The impact of short-term ciclosporin A treatment on insulin secretion and insulin sensitivity in man

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Abstract

Background. The objectives of the present study were to investigate the possible adverse effects of ciclosporin A (CsA, Sandimmun Neoral®) on insulin secretion and insulin sensitivity (IS) in man.

Methods. A total of 11 Caucasian non-diabetic haemodialysis (HD) patients were recruited from the Norwegian transplant waiting list to participate in this study. The patients underwent two consecutive 3h hyperglycaemic glucose clamp procedures, before and following 2 weeks of oral CsA treatment. Statistical analyses included nine patients (7M/2F, mean age 61±14 years) as two patients were withdrawn due to side effects and poor compliance. First and second phase insulin secretion (Secr1.phase and Secr2.phase) were estimated as area under the insulin serum concentration vs time curve (AUC) during the first 10 min and the last hour of the clamp, respectively. The IS index (ISI) was calculated as the glucose disposal rate corrected for insulin levels during the last 60 min of the procedure.

Results. Secr2.phase decreased significantly (30%) following CsA treatment (P = 0.045). In contrast, no significant change was observed in the average Secr1.phase or ISI, although relatively large inter-individual differences were present. Calculation based on C-peptide concentrations gave the same results. No significant changes in body weight, dialysis status, patient medication or safety parameters were observed.

Conclusions. Short-term treatment with CsA at doses used following transplantation seems to impair Secr2.phase, but has no significant effect on Secr1.phase in Caucasian HD patients. The mechanism behind these findings and their possible clinical implications need further study.

Keywords: ciclosporin A; glucose clamp; insulin resistance; pancreatic β-cell function; renal transplantation

Introduction

New-onset post-transplantation diabetes mellitus (PTDM) is frequently observed after renal transplantation [1–3]. We have previously shown that PTDM developed in one-fifth and impaired glucose tolerance (IGT) in one-third of the renal transplant recipients receiving a ciclosporin A (CsA, Sandimmun Neoral®) based triple immunosuppressive regimen [4]. Certain immunosuppressive drugs play a pivotal role in the pathogenesis of PTDM, adversely affecting both insulin sensitivity and pancreatic β-cell function [1–8]. In a recent systematic review, Montori et al. [2] argued that immunosuppressive drugs may explain 75% of newly diagnosed cases of PTDM. The diabetogenic effect of glucocorticoids is primarily caused by insulin resistance [9–11]. However, a small study of 20 young healthy men treated with dexamethasone 2 or 6 mg/day for 3 days, showed that high-dose, but not low-dose steroid treatment was associated with impaired insulin secretion [12]. In vitro and biopsy studies in human and animal models suggest that both CsA and tacrolimus impair pancreatic β-cell function. The exact mechanism for this is poorly understood but may include islet cell damage [13], diminished insulin synthesis or impaired insulin secretion [14–18]. It is generally agreed that tacrolimus has a greater diabetogenic propensity than CsA in clinical use [1–3,6]. There is also some evidence that the adverse effects of tacrolimus on pancreatic β-cell function are dose...
Recent studies [7,19] have shown a lack of significant correlation between CsA dose or CsA blood trough concentration and impaired insulin secretion in dialysis patients. In contrast, Robertson et al. [20] did not find any abnormalities in pancreatic β-cell function as assessed by IVGTTs before and during a 2-year course of ciclosporin therapy in a small group of patients with multiple sclerosis.

These studies were, however, limited by study design with confounding factors such as concurrent steroid treatment and methods examining only the acute insulin response (IVGTT). Importantly, in contrast with the hyperglycaemic glucose clamp technique, the IVGTT does not allow evaluation of the biphasic insulin response, including the $\text{Secr}_2\text{phase}$ [21,22].

In this study we investigated the effects of single treatment with CsA on first and second phase insulin secretion ($\text{Secr}_1\text{phase}$ and $\text{Secr}_2\text{phase}$) in human subjects by the use of the hyperglycaemic glucose clamp technique [23]. In addition, a possible adverse effect of CsA on insulin sensitivity was addressed.

Materials and methods

Approvals and ethics

This study was recommended by the Regional Committee for Medical Research Ethics, approved by the Norwegian Medicines Agency (EUDRA CT no. 2004-004488-31) and registered on ClinicalTrials.gov (NCT00139035). The study was performed in accordance with the Declaration of Helsinki [24].

Study design and eligibility

This was an investigator-initiated, interventional, non-randomized, open-label study. The primary outcome was change in insulin secretion after 2 weeks of treatment with CsA. Since the specific effect of CsA cannot be ascertained in renal transplant recipients due to concomitant use of other immunosuppressant drugs that may affect glucose/insulin metabolism (e.g. steroids), we found it appropriate to recruit haemodialysis (HD) patients from the renal transplant waiting list. From an ethical point of view, taking into account the potentially serious adverse effects of ciclosporin A, we found it unacceptable to ask healthy volunteers to participate. Exclusion criteria were age <18 or >75 years, diabetes mellitus, treatment with glucocorticoids, time in dialysis <2 months, unstable angina pectoris, a recent acute myocardial infarction (<3 months), uncompensated heart failure, anaemia (Hb <10.0 g/dl) and pregnancy. All eligible patients underwent an oral glucose tolerance test (OGTT) before inclusion to secure that patients with diabetes mellitus were not included.

Study drug

The patients were evaluated before and following 2 weeks of treatment with CsA (Sandimmun Neoral®, Novartis, Basel). CsA was administered according to our current dosage scheme for renal transplant recipients in the early post-transplantation period. The initial daily dose of CsA (divided in two doses) was 300 mg in patients with a body weight $\geq 60$ kg, but was limited to 5 mg/kg/day in patients with a body weight $<60$ kg. CsA trough concentration (C0) was measured up to three times per week, and appropriate dose adjustments were performed to target the therapeutic window of 100–200 μg/l. CsA whole blood concentrations were measured with the CEDIA assay (Microgenics Corporation, Fremont, CA, USA). To optimize drug compliance, the patients were instructed to fill out a drug dosage scheme.

Subjects

Informed consent was obtained from 11 non-diabetic HD patients (8 men) attending the dialysis unit at Rikshospitalet University Hospital (n = 4) or Akershus University Hospital (n = 7) two (n = 6) or three (n = 5) times a week. A total of 10 patients completed both clamp procedures, as one female patient withdrew after 3 days of CsA treatment due to nausea. Further, one male patient was excluded due to poor compliance (repeated non-quantifiable levels of whole blood CsA). Thus, data from nine patients were included in the statistical analyses.

Table 1. Results from the oral glucose tolerance tests

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Fasting plasma glucose (mmol/l)</th>
<th>2h plasma glucose (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.0</td>
<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>5.3</td>
<td>8.5</td>
</tr>
<tr>
<td>5</td>
<td>5.0</td>
<td>6.6</td>
</tr>
<tr>
<td>6</td>
<td>4.8</td>
<td>6.5</td>
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<td>8</td>
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<td>5.6</td>
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<td>9</td>
<td>6.1</td>
<td>8.0</td>
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<tr>
<td>10</td>
<td>5.4</td>
<td>7.8</td>
</tr>
<tr>
<td>11</td>
<td>4.6</td>
<td>4.7</td>
</tr>
</tbody>
</table>
arteriovenous fistula (n = 5) were cannulated (BD VenflonTM Pro 1.3*33 mm) for the infusion of glucose (Glucose 200 mg/ml, Fresenius Kabi AB, Uppsala, Sweden), alternatively the dialysis catheter was used (n = 3). Blood glucose concentrations were measured at −15, 0, 2.5, 5, 7.5 and 10 min after the start of the glucose infusion, and thereafter repeated every 5 min for the rest of the procedure. A bolus dose of 150 mg glucose/kg body weight was administered during the first 2 min of the clamp. The glucose infusion was started at 5 min, and the infusion rate was adjusted in order to maintain the blood glucose level as close to 10 mmol/l as possible [23]. Blood samples for measurements of insulin and C-peptide were drawn at −15, 0, 2.5, 5, 7.5, 10, 15, 20, 40, 60, 80, 100, 120, 140, 160 and 180 min.

Methods for analysis of glucose, insulin and C-peptide
Whole blood glucose was analysed using HemocueABTM B-glucose Analyser (Angelholm, Sweden). Blood samples for analysis of insulin and C-peptide were drawn in SST tubes (VacutainerTM), left at room temperature for 20 min to coagulate, and centrifuged at 1800 g for 10 min. Serum was decanted and stored at −20°C. Serum insulin and C-peptide concentrations were analysed in two parallels with Immulite Insulin® and Immulite C-Peptide® kits, respectively, on the Immulite 2000® platform (DPC Los Angeles, CA, USA).

Calculations
Lean body mass (lbm) was estimated using Hume’s formula [27] which correlates well with tritiated water or electrical bioimpedance measures [28]. Secr1.phase was calculated as the area under serum insulin vs time curve (AUC, trapezoidal rule) during the first 10 min of the clamp procedure, and Secr2.phase was calculated as the insulin AUC during the last hour (120–180 min) of the clamp procedure. The same calculations were also performed for C-peptide concentrations. Glucose disposal rate (GDR) was calculated from the amount of glucose infused during the last hour of the clamp. The IS index (ISI) was calculated as GDR [µmol/kg (lbm)min] divided by mean serum insulin (pmol/l) in the same period. Glucose clearance was calculated as ISI divided by mean serum glucose during the last 60 min of the clamp [23].

Statistics
The data are presented as means (SDs, range) or proportions. Skewly distributed variables were log-transformed before statistical analysis. Paired t-tests were used to analyse changes in continuous variables after treatment with CsA. P-values < 0.05 were considered statistically significant. The analysis was implemented using SPSS 12.0 (SPSS, Inc., Chicago, IL, USA). The study had a power of > 80% to detect a 20% change in insulin secretion after institution of CsA (α = 5%) if at least seven patients completed the procedures [29]. To allow for withdrawals or any technical failure, 11 patients were recruited.

Results
Baseline characteristics and safety parameters
Baseline characteristics are presented in Table 2. The patients fulfilling protocol treatment (seven men and two women), were all of Caucasian origin, and had a mean age of 61 (14) years. Median (range) time in dialysis was 14 (3–38) months. Mean (SD, range) daily CsA dose and CsA trough levels were 233 mg (90, 100–400) and 136 µg/l (74, 150–310), respectively, on the day of the second clamp procedure. The median body weight (82, 49–95 kg) did not change significantly during the study. No significant changes in blood pressure, haematological parameters, dialysis status, serum lipids, HbAlc, fasting blood glucose, high sensitivity CRP, sodium, potassium, calcium, phosphate, creatine kinase or concomitant medication (data not shown), were observed during the study. Liver parameters (ASAT, LD, GT, bilirubin) were also stable, with the exception of a slight decline of mean ALAT (from 22 ± 15 to 16 ± 10 U/l, P = 0.017).

Hyperglycaemic clamp studies
As shown in Figure 1, the mean blood glucose concentrations during the clamp (0–180 min) were comparable before and after treatment with CsA, with coefficients of variation of 4.3 and 3.6%, respectively.

The average fasting C-peptide levels increased significantly from 1.65 (0.92) to 1.93 (1.10) nmol/l (P = 0.018) during 2 weeks of CsA treatment, whereas fasting insulin levels did not change significantly [84 (49) vs 80 (41) pmol/l, P = 0.738]. No significant change in the molar ratio of fasting C-peptide to insulin was observed [21 (7) vs 27 (13), P = 0.118].

The serum insulin concentrations vs time curves during the 3 h glucose clamp procedures before and after treatment with CsA are shown in Figure 1. The data are presented as means (SDs, range) or proportions (%).

Table 2. Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>61 (14, 27–74)</td>
</tr>
<tr>
<td>Male gender</td>
<td>7 (78%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 (19.7–33.1)</td>
</tr>
<tr>
<td>Waist–hip ratio</td>
<td>0.93 (0.81–1.08)</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.2 (4.6–6.1)</td>
</tr>
<tr>
<td>2 h plasma glucose (mmol/l)</td>
<td>6.9 (4.7–9.0)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>12.0 (10.4–13.6)</td>
</tr>
<tr>
<td>Glycosylated haemoglobin (%)</td>
<td>5.4 (4.9–6.3)</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>41 (23–77)</td>
</tr>
<tr>
<td>High-sensitivity CRP (mg/l)</td>
<td>7.7 (4.4–28.0)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>145 (128–163)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78 (54–96)</td>
</tr>
</tbody>
</table>

Data are given as means (SD, range) or proportions (%).
after CsA treatment are shown in Figure 2. The curves show no major changes in insulin concentrations during the first 40 min, but the curves clearly diverge during the last 2 h of the clamp procedures (before and after 14 days of treatment with CsA). C-peptide concentrations before and after CsA treatment showed a similar pattern as insulin (Figure 3).

**First-phase insulin secretion**

On average, Secr$_{1\text{-phase}}$ did not change significantly ($\Delta$Secr$_{1\text{-phase}} = -0.4\%$, $P = 0.885$) during the study period. However, inter-individual variations were observed; three patients had a 41–50% decline, whereas another three patients had 36–70% increases in Secr$_{1\text{-phase}}$, respectively.

**First-phase C-peptide secretion**

First-phase C-peptide secretion (AUC$_{0-10\text{min}}$) did not change significantly [5.5 (4.5) vs 3.0 (2.6), $P = 0.129$].

**Second-phase insulin secretion**

Mean Secr$_{2\text{-phase}}$ decreased significantly after treatment with CsA ($\Delta$Secr$_{2\text{-phase}} = -30\%$, $P = 0.045$) (Figure 4). A total of seven patients had a decline in Secr$_{2\text{-phase}}$ ($\Delta$Secr$_{2\text{-phase}} = -17$ to $-57\%$), one had an increased Secr$_{2\text{-phase}}$ (No. 1; $\Delta$Secr$_{2\text{-phase}} = 63\%$), whereas no change ($\Delta$Secr$_{2\text{-phase}} = 0\%$) was observed in patient No. 9. The average decline in Secr$_{2\text{-phase}}$ did not differ significantly between patients with NGT and IGT ($P = 0.165$, individual results shown in Figure 4). No significant correlations were found between $\Delta$Secr$_{2\text{-phase}}$ and CsA dose, CsA trough levels or $\beta$-blocker treatment (data not shown).
The second-phase C-peptide secretion (AUC_{120\text{min}} \text{–} 180\text{min}) also declined significantly (−32%, \( P = 0.016 \)), and eight patients had a lower second phase C-peptide release after CsA treatment (including patient No. 9). One patient (No. 1) showed an increase in both second-phase C-peptide and insulin secretion.

The average second-phase molar ratio of C-peptide to insulin did not change significantly [9.2 (4.3) \text{ vs } 8.8 (3.8), \( P = 0.655 \)] during the study.

**Insulin sensitivity and glucose clearance**

There were no statistically significant differences in mean ISI (\( \Delta \text{ISI} = 19\%, \ P = 0.238 \)) or glucose clearance (\( \Delta = 16\%, \ P = 0.319 \)) during the study.

**Discussion**

It is generally accepted that calcineurin inhibitors adversely affect glucose metabolism, and it has been hypothesized that the main mechanism is inhibition of pancreatic β-cell function. The main and novel finding of the present study is that 2-weeks' single treatment with CsA in doses used following renal transplantation is associated with impaired Secr_2.phase. Treatment with CsA showed no significant effect on Secr_1.phase, as assessed by the hyperglycaemic glucose clamp technique.

**Comparison with clinical studies**

A few studies indicate that use of tacrolimus is associated with a decrease of the acute insulin response in man [2,7]. The only previous study addressing the possible effect of CsA treatment on pancreatic β-cell function in man (patients with multiple sclerosis) did not reveal any significant effect of 3 weeks to 1 year of CsA treatment on the early phase insulin response as assessed by IVGTTs [20]. This is in accordance with our finding that CsA treatment does not significantly affect Secr_1.phase.

Most previous studies examined patients treated with a combination of calcineurin inhibitors and steroids, making it difficult to ascertain the specific effect of the calcineurin inhibitor. In a Korean study of 114 transplant recipients treated with CsA and prednisolone, insulin/glucose parameters were evaluated with an OGTT including measurements of glucose and insulin at 0, 30, 60, 90 and 120 min, before and 9–12 months after living-related renal transplantation. A significant decline in insulin AUC was observed after transplantation, but although the relationship between CsA and insulin release was not addressed in this study, no correlation between CsA dose and diabetic status was revealed [30].

In a Japanese study of 48 dialysis patients, insulin secretion was examined with an OGTT before and after living-related renal transplantation [31]. The mean insulinogenic index [(Insulin conc. at 30 min – Insulin 0)/(Glucose 30 – Glucose 0)] increased significantly in the 28 patients receiving a CsA/prednisolone-based regimen, whereas the insulinogenic index remained unchanged in patients on a tacrolimus/prednisolone-based regimen.

In a study by van Duijnhoven et al. [7], insulin secretion was evaluated with an IVGTT before and after 5 days of treatment with tacrolimus in 17 Caucasian dialysis patients. On average, early-phase insulin secretion decreased 34% (median 20%) and insulin secretion changed <5% in four patients. Second-phase insulin secretion was not assessed in this study since, in contrast with hyperglycaemic glucose clamp studies, the IVGTT does not allow evaluation of the biphasic insulin response including the Secr_2.phase [21].

**Comparison with in vitro studies; possible mechanisms**

**Decreased insulin gene transcription.** Calcineurin inhibitors act by inhibiting the phosphatase activity of calcineurin, which prevents the dephosphorylation and activation of transcription factors such as nuclear factor of activated T cells (NFAT) and cAMP response element-binding protein (CREB) [32–34]. Accordingly, transcription of genes, including insulin, that are regulated by these factors may be impaired by calcineurin inhibitors [35,36].

**Insulin release.** It has been hypothesized that a ‘readily releasable pool’ of insulin-containing granules is responsible for the Secr_1.phase, whereas the Secr_2.phase is determined by the rate of priming stored granules for release [37]. Calcineurin inhibitors may specifically reduce the second-phase insulin release by inhibiting/disrupting the kinesin-dependent intracellular trafficking of insulin secretory granules from deeper stores to the ‘readily releasable pool’ [32,38].

Drachenberg et al. [13] reported cytoplasmic swelling, vacuolization and apoptosis in pancreas allograft biopsies from simultaneous kidney–pancreas transplant recipients treated with either tacrolimus or CsA. The islet cell damage was less pronounced in patients treated with CsA. The degree of vacuolization correlated significantly with average trough levels of CsA and tacrolimus the last 2 weeks before biopsy.

Nielsen et al. [14] examined islets isolated from eight necro-kidney donors and demonstrated, first, that insulin release was reduced by 36% (7–61%) after 5 days in culture with a therapeutically relevant dose of CsA (C_{\text{trough}} \sim 100 \text{ng/ml}), and, second, that glucose-stimulated insulin release was markedly depressed in CsA-treated islets. Fuhrer et al. [18] demonstrated that the addition of CsA to cultures of RIN-5F and HIT-T15 cells resulted in an acute significant glucose-independent exocytosis of insulin. Further, this insulin release was blocked by inhibitors of L-type calcium channels. In line with the results of the present investigation, later glucose-induced insulin secretion was, however, inhibited by CsA. Ebihara et al. [39] showed similar result in MIN6-cells.
Does CsA induce increased hepatic degradation of insulin? In a study of liver transplant recipients, frequently sampled intravenous glucose tolerance tests (FSGITT) were used to examine insulin secretion in patients treated with CsA or tacrolimus monotherapy [40]. Similar results were revealed in both the groups, demonstrating that first-phase insulin secretion did not differ between patients and healthy subjects, whereas second-phase insulin secretion was ~50% higher in patients than controls (P < 0.05). The authors speculate that increased hepatic insulin clearance is compensated by enhanced insulin secretion. Dresner et al. [41] showed in an animal model (seven sheep) that 4 weeks of CsA treatment was associated with impaired insulin secretion, increased insulin clearance, and unaltered insulin sensitivity. We could not verify any CsA effect on insulin clearance in our study, as no significant changes in fasting or second-phase molar ratios of C-peptide to insulin were observed.

Limitations and strengths. The strengths of the present study are, first, that the hyperglycaemic glucose clamp technique was used to assess insulin secretion, and second that we were able to investigate the isolated effect of CsA without the interference of steroids. One important limitation is the inclusion of HD patients, which again may not be the ideal model for transplanted patients. However, since the present study primarily aimed to address changes in insulin secretion before and after treatment with CsA, the impaired insulin degradation caused by renal failure probably does not represent any major flaw. In addition, the possible long-term effects (beyond 14 days) of lower doses of CsA on insulin secretion remains to be shown. Importantly, our study does not give a definitive answer to the question whether the detrimental effect of CsA on Second-phase insulin release is associated with increased risk of diabetes mellitus. However, it is conceivable that the inhibitory effect of CsA on Second-phase insulin release might trigger the development of PTDM in pre-disposed transplant recipients (e.g., older patients with a family history of diabetes treated with glucocorticoids). Finally, the patients were Caucasians, and the results may not be directly extrapolated to non-Caucasian patient populations.

Conclusions

Short-term treatment with CsA at doses used following transplantation seems to impair Secr_{2,phase}, but has no significant effect on Secr_{1,phase} in Caucasian HD patients. The mechanism behind these findings and their possible clinical and therapeutic implications need further study.

Acknowledgements. J.H. has received an educational grant from the Norwegian Foundation for Health and Rehabilitation. The study was investigator driven and fully financed by internal research funds. We acknowledge the professional help from Kirsten Lund and co-workers at the Laboratory for Renal Physiology, Rikshospitalet University Hospital as well as from all involved nurses at the Dialysis units (Akershus University Hospital and Rikshospitalet University Hospital).

Conflict of interest statement: AÅ. has received a research grant for the study of CsA pharmacokinetics in renal transplantation from Novartis. The other authors have declared no conflicts of interest.

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Received for publication: 22.9.06
Accepted in revised form: 20.12.06