Rapid Publications

Increase in Remission Rate in Newly Diagnosed Type I Diabetic Subjects Treated with Azathioprine

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SUMMARY

Azathioprine (2 mg/kg) was given, in addition to routine insulin treatment, to alternate patients presenting with recent-onset type I diabetes. Treated (N = 13) and untreated (N = 11) patients did not differ significantly at diagnosis with respect to age, duration of symptoms, body weight, blood glucose, hemoglobin A_{1c}, or presence of ketosis. Eight patients were treated for 12 mo, three elected to stop treatment at 6 mo, and treatment was stopped in two because of side effects. Seven treated patients had a remission compared with one untreated patient. At 12 mo these seven patients were distinguished by significantly higher basal and glucagon-stimulated levels of C-peptide (1.98 ± 0.52 and 3.88 ± 0.34 μg/L, respectively) compared with the other six treated patients (0.93 ± 0.52 and 1.32 ± 0.85 μg/L, respectively), and by the persistence of islet cell cytoplasmic antibodies. Remissions were not sustained in the 1–2 yr after treatment, although relapsed patients required less insulin for control. These results corroborate those from nonrandomized trials using cyclosporine[1,2] and suggest that protracted treatment with nonspecific immunosuppressive drugs may be necessary to avert insulin dependence. DIABETES 1985; 34:1306–308.

The selective destruction of pancreatic islet beta cells that leads to insulin-dependent (type I) diabetes in man is thought to be an autoimmune-mediated process, evidenced by lymphocytic infiltration of the islets,[3] cell-mediated[4] and humoral[5] anti-islet reactivity, and the association of type I diabetes with other autoimmune diseases.[6] Initial reports on the use of nonspecific immune therapy, given to type I diabetic patients with the aim of preserving residual beta cell function, were inconclusive.[7,8] However, Stiller et al.[1] (Canada) and Assan et al.[2] (France) have reported that cyclosporine, given in a nonrandomized, noncontrolled manner to newly diagnosed type I diabetic patients, is associated with a significant increase in remission rate. We describe the results of a trial, initiated in 1981, of randomized treatment of newly diagnosed type I diabetic subjects with azathioprine given for up to 12 mo.

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MATERIALS AND METHODS

Patients. Azathioprine (2 mg/kg) was given to alternate patients, age 15–50 yr, presenting with acute, symptomatic hyperglycemia requiring insulin treatment. All patients fulfilled the criteria for the diagnosis of insulin-dependent diabetes mellitus.[9] The following exclusions applied: symptoms for longer than 12 wk, pregnancy, lactation, risk of pregnancy, or current or past disease that might contraindicate immunosuppression (e.g., immune deficiency, severe anemia, rheumatic heart disease, chronic infection, cancer). The protocol was approved by the Board of Medical Research of the hospital and each patient gave written consent. Except for a predominance of males in the untreated group, the characteristics of the treated and untreated patients on entering the trial were similar (Table 1).

Patients were given regular and intermediate-acting insulin, initially twice daily, and instructed to monitor finger-prick capillary blood glucose up to four times daily and adjust insulin doses to attain near-normal glucose levels. They were seen monthly and fasting blood glucose, plasma C-peptide, and hemoglobin A_{1c} were measured. Endogenous insulin secretion (plasma C-peptide 6 min after 1 mg of glucagon i.v.) and islet cell cytoplasmic antibodies (ICA) were measured every 3 mo. Every attempt was made to minimize insulin requirements, consistent with the maintenance of near-normal blood glucose and hemoglobin A_{1c} levels. Patients in whom insulin could be withheld for more than 1 wk while fasting blood glucose was maintained at <7.0 mmol/L were classified as having had a remission. Upon relapse they were treated initially with the sulfonylurea gliclazide.

For the first month of treatment full blood counts were performed twice weekly. One patient developed lymphopenia (<1,000/cmm) and another thrombocytopenia (<100,000/cmm) within 2 wk of starting treatment, necessitating tem-

TABLE 1
Characteristics of patient groups at diagnosis (mean values ± SD)

Patients	Treated N = 13	Untreated N = 11
Sex	7M, 6F	9M, 2F
Age (yr)	24.7 ± 7.5	24.8 ± 7.9
Duration of symptoms (wk)	2.2 ± 1.5	3.5 ± 3.5
Weight (kg)	60.5 ± 10.0	61.5 ± 8.7
Blood glucose (mM)	19.3 ± 6.9	30.5 ± 18.3
Hemoglobin A_{1c} (%)	10.8 ± 3.3	12.9 ± 5.2
Ketosis	9/13	7/11

porary withdrawal of azathioprine for 3 wk and reintroduction at half dose (1 mg/kg). One of these patients elected to be treated for 6 mo only; neither had a remission. Otherwise, 8 patients were treated for 12 mo, 2 other patients elected to stop treatment after 6 mo, and in 2 others, treatment was stopped after 2 mo, in one case because of drug-associated diarrhea, and in the other case because of scalp hair loss probably unrelated to treatment.

Methods. Blood glucose was measured by the glucose-oxidase method and hemoglobin A_{1c} with the Corning kit (Corning, Palo Alto, California, normal value <8%). Immunoreactive plasma C-peptide was measured using a Daiichi kit (Daiichi Radioisotope Labs Ltd., Tokyo, Japan), the normal fasting range established on 100 normal subjects age 18–60 yr being 0.3–1.6 μg/L, with an inter- and intraassay coefficient of variation of 10% and 5%, respectively. The increase in plasma C-peptide after glucagon (1 mg i.v.) in young, nondiabetic subjects is in the range 130–377%.[10] HLA-DR typing was performed according to Terasaki and McClelland.[11] ICA in plasma were assayed by indirect immunofluorescence on 4-μm sections of unfixed, frozen group O human pancreas, as described by Bottazzo et al.[5]

RESULTS

The findings in the treated and untreated patients are summarized in Table 2. Both patient groups were similar with respect to the expression of immunogenetic markers, i.e., islet cell antibody and HLA-DR status. At diagnosis there was no difference between their fasting or postglucagon levels of plasma C-peptide, using either nonparametric or parametric tests of statistical signficance. A remission occurred in 7 of the treated patients (all taking 2 mg azathioprine/kg/day) and in 1 untreated patient. All remained in remission at 12 mo except for 1 of 2 treated patients who had elected to stop azathioprine after 6 mo; he was started on gliclazide 8 mo after diagnosis because of postprandial blood glucose levels around 10 mmol/L.

At 12 mo, just before cessation of treatment, both the fasting and postglucagon levels of plasma C-peptide were significantly higher in the treated group ($P < 0.01$). All patients in this group had measurable C-peptide whereas in 4 patients of the untreated group, fasting C-peptide was <0.3 μg/L and was unresponsive to glucagon. The 7 treated patients who had a remission were distinguished by significantly higher fasting and postglucagon levels of C-peptide at 12 mo (1.98 ± 0.52 and 3.88 ± 0.34 μg/L) compared with the other 6 treated patients taking insulin (0.93 ± 0.52 and 1.32 ± 0.85 μg/L, respectively) ($P < 0.01$). In addition, 5 of these 7 patients remained islet cell antibody-positive at 12 mo (6 were initially positive), in contrast to none of the other 6 treated patients taking insulin (4 were initially positive). In the untreated group, no patient who was initially antibody positive remained so at 12 mo.

Despite these differences between the groups at 12 mo, in the following year, 4 of the treated patients in remission relapsed (one relapsed in the subsequent year). All relapsed patients are now taking insulin, although they require a relatively small amount for optimal control (14.0 ± 5.7 U/day). One patient, a woman who stopped full-dose azathioprine after 6 mo, remains in remission 35 mo after diagnosis. This patient, and a treated male who went into remission, had histories of Graves' hyperthyroidism and were the only patients in the trial with evidence of polyglandular autoimmunity.

DISCUSSION

In this randomized study azathioprine treatment of newly diagnosed type I diabetic patients was associated with a significant increase in remission rate, although remissions were not sustained in the year or two after treatment. The finding

TABLE 2
Randomized treatment of newly diagnosed diabetes with azathioprine: summary of results (mean values ± SD)

	Treated (N = 13)	Untreated (N = 11)
HLA-DR 3, 4, or 3/4	11/13	9/11
Islet cell antibody (+)	10/13	5/9
Plasma C-peptide (μg/L)		
Fasting/6′ after glucagon i.v.		
At diagnosis	1.28 ± 0.48/1.75 ± 0.82	0.95 ± 0.23/1.50 ± 0.78
At 12 mo	1.47 ± 0.76/2.50 ± 1.43	0.55 ± 0.16/0.88 ± 0.22*
Remission	7/13	1/11
Duration of treatment before remission (wk)	11.6 ± 11.4	12.0
Treatment at 12 mo		
None	6	1
Gliclazide	1	—
Insulin (total daily dose)	6 (35.2 ± 16.8 U)	10 (33.7 ± 10.5 U)
Current follow-up (mo)	36.2 ± 9.4	30.5 ± 12.2
Relapse (mo)		
0–12	1	0
12–24	4	1
24–36	1	0

*Not detected in 4 patients.

that about half the patients on treatment had a remission is remarkably similar to that reported from each of the two non-randomized trials of cyclosporine.[1,2] We used alternate, untreated patients as controls and although they cannot be said to represent the ideal control group, their low frequency of remission was of the order generally recognized[12,13] and quoted for historical controls in the cyclosporine reports.[1,2]

Several findings warrant comment in comparison to the cyclosporine trials. In the Canadian trial[1] there was an indication that remission was more likely to occur the earlier treatment was instituted. This is a logical expectation since intervention is aimed at preserving residual beta cell function. However, initial C-peptide levels did not discriminate patients with and without remission. On the other hand, in the French trial,[2] although the interval between clinical presentation and treatment was not analyzed, initial C-peptide levels were higher in patients who subsequently had a remission. We could not discriminate response to treatment on the basis of initial duration of symptoms or C-peptide levels. However, in contrast to the Canadian results, patients who had a remission had significantly higher C-peptide levels on cessation of treatment, consistent with the notion that the preservation of beta cell function was the responsible factor. We also noted that these patients, perhaps unexpectedly, had a higher frequency of islet cell antibody after treatment, which could reflect persistence of the autoantigen.

We chose azathioprine as an immunosuppressive agent because wide experience with it in autoimmunity and transplantation has generally been favorable,[14,15] because we believed that its short-term use would be associated with fewer side-effects (even minor ones with cyclosporine, such as hirsutism or gum hypertrophy might not be acceptable in young diabetic patients), and because it is given conveniently without the need to monitor blood levels. The only definite side-effects encountered were reversible dose-related marrow depression in two patients and diarrhea in a third.

The apparent success of azathioprine in inducing remissions in patients with newly diagnosed type I diabetes attests to the underlying autoimmune pathogenesis of this disease and provides justification for a larger, double-blind controlled trial, which we are currently undertaking. Such a trial is necessary to exclude any possibility of bias in the selection or management of patients. The long-term follow-up of patients treated with cyclosporine has not yet been reported, but our finding that remissions on azathioprine were not sustained suggests that treatment may have to continue for longer in many patients to prevent the development of insulin dependence.

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