 (61), erythropoietin, and statins (62,63) as promising agents that warrant clinical investigation.

Effects of rapamycin on insulin resistance. In addition to the direct effects of rapamycin on β-cells described above, there is evidence that rapamycin has effects on peripheral insulin resistance (64–66), which could also contribute to the changes in glucose homeostasis seen in animal models and human studies. Rapamycin has been shown to inhibit phosphorylation of IRS-1 and IRS-2 in human adipocytes, mimicking changes in type 2 diabetes (67,68), and in human peripheral blood monocytes with associated insulin resistance (69). Interestingly, recent evidence suggests that this insulin resistance is mediated predominantly via mTORC2 rather than mTORC1 (70–72).

Conclusions. Rapamycin is a key immunosuppressant, particularly in islet cell and kidney transplantation. However, there is a good body of both in vitro and in vivo evidence strongly suggesting that rapamycin has detrimental effects on pancreatic β-cells and peripheral insulin sensitivity. This has implications for islet transplant function and the development in NODAT in solid-organ transplantation. This toxicity is perhaps unsurprising, given that rapamycin inhibits mTOR, which via mTORC1 and mTORC2 is part of complex signaling pathways controlling a host of important cellular functions including mRNA translation, cell proliferation, cell growth, differentiation, protein synthesis, angiogenesis, and apoptosis.

This has important implications and brings into serious question the use of rapamycin as a primary immunosuppressant in islet transplantation and as a second-line agent in transplant recipients developing NODAT with calcineurin inhibitors. However, there are limited alternatives. One alternative is MMF, another antiproliferative agent that exerts its immunosuppressive effects via inhibition of inosine monophosphate dehydrogenase (73). However, studies in human islets have shown MMF treatment to result in significant reductions in GSIS (61). Despite this,

MMF is now in increasing use in clinical islet programs, while the use of rapamycin has declined. Of note, this change in immunosuppressive strategy has coincided with an improvement in primary efficacy seen from the Collaborative Islet Transplant Registry (74). Indeed the Edmonton group, who was the original proponent of rapamycin in islet transplantation, no longer uses it, although this choice was because of the side effects of rapamycin rather than perceived toxicity to β-cells.

A promising new immunosuppressant is sotrastaurin, a PKC inhibitor, which has been used in phase II trials in kidney transplantation (75). Of particular note, sotrastaurin does not appear to have detrimental effects on cultured human islets or those transplanted into immunodeficient mice (76).

Given the suggestion that both rapamycin β-cell toxicity and insulin resistance may be mediated predominantly via mTORC2 rather than mTORC1 (36,55), the question arises whether an mTORC1-specific inhibitor would retain the immunosuppressive effects of rapamycin without any mTORC2-mediated toxicity. However, this involves the assumption that the immunosuppressive effects of rapamycin are indeed mediated predominantly via mTORC1, which is not currently known.

Further clinical investigation is also required regarding glucose homeostasis in patients receiving rapamycin to establish the relative influence of β-cell dysfunction and peripheral insulin resistance in the development of hyperglycemia.

In conclusion, the body of evidence from in vivo and in vitro studies strongly suggests that rapamycin has profound effects on glucose homeostasis. Potential mechanisms involved in this include reductions in β-cell size, mass, proliferation, and insulin secretion, alongside increases in apoptosis, autophagy, and peripheral insulin resistance.

ACKNOWLEDGMENTS

No potential conflicts of interest relevant to this article were reported.

A.D.B. conceived the manuscript, reviewed the literature, and wrote and edited the manuscript. M.L.N. conceived, wrote, and edited the manuscript. T.P.H. conceived the manuscript, reviewed the literature, and wrote and edited the manuscript.

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