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ORIGINAL ARTICLE

Cyclosporin and methotrexate therapy induces remission in type 1 diabetes mellitus

Douglas O. Sobel · Annette Henzke · Val Abbassi

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Abstract Cyclosporin and methotrexate administration induces remission of type 1 diabetes mellitus. Administration of high-dose cyclosporin (cyclo) has been demonstrated to induce remission of type 1 diabetes mellitus (T1D). Its usefulness was limited by its toxicity. Since methotrexate (mtx) and cyclo synergistically inhibit autoimmune processes, we postulated that low doses of cyclo and mtx could safely induce remission of T1D. In a pilot study, insulin dose requirements and glycemic control were compared in 10 new onset T1D control children with seven children who were administered cyclo at 7.5 mg/kg/day for 6 weeks and then 4 mg/kg/day in addition to mtx 5 mg/kg/wk for 1 year. After 6 weeks, cyclo doses were adjusted to maintain blood cyclo levels 110–220 ng/ml. All children were treated with two daily injections of insulin. Clinical and biochemical toxicity of drug therapy was assessed. There were only very minor adverse effects and no drug induced biochemical test abnormalities. Mean HbA1c levels were similar in the experimental and control groups at baseline and at 3, 6, and 9 months but was lower in the cyclo + mtx group at 12 months. Daily insulin requirements of the groups were similar at baseline but lower in the cyclo + mtx group at 3, 6, 9, and 12 months. Although no control subjects became non-insulin requiring, four of seven cyclo + mtx-treated subjects were entirely off insulin therapy for 2.5, 4.5, 8, and 12 months. Low-dose cyclo and mtx treatment of subjects with new onset T1D can safely induce remission of disease and decrease the amount of required insulin.

D. O. Sobel (✉) · A. Henzke · V. Abbassi
Department of Pediatrics, Georgetown University Medical Center, 3800 Reservoir Road NW, Washington, DC 20007, USA
e-mail: sobeldo@georgetown.edu

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Introduction

Evidence that type 1 diabetes mellitus (T1D) is an autoimmune mediated disease [1] associated with other autoimmune disorders [2] has led to research aimed at altering the immunologic disease process in order to affect a cure. Earlier attempts to induce remission with plasmaphoresis, prednisone, and azathioprine were either unsuccessful or the claim could not be confirmed [3–5].

Since T1D is now thought to be a T-cell-mediated disorder [6–8], treatment with drugs that affect T-cell function has become a focus of interest [9, 10]. Since cyclosporine (cyclo) is a modulator of T-cell activity [11], the effect of administering this drug was studied and found to inhibit the development of diabetes in animal models of diabetes [12, 13]. Human studies were then performed and were initially promising [14, 15]. However, it appeared that the high doses of cyclo which are needed to induce remission resulted in significant adverse effects, most concerning nephrotoxicity [14, 16]. In fact, biopsy-proven renal toxicity occurred in some diabetic subjects after only a 12-month course of 8–10 mg/kg/per day cyclo [15].

Since long-term cyclo therapy of T1D using previously described doses would involve undertaking an unwise risk of significant adverse effects, particularly with nephrotoxicity [17], further investigation may focus on means to more safely administer cyclo to induce and maintain remission of disease. It has been proposed that the combination of lower and less toxic doses of cyclo with non-nephrotoxic drugs that are synergistically

active with cyclo may be effective therapy and without the potential nephrotoxic side effects associated with cyclo [18].

Methotrexate (mtx) may be a good candidate to utilize in combination with cyclo since it has low toxicity and no nephrotoxicity at low dosages [19]. Further, mtx acts synergistically with cyclo in the treatment of rheumatoid arthritis and psoriasis, and in the prevention of graft versus host disease [20–22]. Furthermore, the addition of mtx to cyclosporin is also particularly attractive since each drug has been individually found to be effective therapy for other autoimmune diseases, such as rheumatoid arthritis and psoriasis [23–27].

The aim of this pilot study is to determine whether the combination of cyclo and mtx at low dosages can safely induce non-insulin requiring state of remission from diabetes.

Research design and methods

Subjects

The protocol was approved by the University Institutional Review Board, and informed written consent was obtained from each subject and parent prior to initiating the study. The study was performed from February 1990 to June 1994.

The experimental design was of an open non-randomized pilot study. Subjects from families who wished to be part of the Experimental (cyclo + mtx) group were assigned to that group. To be included into the study, children had to be diagnosed with T1D, be between the ages of 8 and 18 and be obtaining insulin therapy for less than 4 weeks prior to study entry. Exclusion criteria included ketoacidosis, weight over 110% of ideal body weight, the presence of a condition in which immunosuppression was contraindicated, any severe chronic illness, abnormal serum kidney or liver function tests, or an abnormal renal ultrasound or chest X-ray. Seven children were selected for the study group and were treated with low-dose cyclo and mtx for 1 year, and ten children served as controls (Table 1).

Management of glycemic control

At diagnosis of diabetes, each patient was hospitalized and received an intensive educational program. Methods to establish normal or near normal glycemic control with aggressive insulin therapy were identical for all subjects. A standard ADA diet for age, which included three meals and two snacks, was provided. Treatment with twice daily injections with a fast acting and an intermediate acting insulin was instituted and utilized throughout the study. None of the subjects was placed on multiple daily insulin injections or an insulin pump.

Blood glucose was monitored at home utilizing a strip analyzer with a minimum of 3–4 pre-prandial measurements per day. Insulin dosages were adjusted to maintain pre-prandial home fasting blood glucose of less than 130 mg% while also avoiding hypoglycemia.

Glycemic control was assessed in all subjects in the Diabetes Clinic at 2 weeks, 1, 3 months, and then every 3 months after initiation into the study. Telephone contacts occurred between visits as needed in all subjects in order to establish good glycemic control.

Induction and maintenance experimental drug therapy

The induction phase of therapy was begun within 4 weeks after initiating insulin treatment and lasted 6 weeks. It was then followed by maintenance phase therapy for a total of 12 months of experimental drug treatment. Because adequate cyclosporine blood levels in the first month of therapy have been shown to be important in the induction of remission [28], the cyclo (Sandimmune) dose was higher in the induction phase, at 7.5 mg/kg/day. Cyclo dosages were adjusted to maintain trough whole blood cyclo levels between 275 and 385 ng/ml by TDX immunoassay which correlates with 220–308 ng/ml by RIA assay [29]. In the 'maintenance phase', the cyclo dose was lowered to 4 mg/kg/day and adjusted to maintain trough whole blood cyclo levels between 110 and 220 ng/ml which correlates with 88–176 ng/ml by RIA. Mtx was initiated 1 week after the start of cyclosporin and was administered at the dose of 5 mg/m^2/week during the entire study. Mtx and cyclo dosages were to be adjusted to reduce drug toxicity.

Table 1 Characteristics of subjects at study entry [mean (SD)]

Subjects	Mean age (year)	*N*	Gender F/M	Initial BMI	HbA1c (%)	Insulin dose (u/kg/day)	Prior to diagnosis	
							Wt loss (kg)	Symptoms (days)
Cyclo + Mtx	13.7 (3.5)	7	1, 6	20.2 (3.9)	11.5 (1.3)	0.48 (0.19)	3.86 (2.71)	37 (29)
Control	12.5 (2.4)	10	5, 5	18.8 (3.0)	12.1 (2.4)	0.56 (0.12)	2.63 (1.6)	18 (9)

Surveillance for adverse immunosuppressive drug effects on subjects receiving experimental therapy

Clinical evaluation

Subjects were assessed for drug toxicity with monthly vital signs and physical examination at study entry, after 2 weeks; and then at 1, 3, 6, and 12 months into the study.

Laboratory evaluation

1. Blood cyclo levels: During the 'induction phase', whole blood trough cyclo levels were determined at weekly intervals until stabilized within the required range and then every 2 weeks. In the 'maintenance phase', blood cyclo levels were evaluated every month. Cyclo values were measured by fluorescence polarization immunoassay (TDX, Abbott Laboratories). Cyclo levels by TDX assay are approximately 10–20% higher than those measured by RIA [29, 30].
2. CBC and differential and platelet counts were obtained at entry, weekly for the first month, biweekly for the second month, and then monthly thereafter. These tests were also performed at times of fever or illness at the discretion of the physician.
3. Serum electrolytes, SGPT, alkaline phosphatase, bilirubin, total CO2, BUN, uric acid, and creatinine were obtained at study entry and then biweekly for the first month and then monthly for the remainder of the study.
4. HbA1c was assessed by column chromatography by Bio-rad. The intra-assay CV was 2.2% and the interassay CV was 3.6%. HbA1c was obtained at study entry and at 3-month intervals in all subjects.

Definition of remission

'Complete' remission was defined when insulin therapy was no longer required to maintain excellent glycemic control as outlined above and the HbA1C was less than 7.5% (a level within 125% of the upper limits of normal). Partial remission was defined when the insulin requirement was less than or equal to 0.25 units/kg/day and the HbA1C was less than 7.5%.

Statistical analysis

Group means were compared by Student's *t* test. Correlations were assessed by chi square analysis. The statistical program used was GraphPad Software. The product-limit method of Kaplan–Meier was used to estimate the remission function. Gehan's Wilcoxon's test compared the product limit functions.

Results

Characteristics of subjects at entry

The mean age, BMI, weight loss, and duration of symptoms prior to diagnosis and initiation of insulin therapy, initial daily insulin requirement, and HbA1c levels were similar in the experimental drug-treated and control groups at study entry (Table 1). Further, there was no statistical difference in proportion of girls versus boys in each group. All experimental drug-treated subjects had a positive test for either serum islet cell antibody, insulin antibody, or GAD antibody. The mean time from diagnoses of diabetes to initiation of experimental treatment was 19.6 days.

Effect of experimental treatment on glycemic control, daily insulin requirement, and on the induction of remission

The mean HbA1c values were similar in the two study groups at entry and at 3, 6, and 9 months but was slightly lower in the experimental group at 12 months into the study (Fig. 1).

The mean daily insulin requirements were similar in the two study groups at entry but were significantly lower in the experimental group at 3, 6, 9, and 12 months into the study (Fig. 2).

The frequency of subjects who attained either a partial or total remission was greater in the experimental drug-treated group from 4 to 12 months into the study by chi square

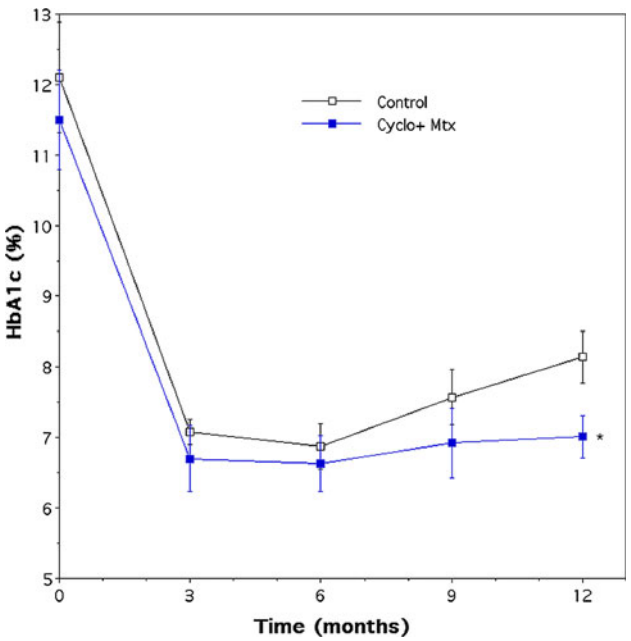

Fig. 1 The mean HbA1c values in the cyclo + mtx treated and control groups at entry and at 3, 6, 9, and 12 months into the study. *$P < 0.05$ versus control group

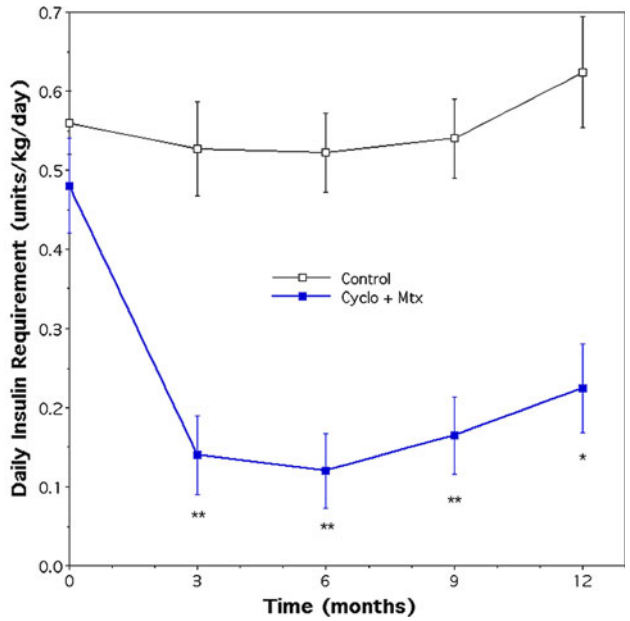

Fig. 2 The mean daily insulin requirements (units/kg/day) of the cyclo + mtx treated and control groups at entry and at 3, 6, 9, and 12 months into the study. *$P < 0.05$, **$P < 0.01$ versus control group

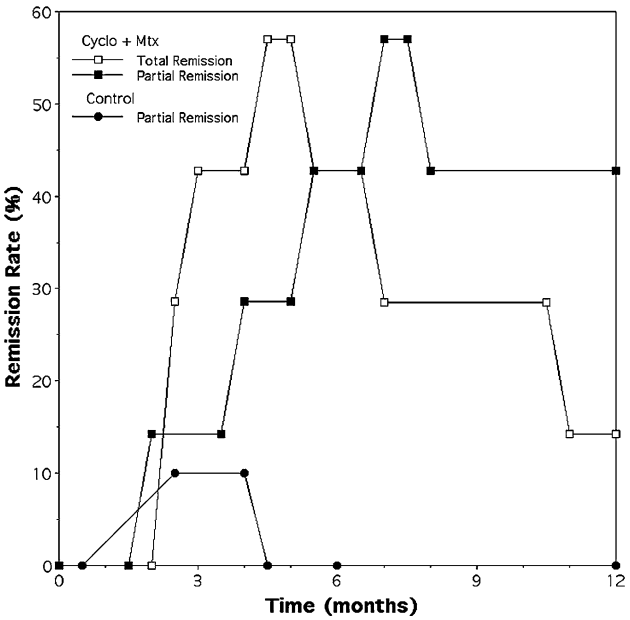

Fig. 3 The frequency of subjects who attained either a partial or total remission in the experimental drug treated and control groups. Cyclo + mtx treated group had a greater frequency of total remission from 3 to 6.5 months ($P < 0.5$ from 3 to 4 months; $P < 0.02$ from 4 to 6.5 months), a greater frequency of partial remission from 5.5 to 12 months ($P < 0.05$), and greater frequency of either partial or complete remission from 4 to 12 months than the control group ($P < 0.002$ from 4 to 11 months; $P < 0.05$ from 11 to 12 months)

analysis (Fig. 3). Further, the development of a complete or partial remission was greater in the experimentally treated group by survival curve analysis ($P < 0.001$).

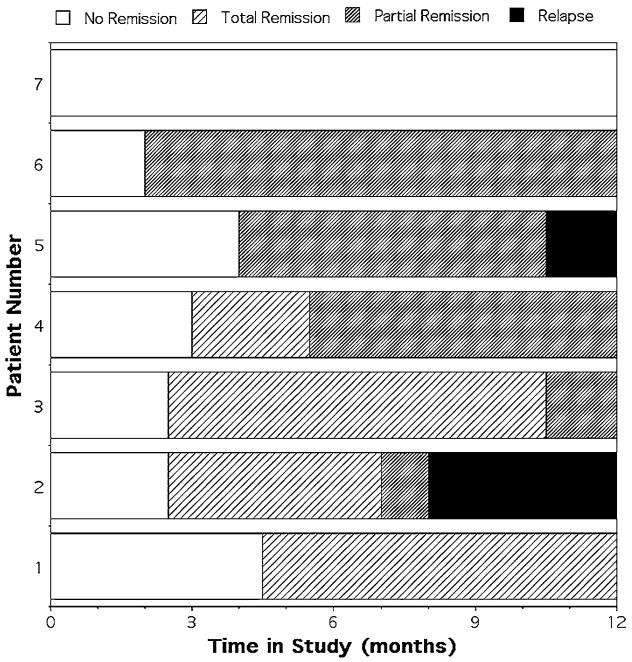

Fig. 4 The duration and time of onset of complete and partial states of remission in each of the seven cyclo + mtx treated subjects

No control subjects attained complete remission, and only one control subject developed a partial remission. That remission lasted only 1.5 months (Fig. 3).

Complete remission was attained between months 2.5 and 4.5 in four experimental drug-treated subjects (Figs. 3, 4). The frequency of complete remission was significantly greater in the experimental drug group from 3 to 6.5 months into the study by chi square analysis. By survival curve analysis, the development of complete remission was also greater in the experimental treated group than in the control group where no subject attained a complete remission ($P < 0.01$). The duration of complete remission in the four experimentally treated subjects was 2.5, 4.5, 7.5, and 8 months during the 12-month time of the study (Fig. 4). One of these children continued to be in total remission 4.5 months after the study was over for a total of 12 months of complete remission. Of the four subjects who attained complete remissions, three continued to have either a complete or partial remission for the rest of the study. The other subject who attained a complete remission subsequently had a very short (30 day) partial remission followed by relapse. This subject, however, was found to have low blood cyclo levels (below 100 ng/ml) starting at 6 months into the study which was thought to be due to non-compliance in taking the drug which in turn could be related to his relapse. During times of complete remission, HbA1c levels were within normal limits in all subjects save for one HbA1c level, which was 6.7%, a very near normal value.

Two experimental drug-treated subjects who did not attain a complete remission developed a partial remission at 2 and 4 months into the study which lasted 10 and 6.5 months, respectively (Fig. 4).

Cyclosporin dose and blood levels

The mean trough blood cyclosporine levels are depicted in Table 2. The mean cyclo levels were not significantly different at any time point in subjects that attained a complete remission and those who did not up until the last month of the study. At 12 months, subjects attaining complete remission had a lower mean blood cyclo level (Table 2).

Factors predictive of remission

Age, time from diagnosis to study entry, time from clinical onset of symptoms to study entry, cyclo dose, initial HbA1c, daily insulin requirement at study entry, or blood cyclo level at any time point did not correlate with attaining complete remission (data not shown).

Adverse effects of experimental therapy

Clinical side effects

There were no severe clinical side effects. Mild hypertrichosis occurred in two subjects and mild gingival hypertrophy in three subjects which were attributed to cyclo therapy. One subject developed mild oral mucositis, a side effect attributed to mtx which quickly resolved with cessation of drug. Mtx was resumed 1 week later without further adverse effects.

Biochemical side effects

Serum creatinine did not statistically increase at any time point over baseline levels and all serum creatinine levels remained within normal range. The serum creatinine level transiently increased over baseline value in one subject from 0.8 to 1.1 mg/dl (still a normal value) at 4 months into the study. However, the creatinine level decreased to 0.7 mg/dl upon repeat testing without a change in the cyclo dose and the serum creatinine level remained under 1.0 for the remainder of the study in this subject. There were no alterations in levels of bilirubin, hemoglobin, WBC, granulocyte count, lymphocyte count, or serum electrolytes, SGPT, alkaline phosphatase, total CO2, BUN, and uric acid (data not shown).

Discussion

This study demonstrates that the combination of low-dose cyclo and mtx therapy in children with new onset T1D

Table 2 Blood cyclosporin levels (ng/ml) in all subjects and in subjects who attained (responders) and not attained (non-responders) a complete remission

Time	1 weak	2 weak	3 weak	1 month	2 month	3 month	4 month	5 month	6 month	7 month	8 month	9 month	10 month	11 month	12 month
All ($n = 7$)	418 (82)	360 (35)	342 (31)	433 (56)	252 (20)	262 (21)	206 (20)	220 (26)	194 (28)	170 (20)	154 (21)	219 (16)	179 (24)	188 (14)	218 (38)
Responders ($n = 4$)	300 (52)	360 (56)	282 (27)	411 (45)	236 (8)	247 (16)	202 (32)	233 (34)	160 (27)	149 (20)	128 (7)	186 (22)	169 (11)	162 (21)	291*(30)
Non-responders ($n = 3$)	585 (95)	375 (24)	404 (30)	464 (104)	274 (37)	282 (37)	210 (4.9)	202 (16)	264 (27)	198 (24)	134 (27)	183 (20)	193 (37)	205 (7)	146 (20)

Data are means (SEM)

* $P < 0.05$ versus non-responders

causes only minor clinical side effects. Transient mucositis was attributed to mtx therapy and mild hypertrichosis and gingival hyperplasia were attributed to cyclo treatment. Whereas, paresthesias, epigastric pain, nausea, and hypertension occurred in previous studies when higher doses of cyclo had to be utilized to induce clinical remission [15, 16, 31–33].

Further, previous studies aimed at inducing remission from diabetes found that cyclo administration caused significant biochemical toxicities including nephrotoxicity, hypokalemia, anemia, and elevations of alkaline phosphatase, bilirubin, and uric acid [14, 16, 31, 32]. However, in the present study, there was no significant drug-induced biochemical toxicity. Most importantly, there was no evidence of nephrotoxicity which was a major limiting factor in previous studies attempting to induce remission of diabetes. Serum creatinine levels were not altered by experimental drug therapy. Since biopsy-proven nephrotoxicity is generally not found in cyclo-treated diabetic subjects whose serum creatinine levels are not increased over 30% of baseline levels [34], as found in this study, no significant nephrotoxicity was apparent in our treated subjects.

Significant cyclo-related nephrotoxicity was not expected in the present study since cyclo trough concentrations were maintained at a level much lower than 350 ng/ml, a level found to be safe for children with diabetes[34]. Further, in other autoimmune disorders, maintenance cyclo doses comparable to those used in the present study did not cause nephrotoxicity in adults [35, 36]. There is, however, a report demonstrating biopsy-proven nephropathy in adults with psoriasis who were obtaining cyclo doses similar to that used during maintenance phase in our study. However, all but one subject was reported to have developed elevations of serum creatinine prior to the onset of nephropathy. In addition, all subjects were adults who are at more risk than children to develop nephrotoxicity [37].

This is the first report to demonstrate that the administration of low-dose cyclosporine and mtx has a dramatic effect on the clinical state of T1D. This experimental therapy significantly and markedly decreased daily insulin requirements by 3 months after the initiation of therapy. This effect persisted for the entire duration of this 12-month study.

Further, remission of disease was also significantly induced by cyclo and mtx therapy. The rate of developing either a partial or complete remission was significantly greater in the experimental treated group from 3 months to the end of the study. A total of 86% of cyclo + mtx-treated children developed one form of remission and 71% were in some form of remission by 12 months into the study, whereas only one control subject (10%) developed a partial remission which lasted for only 1.5 months.

Most importantly, experimental treatment induced a complete remission in 65% of the subjects at which time they were all in excellent glycemic control with normal or near normal HbA1c values. This was in stark contrast to the children in the control group where no complete remissions were attained.

The ameliorating effect of experimental drug therapy appeared long lasting since the frequency of attaining either complete or partial remission was significantly higher in the experimental group at 1 year. In addition, one subject continued to be in complete remission for an additional 4.5 months after the cessation of drug therapy at 1 year for a total of 12 months of complete remission. However, most subjects attaining complete remission reverted to a partial remission state during the time of the study.

Although subjects attaining partial remission are still insulin requiring, attaining a state of partial remission is clinically very important and a relevant endpoint since patients with diabetes with very low insulin requirements more easily attain excellent glycemic control and in turn have a reduced risk of long-term diabetic complications [38, 39].

Although this was an open study and thus subject to investigator bias to decrease the insulin dose of test subjects, the excellent and similar glycemic control, measured by HbA1c, of the experimental drug-treated and control subjects supports the contention that both groups were similarly treated with insulin. Further, the ‘state of complete remission’ not only requires a non-insulin requiring state but also includes an important objective criterion of having a near normal HbA1c. Not only were the HbA1c levels of drug-treated subjects in complete remission in that defined very good HbA1c range, the levels were far lower and almost entirely within normal range for a non-diabetic person, again supporting the aggressive insulin treatment given to these subjects.

The complete remission rate induced by low dose cyclo and mtx in the present study compares favorably with previous studies utilizing higher doses of cyclosporine as single drug therapy. The definition of remission in the present study is similar or more conservative than with definition that was used in the below cited studies.

Using a 10 mg/kg/day cyclo dose, Stiller reported a complete remission rate of only 27.3% in subjects under 16 years of age and 53% in their entire group of subjects with new onset T1D [14, 40]. However, these high doses of cyclo caused elevations in serum creatinine, bilirubin, uric acid, and alkaline phosphatase.

A Canadian European double-blinded placebo control trial using the same 10 mg/kg/day dose induced a 51% remission rate in adolescent and adult subjects [16].

However, serum creatinine again rose 40% in the cyclo-treated group.

A placebo-controlled double-blind trial by the French Study Group using a lower dose of cyclo of 7.5 mg/kg/day induced only a 24.1% remission rate in adult subjects at 9 months. Further, plasma creatinine rose in 26% of the cyclo-treated subjects [32].

Assan described only a 33% remission rate in a group of 12 adult patients with diabetes who were treated for 2–8 months with 5–10 mg/kg/d cyclosporin [15].

In an open trial, Bougneres reported that 7.5 mg/kg/day dose of cyclo induced 65% overall remission rate in children with newly diagnosed T1D [33]. However, this study not only used a higher dose of cyclo with concomitant higher blood levels of cyclo than in the present trial but also incorporated children that may be expected to respond better to cyclo treatment, children with much shorter durations of disease prior to study entry than those in the present study. In addition, the mean serum creatinine level increased by 3 months of therapy which may be reflective of the higher cyclo dosage.

It was difficult to assess what parameters are predictive of remission since this was a pilot study and the number of subjects was small. Nonetheless, there was no correlation between weight loss or duration of symptoms prior to diagnosis of diabetes, insulin requirement or HbA1c at initiation of study, cyclo blood levels in the early part of the study with the induction of remission or insulin requirement at any time points.

It is unclear how the combination of cyclo and mtx drug therapy induces remission of disease. Previous studies suggest that cyclosporine induces remission by preserving some beta cell capacity to secrete insulin [16, 40]. We suspect this is the case in this study. However, we have no data comparing the insulin secretory capacities of the test and control groups. Alternatively, the combined drug regimen could be increasing insulin sensitivity or decreasing hepatic glucose production. This is unlikely since there is no evidence that either of the drugs possesses any of these properties. In fact, cyclo administration has been shown to increase peripheral insulin resistance [41].

Since this was a pilot trial and there were no individual treatment arms of the study utilizing cyclo and mtx alone, one must consider the possibility that the very low dosages of either cyclo or mtx alone caused the diabetes ameliorating effect and induced the remissions. However, we strongly doubt either of these cases. First, a previous double-blind trial studying subjects with similar duration of diabetes prior to study entry, an important determinant on who is more likely to achieve remission, demonstrated that treatment with cyclo at doses which provide blood trough levels below 300 ng/ml, as in the present study, did not significantly induce remission of disease [32].

Secondly, cyclo was ineffective in inducing remission of diabetes when doses were lowered to below 5–7.5 mg/kg/day in order to reduce elevated serum creatinine levels [42].) Thirdly, subjects placed into remission with the administration of 8–10 mg/kg/day of cyclo frequently relapsed when the dose was lowered to 3.5 mg/kg/d, a dose similar to that used in the maintenance phase of the present study [43]. Furthermore, mtx administration as single drug therapy at doses used in the present study did not induce a non-insulin requiring state or even lower the insulin requirements in newly diagnosed diabetic children [44].

In conclusion, this study demonstrates that the combination of low-dose cyclo and mtx therapy safely and significantly induces the remission of disease and in turn uncovers a potential new means to avert the toxic adverse effects of single-drug high-dose cyclo therapy. Further studies would be needed to more clearly evaluate the synergistic effect of mtx and cyclo to inhibit the disease process. Potential drug toxicity at even very low doses of cyclo and mtx must still be further examined.

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