Follow-up of cyclosporin A treatment in Type 1 (insulin-dependent) diabetes mellitus: lack of long-term effects*

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Summary. In the Canadian/European randomized controlled study on cyclosporin A (CsA) in recent onset Type 1 (insulin-dependent) diabetes, treatment with the immunosuppressive drug had increased and maintained Beta-cell function and clinical remission during the first 12 months. Following discontinuation of the study drug and double-blinding after a mean of 13.8 months former CsA patients doubled the daily insulin dose within 6 months reaching the level of former placebo patients. The difference in Beta-cell function between the two groups was also lost. Metabolic control (HbA_{1c}) was transiently worse in the former CsA

group. Adverse effects of cyclosporin A on systolic blood pressure, haemoglobin levels, serum potassium and creatinine levels also remitted during that time. We conclude that treatment with cyclosporin A for a mean of 13.8 months had no long-lasting effect on the course of Type 1 diabetes persisting beyond drug discontinuation.

Key words: Cyclosporin A, Type 1 (insulin-dependent) diabetes mellitus, immunotherapy, C-peptide, islet function, remission of Type 1 diabetes.

When patients with recent onset Type 1 (insulin-dependent) diabetes mellitus were treated with the immunosuppressive drug cyclosporin A an increase in the rate and lengths of remissions was observed [1–3] with concomitant improvement and preservation of Beta-cell function [3].

In these as well as in other studies the administration of cyclosporin A was discontinued within two years because of loss of clinical remission, because of risk of side-effects or at the patients' request [4–7]. However, little is known about the further course of Type 1 diabetes in these patients. Animal studies [8–10] and data from a small numbers of patients [11] have indicated that a limited period of CsA administration may have a long-lasting beneficial effect in Type 1 diabetes. Follow-up data of a large cohort from the Canadian/European randomized controlled trial were analysed for long-term effects on the course of Type 1 diabetes after discontinuation of CsA therapy.

Subjects and methods

The patients and methods used for this analysis have been described elsewhere in more detail [3]. In brief, 188 recent onset Type 1 diabetic patients (age 9–35 years, duration of classic symptoms before onset < 14 weeks) were randomized in two groups. The initial cyclosporin A dose was 10 mg/kg divided in 12 h intervals, subsequently the dose was modified to maintain 12 h trough concentrations in serum of 100–200 ng/ml or in whole blood of 400–800 ng/ml (determined by radioimmunoassay with the polyclonal antibody provided by Sandoz, Basel, Switzerland). An increase of serum creatinine to > 150% of baseline values called for cyclosporin A dose reduction.

Insulin was administered with the objective of mean capillary blood glucose levels less than 7.8 mmol/l with no single blood glucose value more than 9.0 mmol/l before meals and before bedtime. Endogenous secretion of insulin was tested before and 6 min after 1 mg glucagon i.v. in the overnight-fasted state without prior administration of insulin and with blood glucose levels below 7.8 mmol/l.

Twin analysis

To evaluate the follow-up after cyclosporin discontinuation patients still on drug treatment or with an incomplete record had to be excluded. Of the cyclosporin A group 63 patients with complete record

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Fig. 1 A,B. Number of patients before and after discontinuation of double-blinding. During the double-blind phase of the study some patients were switched to placebo in order to minimize risk from toxicity if there was no prospect of benefit (basal C-peptide < 0.1 nmol/l on two consecutive months)

after drug discontinuation were available and for each a matching statistical twin was searched in the placebo group. The following criteria were defined for matching: same sex, age difference less than 4 years, initial glucagon stimulated C-peptide difference less than 0.15 nmol/l and difference in duration of diabetic symptoms before therapy less than 3 weeks. Following these criteria 46 statistical twin pairs could be formed. Between the former CsA and placebo twins only corresponding data were compared, i.e. with identical duration in the study.

Statistical analysis

Statistical analyses were performed by using Student's *t*-test (two-tailed), Wilcoxon test and Fisher's-test with the level of significance at $p < 0.05$.

Results

The follow-up of all patients in the Canadian/European cyclosporin A trial is shown in Figure 1. Double blinding and placebo treatment were discontinued 12–15 months after entry when patients and physicians were informed about the nature of the individual study drug. Cyclosporin A administration was continued at the patient's request as long as target control of glycaemia was maintained (blood glucose levels ≤ 7.8 mmol/l before meals) and the daily insulin dose was ≤ 0.15 IU/kg. All patients were asked to participate in the follow-up analyses. As shown in Figure 1 this was the case for the majority of patients remaining in the study.

In Table 1 a summary of all available data, including patients who did not discontinue CsA is given. At 24 months after entry to the study patients of the former CsA group showed similar insulin requirements, glycosylated HbA_{1c} levels, basal and stimulated C-peptide concentrations as was found for former placebo patients.

As described above, 46 statistical twin pairs with complete records were formed to analyse the long-term effects of CsA after drug discontinuation. The statistical twins did not differ in entry characteristics from the total group of randomized patients and thus can be regarded as representative (Table 2).

Cyclosporin A administration was discontinued at a mean of 13.8 ± 7.4 months (SD). Within 6 months the mean insulin dose more than doubled ($+111\%$) compared to a modest increase in the former placebo patients ($+21\%$) (Fig. 2a). Similarly the significantly higher mean basal and stimulated C-peptide values of the CsA patients fell within half a year to the level of the former placebo patients. Glycaemic control was transiently worse in the former CsA patients (Fig. 2a).

Of a large number of biochemical and other parameters studied during the double blind phase of the study [3] four (serum creatinine, potassium, haemoglobin, systolic blood pressure) had shown a significant adverse effect of CsA. During follow-up after drug discontinuation all of the parameters remitted to the range of the former placebo group within 6 months (Fig. 2b).

Because of the individual variation in serum creatinine values a single patient analysis was performed. As shown in Figure 3 each patient approached his pre-CsA level of serum creatinine after discontinuation of CsA.

Discussion

After termination of double-blinding and discontinuation of the study drug, compliance of patients allowed a follow-up in the large majority of study participants. Compared to metabolic data shortly before termination of CsA therapy there was a striking increase in insulin dose, loss of Beta-cell function and worsening of glycaemic control, i.e. basic parameters of the disease rapidly approached the level of the former placebo group. The twin analysis did not consider the small fraction of patients who had not discontinued CsA during the follow-up. However, when these were included and compared to the total of former placebo patients, again no difference in metabolic control between the two groups existed (Table 1).

We therefore conclude that the administration of CsA in recent onset Type 1 diabetes for a mean of 13.8 months did not have a long-lasting impact on the course of diabetes persisting beyond drug discontinuation. This conclusion may be at variance with follow-up data of for-

Table 1. Descriptive data of all patients studied

	Before start of therapy	After start of therapy		
		12 months	18 months	24 months
Insulin dose ($\text{IU} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$)				
Cyclosporin A	0.53 ± 0.27	0.30 ± 0.27	0.41 ± 0.33	0.50 ± 0.34
Placebo	0.47 ± 0.21	0.35 ± 0.24	0.43 ± 0.25	0.48 ± 0.24
Basal C-peptide (nmol/l)				
Cyclosporin A	0.22 ± 0.09	$0.26^b \pm 0.12$	$0.23^a \pm 0.13$	0.18 ± 0.22
Placebo	0.23 ± 0.11	0.21 ± 0.12	0.19 ± 0.12	0.18 ± 0.24
Stimulated C-peptide (nmol/l)				
Cyclosporin A	0.33 ± 0.16	$0.43^a \pm 0.21$	$0.35^a \pm 0.21$	0.25 ± 0.16
Placebo	0.39 ± 0.22	0.36 ± 0.23	0.29 ± 0.21	0.31 ± 0.32
HbA _{1c} (%)				
Cyclosporin A	10.5 ± 2.2	7.4 ± 2.2	8.3 ± 2.1	8.6 ± 2.4
Placebo	10.2 ± 2.3	7.3 ± 2.0	7.8 ± 2.0	8.0 ± 2.3
Creatinine ($\mu\text{mol/l}$)				
Cyclosporin A	76 ± 15	$107^c \pm 33$	$93^c \pm 25$	$91^b \pm 22$
Placebo	79 ± 13	80 ± 13.8	81 ± 14	81 ± 11
Haemoglobin (mmol/l)				
Cyclosporin A	8.8 ± 0.9	$8.2^c \pm 0.9$	$8.5^c \pm 0.9$	8.8 ± 1.0
Placebo	8.8 ± 0.8	9.1 ± 0.9	9.1 ± 0.9	9.1 ± 0.9
Systolic blood pressure (mmHg)				
Cyclosporin A	117 ± 13	119 ± 15	119 ± 14	122 ± 17
Placebo	116 ± 11	117 ± 14	115 ± 15	117 ± 14
Potassium (mmol/l)				
Cyclosporin A	4.3 ± 0.4	$4.7^c \pm 0.5$	4.3 ± 0.4	4.3 ± 0.4
Placebo	4.3 ± 0.4	4.3 ± 0.3	4.2 ± 0.3	4.3 ± 0.3

The numbers of patients available at the different dates are given in Figure 1. All the patients are documented, including those who did not discontinue CsA by 24 months. Given are mean values $\pm$ SD. ^a $p < 0.05$; ^b $p < 0.01$; ^c $p < 0.001$ between CsA and placebo groups

Table 2. Characteristics at entry of all patients and of statistical twin pairs

	All patients		Statistical twins	
	Cyclosporin A	Placebo	Cyclosporin A	Placebo
Number (<i>n</i>)	93	95	46	46
Sex				
Male (<i>n</i>)	74	72	36	36
Female (<i>n</i>)	19	23	10	10
Age at entry (mean $\pm$ SD)	21.5 ± 6.4	22.4 ± 5.0	21.0 ± 6.1	21.4 ± 4.8
Duration of symptoms (weeks) (mean $\pm$ SD)	3.9 ± 2.7	4.0 ± 2.6	4.1 ± 2.8	4.0 ± 2.7
Insulin dose ($\text{IU} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) (mean $\pm$ SD)	0.53 ± 0.27	0.48 ± 0.22	0.55 ± 0.32	0.46 ± 0.21
HLA-type				
DR 3,x n (%)	18 (19)	25 (26)	7 (15)	8 (17)
DR 4,x n (%)	36 (39)	43 (45)	22 (48)	24 (52)
DR 3,4 n (%)	29 (31)	17 (18)	13 (28)	9 (20)
DR x,x n (%)	10 (11)	10 (11)	4 (9)	5 (11)
HbA _{1c} (%) (mean $\pm$ SD)	10.8 ± 2.1	10.4 ± 2.6	10.2 ± 2.0	10.0 ± 2.8
C-peptide (nmol/l) basal (mean $\pm$ SD)	0.20 ± 0.09	0.23 ± 0.11	0.21 ± 0.07	0.20 ± 0.09
stimulated (mean $\pm$ SD)	0.33 ± 0.21	0.40 ± 0.21	0.35 ± 0.15	0.35 ± 0.14

mer CsA patients in a single centre of the French CsA multicentre trial [11]. In that study the better Beta-cell function in the CsA group (but not the lower insulin dose) seems to have been preserved for at least 2 years after drug discontinuation. However, these data come from a small cohort and no attempt was made to select statistical twins. An earlier open study had indicated a rapid increase in insulin requirements after drug CsA discontinuation [4].

After 12 months of CsA treatment dose-dependent side-effects were observed as has been reported previously [3]. Follow-up showed normalization of all abnormal parameters within 6 months of discontinuation. The four major parameters are documented in this report. However, previous kidney biopsies had shown structural damage (moderate arteriolopathy, tubular atrophy or interstitial fibrosis) in specimens from some CsA patients [3]. Kidney function studies in the Danish cohort of the study indicated incomplete normalisation in three out of nine patients [12]. Subsequent trials with lower CsA doses than used in this study did not show renal injury in diabetic patients [13–15].

In conclusion, the data presented suggest that CsA administration transiently delayed the progression of Type 1 diabetes by its protective action on Beta-cell function. However, this effect was rapidly lost after discontinuation of cyclosporin A. We conclude that the drug did not correct the immunoregulatory imbalance between Beta-cell destroying and protective activities in Type 1 diabetes.

Fig. 2a, b. Effects and side-effects before and after discontinuation of study drug in 46 representative statistical twins with complete record. **a** Follow-up of metabolic parameters. **b** Follow-up of systolic blood pressure, serum creatinine, potassium and haemoglobin. Open columns, cyclosporin A (CsA) group; closed columns, placebo group; A, before start of therapy; B, 1–3 months before discontinuation of CsA; C, 6 months after CsA discontinuation; D, 15 months after CsA discontinuation. Bars indicate SD; *, $p < 0.05$; **, $p < 0.01$

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Fig. 3. Follow-up of serum creatinine levels in 46 statistical twins of the cyclosporin A (CsA) group divided in four panels for easier

reading. Dotted lines indicate the normal range for the 46 twins of the placebo group

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