Beta cell function during rapamycin monotherapy in long-term type 1 diabetes

L. Piemonti · P. Maffi · L. Monti · V. Lampasona ·
G. Perseghin · P. Magistretti · A. Secchi · E. Bonifacio

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Abstract

Aims/hypothesis Type 1 diabetes is considered non-reversible at end-stage disease when there is no measurable insulin production. However, there are indications that insulin-producing beta cells could be present or return if autoimmunity could be controlled. We therefore sought to determine whether immunosuppression therapy can reinstate beta cell function in patients with long-term type 1 diabetes.

Methods We examined pancreatic beta cell function in 22 patients with long-term type 1 diabetes (median disease duration 27 years), who had been receiving rapamycin

monotherapy (0.1 mg/kg; target trough levels 8–10 ng/ml; 26–314 days) as pre-conditioning for islet transplantation. As control, beta cell function was measured in 14 patients (median disease duration 17 years) who were waiting for an islet transplant without rapamycin pre-conditioning.

Results Fasting C-peptide increased from <0.03 nmol/l (0.0066 nmol/l, interquartile range [IQR] 0.0003–0.023) at baseline to 0.039 nmol/l (IQR 0.0066–0.096) at end of rapamycin monotherapy ($p<0.005$). In 12 patients, fasting C-peptide increased to >0.076 nmol/l (C-peptide responders). Exogenous insulin requirement decreased from 0.64 U/kg daily (IQR 0.56–0.72) to 0.57 U/kg (IQR

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L. Piemonti (✉) · P. Maffi · P. Magistretti · A. Secchi ·
E. Bonifacio
Diabetes Research Institute (HSR-DRI),
San Raffaele Scientific Institute,
Via Olgettina 60,
20132 Milan, Italy
e-mail: piemonti.lorenzo@hsr.it

L. Monti
Cardiometabolic and Core Lab, Division of Metabolic and
Cardiovascular Sciences, San Raffaele Scientific Institute,
Milan, Italy

V. Lampasona
Unit of Genomics for the Diagnosis of Human Pathologies,
Center for Genomics, Bioinformatics and Biostatistics,
San Raffaele Scientific Institute,
Milan, Italy

G. Perseghin
Unit of Obesity and Metabolic Related Diseases,
Division of Metabolic and Cardiovascular Sciences,
San Raffaele Scientific Institute,
Milan, Italy

G. Perseghin
Department of Sport, Nutrition and Health Sciences,
Università degli Studi di Milano,
Milan, Italy

A. Secchi
Unit of Clinical Transplant, Division of Immunology,
Transplantation and Infectious Diseases,
Università Vita-Salute San Raffaele,
Milan, Italy

E. Bonifacio
Center for Regenerative Therapies Dresden,
Dresden University of Technology,
Dresden, Germany

0.45–0.70; $p=0.01$), but this reduction was significant only in the 12C-peptide-responsive patients. Rapamycin monotherapy was also associated with a decrease in insulin antibody titre (median decrease 110 to 35.9 U/ml; $p<0.001$) and fasting serum proinsulin (median decrease 0.51 to 0.28 pmol/l; $p=0.001$). All variables remained unchanged in the 14 control patients.

Conclusions/interpretation Therapies to reinstate beta cell function may be applicable to patients with long-term C-peptide-negative type 1 diabetes.

Trial registration ClinicalTrials.gov NCT01060605

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Keywords C-peptide · Humans · Long-term type 1 diabetes · Rapamycin

Abbreviations

EIR	Exogenous insulin requirement
FOXO1	Forkhead box O1
GADA	Antibodies to GAD
IA	Antibodies to insulin
IQR	Interquartile range

Introduction

Several studies in patients with type 1 diabetes support the hypothesis that beta cells survive in the long term or that the pancreas has an underappreciated capacity for functional beta cell recovery [1–5]. A significant subset of patients with type 1 diabetes continue to produce C-peptide, years after their diagnosis [4–8]. Moreover, pancreatic cells staining for insulin are generally reported by autopsy studies of patients with long-term type 1 diabetes [9–13]. Finally, the persistence of beta cell autoimmunity in patients years after diabetes onset indirectly suggests the permanence of beta cell-derived antigens [14–18]. The notion that beta cells (if not function) remain for decades after the onset of type 1 diabetes begs the question of what would happen to beta cell function if we were able to halt immune disease?

Since 2002, we have been using two clinical protocols in which the immunosuppressive drug rapamycin is given as monotherapy to type 1 diabetes patients before solitary islet transplantation. These studies have provided the opportunity to investigate the effect of immunosuppression with rapamycin alone on beta cell function in patients with long-term type 1 diabetes.

Methods

Enrolment Patients with type 1 diabetes and on the waiting list for islet transplantation alone at the San Raffaele Diabetes Research Institute were eligible for clinical protocols in which rapamycin at a dose of 0.1 mg/kg (target trough levels 8–10 ng/ml) was prescribed as monotherapy for at least 4 weeks before the first islet infusion (ClinicalTrials.gov NCT01060605). The study protocols were approved by the Ethics Committee of the San Raffaele Scientific Institute and all patients gave informed consent before entering the study. Between February 2002 and March 2009, 23 patients aged 30 to 48 years (mean 38.5 years) were enrolled and started pre-treatment with rapamycin. Measurements during the pre-transplant preconditioning therapy were obtained on 22 of the 23 patients and included: (1) fasting serum C-peptide, exogenous insulin requirement (EIR), creatinine, GFR (calculated using the Modification of Diet in Renal Disease formula [19]), weight, serum rapamycin concentration, fasting glycaemia, antibodies to insulin (IA) and antibodies to GAD (GADA), each measured every week for the 1st month and monthly thereafter; and (2) HbA_{1c}, fasting serum proinsulin and fasting plasma glucagon, measured monthly. The median duration of pre-transplant rapamycin treatment was 111.5 days (minimum 26, maximum 314). Of the 23 patients enrolled, 18 completed the pre-transplant therapy and received an islet infusion. Of the remaining five patients, two discontinued therapy after 38 days because of a sustained increase in urinary protein excretion which resolved after drug withdrawal. Two others withdrew voluntarily after 32 and 78 days because of the appearance of mouth ulcers, acne and irregular menstrual cycles, and one patient was withdrawn after 69 days because of a sustained increase (>0.1665 nmol/l) in fasting C-peptide. The control group consisted of 14 long-term type 1 diabetes patients who were aged 20 to 68 years (mean 37.5 years) and had been recruited to the waiting list for islet transplant during the same period, but did not receive pre-treatment with rapamycin. Although not matched, we considered these as informative, since they were recruited over a similar period, fulfilled islet transplantation entry criteria and had a similar follow-up time (median 173 days; minimum 31, maximum 396). General characteristics of all patients are reported in Table 1.

Assays Serum C-peptide concentrations were determined using a commercially available assay (DakoCytomation, Ely, UK) that has a lower detection limit of 0.03 nmol/l (0.09 ng/ml). The normal range in healthy individuals was 0.331 to 0.662 nmol/l, with an interassay precision of 3.8% at 0.585 nmol/l. Serum proinsulin was measured with a kit (Proinsulin Enzyme Immunoassay; DRG

Table 1 Patient characteristics at start of follow-up

Characteristic	Control group	Rapamycin group	<i>p</i> value
<i>n</i>	14	22	
Age (years)	37±13	38±8	0.75
Sex			
Men (<i>n</i>)	8	11	0.63
Women (<i>n</i>)	6	11	0.63
Weight (kg)	64±6	62±10	0.48
BMI (kg/m ²)	22.3±1.0	21.8±2.6	0.54
Creatinine (μmol/l)	76.02 (68.06–83.98)	69.83 (56.57–84.86)	0.37
GFR (ml/s)	1.51 (1.25–1.71)	1.70 (1.58–1.85)	0.055
Diabetes			
Age of onset (years)	15 (9.5–23.7)	9.5 (5.0–19.7)	0.23
Duration (years)	17 (11.8–22.0)	27 (17.7–35.0)	0.013
EIR (Ukg ⁻¹ day ⁻¹)	0.65 (0.55–0.78)	0.64 (0.56–0.72)	0.73
HbA _{1c} (%)	8.2 (7.5–8.7)	8.6 (8.0–9.3)	0.16
Fasting C-peptide			
Median (nmol/l)	0.011(0.0003–0.02648)	0.0066 (0.0003–0.023)	0.76
Detectable (<i>n/n</i>)	3/14	4/22	1
Fasting proinsulin			
Median (pmol/l)	0.23 (0.12–1.22)	0.51 (0.11–4.10)	0.28
Detectable (<i>n/n</i>)	5/14	10/22	0.67
Glucagon (ng/l)	NT	53.9 (37.1–63.8)	
IA-positive (<i>n</i>)	10/14	20/22	0.18
GADA-positive (<i>n</i>)	6/14	5/22	0.27

Data are expressed as median (IQR) or mean ± SD
NT, not tested

Instruments, Marburg, Germany) that has a lower detection limit of 0.5 pmol/l. The normal range was 5 to 15 pmol/l, with an interassay precision of 6.8% at 7.32 pmol/l. No cross-reactivity is reported for human insulin (17,000 pmol/l), human C-peptide (33,000 pmol/l), proinsulin of somatomedin-C (10 μg/ml) and somatomedin-C (1 μg/ml). Plasma glucagon was measured with a kit (Glucagon RIA; Linco Research, St Charles, MO, USA). The normal range was 50 to 150 ng/l, with interassay precision of 5% at 60 ng/l. HbA_{1c} was measured by a program (Variant II Hemoglobin A_{1c}) that uses the principle of ion exchange HPLC (Bio-Rad Laboratories, Hercules, CA, USA). Serum creatinine was determined with a chemistry system (ADVIA 2400; Bayer Healthcare, Tarrytown, NY, USA). Rapamycin was measured in whole blood using IMx sirolimus MEIA (Abbott Laboratories, Abbott Park, IL, USA). IA and GADA were determined by immunoprecipitation of recombinant antigens as previously described [20] and validated in the Diabetes Autoantibody Standardization Program (Laboratory 153) [21].

Statistical analysis Continuous variables were compared using the two-sided, paired or unpaired Student's *t* test for normally distributed variables, and Wilcoxon's signed-rank test or Mann–Whitney *U* test for non-normally distributed variables. Categorical variables were compared

by Fisher's exact test. Comparison of continuous variables at different time points after rapamycin treatment was done by generalised linear model for the analysis of repeated measures testing for within-participants effects (vs time 0) or between-participants effects (non-responders vs responders). Non-normally distributed variables (creatinine, GFR, EIR, HbA_{1c}, C-peptide, proinsulin, glucagon, IA, GADA) were used after log transformation. Data are expressed as median with interquartile range (IQR) in parenthesis or mean±SD. Statistical analyses were performed using SPSS 13.0 for Windows (SPSS, Chicago, IL, USA).

Results

Before treatment with rapamycin, 4 of the 22 patients studied had detectable C-peptide concentrations (>0.03 nmol/l) up to a maximum of 0.073 nmol/l. Unexpectedly, ten also had detectable proinsulin concentrations >0.5 pmol/l. Patients with detectable C-peptide prior to treatment were older (44±4 vs 37±5 years, *p*=0.03) and also older at diabetes onset (23±8 vs 9±7 years, *p*=0.004). Of the 14 control patients, three had detectable C-peptide and five detectable proinsulin at start of follow-up. Only 4 of 36 patients studied (both groups) had detectable C-peptide and proinsulin.

Fig. 1 Rapamycin treatment in long-term type 1 diabetes. **a** Fasting C-peptide in 14 patients who were on the waiting list for an islet transplant (controls; white circles) and in 22 patients with long-term type 1 diabetes treated with rapamycin (0.1 mg/kg daily, targeting serum levels of 8–10 ng/ml) as monotherapy at least 1 month before islet transplantation (rapamycin-treated; black circles). Fasting C-peptide concentrations are shown at the start (controls first measurement while on waiting list, rapamycin-treated day 0 prior to start of treatment) and end of follow-up. For rapamycin treated patients, fasting C-peptide concentrations are also shown at 1, 2, 3 and 4 weeks (W) after initiating rapamycin therapy. NS = $p > 0.05$, * $p \leq 0.05$, ** $p < 0.01$ and *** $p < 0.005$ vs start values (two-sided Wilcoxon's signed-rank test). Dashed line, threshold of preserved beta cell function as defined in the Diabetes Control and Complications Trial. **b** Daily EIR per kg body weight, IA titre and serum proinsulin concentration as labelled, at start and end of follow-up in control patients and rapamycin-treated patients. The latter are categorised as non-responders and responders according to whether they reached C-peptide concentrations ≥ 0.076 nmol/l at least one time during follow-up. p values, calculated by two-sided Wilcoxon's signed-rank test, left to right: EIR $p = 0.35$, $p = 0.39$, $p = 0.003$; IA: $p = 0.9$, $p = 0.03$, $p = 0.003$; proinsulin $p = 0.72$, $p = 0.09$, $p = 0.008$

During rapamycin treatment (Fig. 1, Table 2) the median fasting C-peptide in the 22 patients increased from <0.03 nmol/l (0.007 nmol/l; IQR 0.0003–0.023) at baseline to 0.036 nmol/l (IQR 0.006–0.059) at 4 weeks of treatment ($p = 0.007$) and 0.039 nmol/l (IQR 0.007–0.096) at the last time point available before transplant ($p = 0.005$). The proportion of patients with detectable fasting C-peptide increased from 4 of 22 prior to rapamycin therapy to 13 of 22 ($p = 0.01$). Over the same period, median EIR decreased from 0.64 U/kg daily (IQR 0.56–0.72) to 0.57 U/kg daily (IQR 0.45–0.70) at the last follow-up ($p = 0.04$), while HbA_{1c} did not change (8.6% [IQR 8.0–9.3] vs 8.5% [IQR 7.9–9.3]). Median fasting proinsulin decreased from 0.51 pmol/l (IQR 0.11–4.1) to 0.28 pmol/l (IQR 0.02–0.86) at last follow-up ($p = 0.001$). The median IA titre decreased during rapamycin treatment from 110 U (IQR 13.8–382) to 35.9 U (IQR 5.8–122) at last follow-up sample ($p < 0.001$). GADA, weight, serum creatinine and GFR did not change during rapamycin treatment, but a trend towards increasing fasting glucagon was observed ($p = 0.05$). The control group did not change with respect to these variables during follow-up.

The 22 patients receiving rapamycin were categorised according to whether they reached C-peptide concentrations above the threshold of preserved beta cell function as defined in the Diabetes Control and Complications Trial [22] (Electronic supplementary material [ESM] Fig. 1). Patients classified as responders were those ($n = 12$) with at least one follow-up C-peptide concentration ≥ 0.076 nmol/l. The remaining ten patients were classified as non-responders. Interestingly, within the responder group only seven patients had C-peptide >0.076 nmol/l at the last follow-up, while two patients had increased C-peptide concentrations throughout follow-up. Responders were more likely to be GADA-positive (5/12 vs 0/10, $p = 0.04$; ESM Table 1) and have detectable fasting proinsulin concentrations (8/12 vs 2/10, $p = 0.04$; data not shown) prior to rapamycin treatment. Pre-treatment detectable fasting C-peptide did not discriminate responders vs non-responders (3/12 vs 1/10, $p = 0.59$). During follow-up, EIR ($p = 0.003$), IA ($p = 0.003$) and proinsulin ($p = 0.008$) decreased in responders, whereas only IA decreased in non-responders ($p = 0.03$; Fig. 1b, ESM Table 1).

Discussion

As part of an islet transplant protocol, we were able to evaluate whether a course of rapamycin monotherapy prior to transplant improved pancreatic beta cell function in patients with long-term type 1 diabetes and negligible fasting C-peptide. Increases in C-peptide to concentrations above those classified as preserved in the Diabetes Control

Table 2 Follow-up values in rapamycin-treated and control patient groups

Values	Weekly values, rapamycin group (<i>n</i> =22)							Control group (<i>n</i> =14)		
	Start	1 ^a	2 ^a	3 ^a	4 ^a	End	<i>p</i> value ^b	Start	End	<i>p</i> value
Rapamycin (ng/ml)	0	12.2	13	7.8	6.8	9.1	<0.001	0	0	1.0
EIR (Ukg ⁻¹ day ⁻¹)	0.64	0.61	0.57	0.58	0.56	0.57	0.039	0.65	0.64	0.35
C-peptide (nmol/l)	0.0066	0.0198	0.0115	0.0132	0.036	0.039	0.004	0.011	0.011	0.75
Proinsulin (pmol/l)	0.51	NT	NT	NT	0.39	0.28	0.001	0.23	0.20	0.31
Glucagon (ng/l)	46.5	NT	NT	NT	56.1	52.9	0.050	NT	NT	
HbA _{1c} (%)	8.6	NT	NT	NT	8.6	8.4	0.24	8.2	8.1	0.72
Creatinine (μmol/l)	69.83	66.3	68.01	66.3	69.8	69.8	0.45	76.02	NT	
GFR (ml/s)	1.70	1.76	1.8	1.81	1.78	1.78	0.75	1.51	NT	
Weight (kg)	64	64	63.5	63.5	64.5	64	0.61	64	63	1.0
IA (U)	110	97	67	60	60	36	0.001	13	13	0.9
GADA (U)	0.91	0.96	0.88	0.86	0.96	1.05	0.32	2.1	1.8	0.8

Data are expressed as median

^a Week number

^b Comparisons in the rapamycin treated group were done by generalised linear model for the analysis of repeated measures testing within-participants effects vs start

NT, not tested

and Complications Trial [21], along with decreased insulin usage were observed at least transiently in 12 of 22 patients. Consistent with an immunosuppressive effect, titres of antibodies to injected insulin, but not to the autoantigen GAD, decreased under rapamycin therapy.

Our findings highlight the fact that beta cell function can be increased in patients with long-term type 1 diabetes. This supports the recent report of measurable native pancreatic C-peptide in long-term patients who had undergone beta cell replacement therapy either in the form of islet or whole-pancreas transplantation [1]. Similarly, biopsy data from a pancreas allograft recipient suggest that transplant-associated immunosuppression and euglycaemia may have promoted ‘beta-like’ cells to recover within the patient’s native pancreas [3]. On the basis of these findings, an intervention combining an anti-IL-2 receptor antibody with the glucagon-like peptide 1 agonist exenatide was performed in long-term type 1 diabetes patients. Although that study demonstrated that such patients have beta cells able to secrete insulin in a physiologically regulated manner [2], there was no improvement in beta cell function after treatment.

The data from our study are likely to be of high interest to the large number of long-term type 1 diabetes patients. Nevertheless, there are some limitations. Since the study was not designed to fully test beta cell function in the pre-transplant period, C-peptide measurements after stimulation are unavailable. Second, the study had an open design and the control group data are not matched. Thus, although unlikely, the rises in C-peptide could be explained by the

random variation that may be observed with frequent measurement. Third, we cannot be sure that the C-peptide detected in circulation was secreted from beta cells, as non-pancreatic cells have been reported to produce insulin [23]; moreover, although we did not see a decline in renal function as measured by GFR, it is possible that subtle effects of rapamycin on the kidney could have accounted for the rises in serum C-peptide. Fourth, we do not know whether the effect is due to general immunosuppression or specific to rapamycin effects on metabolism and nutrition, including possible nausea or loss of appetite, although the latter was not corroborated by changes in patient weight (Table 1). Finally, the most striking changes in C-peptide levels happened at week 1, after which levels fluctuated, with only seven responders having increased C-peptide at end of follow-up. This could reflect any of the above considerations, but also the possibility that initial rises are blunted by: (1) autoimmunity or negative effects of rapamycin, which may include diabetogenicity [24–26]; (2) reduced insulin uptake by adipocytes [27]; and (3) reduced beta cell mass expansion [28].

Of considerable interest with respect to potential mechanisms were the proinsulin data. Ten patients had detectable serum proinsulin prior to treatment. Upon treatment, proinsulin declined along with the rise in C-peptide. Moreover, C-peptide responder status was associated with detectable proinsulin pre-treatment as well as GADA positivity. These data suggest that defective processing of proinsulin or immature beta cells may be present in these patients and that rapamycin treatment could

restore the correct function. It has also been shown that the transcription factor, forkhead box O1 (FOXO1), affects carboxypeptidase E processing of hormone in the hypothalamus and that the mechanistic target of rapamycin (mTOR) pathway affects this process [29]. It would, therefore, be interesting to determine the effects of rapamycin on FOXO1 translocation and carboxypeptidase levels in the beta cell.

In conclusion, our study suggests that residual beta cells or beta-like cells may play an important role as a potential source of renewed beta cell function in long-term type 1 diabetes patients. Trials in such patients could be considered, in particular to confirm the effects seen here with rapamycin. These trials will need to address the numerous pitfalls in examining beta cell function in patients with no or minimal detectable C-peptide (discussed above) and will hopefully increase our current limited knowledge of beta cell function in patients with long-term type 1 diabetes.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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