Diabetologia (2000) 43: 1000–1004

Diabetologia

Rapid communication

No effect of oral insulin on residual beta-cell function in recent-onset Type I diabetes (the IMDIAB VII)

P. Pozzilli, D. Pitocco, N. Visalli, M. G. Cavallo, R. Buzzetti, A. Crinò, S. Spera, C. Suraci, G. Multari, M. Cervoni, M. L. Manca Bitti, M. C. Matteoli, G. Marietti, F. Ferrazzoli, M. R. Cassone Faldetta, C. Giordano, M. Sbriglia, E. Sarugeri, G. Ghirlanda and the IMDIAB Group*

[1] Rome, Italy
[2] Medical Clinic, University of Palermo, Palermo, Italy
[3] San Raffaele Scientific Institute, University of Milan, Milan, Italy

Abstract

Aims/hypothesis. Induction of tolerance to insulin is achievable in animal models of Type I (insulin-dependent) Diabetes mellitus by oral treatment with this hormone, which can lead to prevention of the disease. In the Diabetes Prevention Trial of Type I diabetes (DPT-1), oral insulin is given with the aim of preventing disease insurgence. We investigated whether if given at diagnosis of Type I diabetes in humans, oral insulin can still act as a tolerogen and therefore preserve residual beta-cell function, which is known to be substantial at diagnosis.
Methods. A double-blind trial was carried out in patients (mean age ± SD: 14 ± 8 years) with recent-onset Type I diabetes to whom oral insulin (5 mg daily) or placebo was given for 12 months in addition to intensive subcutaneous insulin therapy. A total of 82 patients with clinical Type I diabetes (< 4 weeks duration) were studied. Basal C peptide and glycated haemoglobin were measured and the insulin requirement monitored every 3 months up to 1 year. Insulin antibodies were also measured in 27 patients treated with oral insulin and in 18 patients receiving placebo at the beginning of the trial and after 3, 6 and 12 months of treatment.
Results. The trial was completed by 80 patients. Overall and without distinction between age at diagnosis, at 3, 6, 9 and 12 months baseline mean C-peptide secretion in patients treated with oral insulin did not differ from that of those patients treated with placebo. In patients younger than 15 years a tendency for lower C-peptide values at 9 and 12 months was observed in the oral insulin group. Insulin requirement at 1 year was similar between the two groups as well as the percentage of glycated haemoglobin. Finally, IgG insulin antibodies were similar in the two groups at each time point.
Conclusion/interpretation. The results of this study indicate that the addition of 5 mg of oral insulin does not modify the course of the disease in the first year after diagnosis and probably does not statistically affect the humoral immune response against insulin. [Diabetologia (2000) 43: 1000–1004]

Keywords Type I diabetes, oral insulin, insulin antibodies, prevention.

Received: 28 February 2000 and in revised form: 14 April 2000

Corresponding author: Professor P. Pozzilli, Libera Università Campus Biomedico, Via Emilio Longoni, 83, 00155 Rome, Italy
Abbreviations: DPT-1, Diabetes Prevention Trial of Type I diabetes; IA, insulin antibodies

* *Members of the group also include*: C. A. Mesturino, A. Signore, M. G. Baroni, G. Coppolino, L. Valente, L. Lucentini, P. Patera, S. Corbi, C. Teodonio, R. Amoretti, L. Pisano, N. Sulli, A. Cantagallo, S. Piccinini, C. Bizzarri, A. Tempera, S. Petrucci, G. De Mattia

The use of insulin, given either subcutaneously or orally, in subjects at risk for Type I diabetes has been recently introduced in the Diabetes Prevention Trial of Type I diabetes (DPT-1) trial, a large multinational trial in the United States, with the aim of preventing the destruction of beta cells and the clinical onset of the disease [1]. The rationale for the use of insulin in these patients is to induce beta-cell rest and/or tolerance to the hormone and its peptides [2] which are thought to be important targets of the autoimmune response leading to beta-cell destruction [3]. In ani-

Table 1. Baseline clinical characteristics and metabolic control at entry of the trial in the two groups of patients

	Oral insulin	Placebo
Number of patients	46	36
Sex (number of males)	25	17
Age (years ± SD)	14.1 ± 7.9	13.8 ± 7.6
Duration of symptoms before diagnosis (days)	34.5 ± 34.6	38.8 ± 42.5
Blood glucose at diagnosis (mmol/l)	20.7 ± 9.4	22.1 ± 8.3
Insulin dose ($U \cdot kg^{-} \cdot day^{-1}$) ± SD	0.63 ± 0.3	0.55 ± 0.3
Glycated haemoglobin (%) ± SD	9.9 ± 0.5	9.0 ± 0.5
Basal C peptide (nmol/l) ± SD	0.23 ± 0.3	0.17 ± 0.15

Values between groups are not statistically different

mal models, this approach was shown to be effective in halting the process leading to Type I (insulin-dependent) diabetes mellitus [4]. Furthermore, oral insulin was able to reduce the extent and modify the type of lymphocytic infiltration in the pancreas of susceptible mice [5].

The International Diabetes Immunotherapy Group suggested that approaches to prevent Type I diabetes should first be tested in recent-onset Type I diabetic patients and, if effective, applied to pre-diabetic people [6]. In our study we evaluated the effects of oral insulin treatment at clinical onset of Type I diabetes, which could aid in reducing the further destruction of beta cells that generally occurs within the first 12 months after diagnosis. In patients with recent-onset Type I diabetes, simultaneous treatment with subcutaneous and oral insulin might have considerable effects, as the former could improve metabolic control and the latter induce tolerance. As disturbances in the gut immune reactivity could be relevant in the pathogenesis of Type I diabetes [7], induction of oral tolerance with a specific antigen, such as insulin, could be appropriate for this disease. It is therefore the aim of this double-blind randomized trial to find out whether treating patients who have recent-onset Type I diabetes with oral insulin in addition to identified subcutaneous insulin therapy [8] could improve metabolic control, as measured by glycated haemoglobin value, insulin dose and C peptide concentration. The effects of such treatment on the rate of spontaneous clinical remission (suspension of insulin therapy) and on the extent of humoral immune response against insulin were also evaluated.

Subjects and methods

Selection of patients. Patients with recent-onset Type I diabetes ($n = 82$) were recruited by 8 participating centres of the IMDIAB Group and 1 affiliated centre. Each centre contributed with nearly equal numbers of patients to the study. Inclusion criteria were the following: (1) diagnosis of the disease according to the World Health Organisation (WHO) criteria, with age at presentation between 5 and 35 years, (2) duration of clinical disease (since the beginning of insulin therapy) less than 4 weeks, (3) no medical contra-indications (including pregnancy) or any other major chronic disease, (4) willingness and capability to participate in regular follow-up.

Patients' baseline clinical characteristics and metabolic control at entry of the trial are shown in Table 1.

Study design and treatment protocol. The study was endorsed by the Italian Ministry of Health and approved by the central ethics committee at the Gemelli Policlinic, The Catholic University of the Sacred Heart, Rome. After informed consent had been obtained and baseline measurements completed, a permuted-block design was used to blindly assign patients to each of the two treatment groups. A random number table was adopted with a prepared list and a randomization code was assigned to each participating centre. Of the patients 46 received 5 mg daily of oral insulin and 36 placebo. Oral treatment began within 4 weeks of diagnosis in both groups and lasted 12 months. All patients also received intensive subcutaneous insulin therapy as soon as possible after diagnosis to optimize metabolic control and maintain blood glucose concentrations as near to normal as possible (see below).

Guidelines for insulin therapy. All participating centres used the same treatment protocol as in our previous IMDIAB trials [9, 10] based on the following rules: if pre-prandial blood glucose values were below 6.5 mmol/l, the insulin dose was decreased by 10%; if blood glucose concentrations were consistently below 4.5 mmol/l for more than 3 days the insulin dose was decreased by 20%. Insulin therapy was not discontinued unless 2-h postprandial blood glucose concentrations measured at home were consistently below 7.5 mmol/l. Patients with blood glucose above 10 mmol/l received a 10% increase in insulin dose or had their insulin regimen modified. Frequent telephone consultations were arranged with patients to adjust the insulin dose as required.

Investigations and follow-up. Patients included in the study were followed up by the staff of the centre where they were enrolled. Patients were started on a 55% carbohydrate diet and received three to four injections daily of regular plus intermediate insulin. Each patient recorded capillary glucose concentration at fasting and before and after meals daily, for a total of at least 20 weeks. The subcutaneous insulin dose was adjusted to obtain near-normal blood glucose concentrations.

Patients were examined weekly for the first month of therapy and then monthly by the same team of physicians in each participating centre. Drug toxicity was evaluated at follow-up visits, by liver and renal function tests and total blood count. Glycated haemoglobin (HbA_{1c}) (normal range 4–7%) was measured every 3 months by a column assay (Bio-Rad, Milan, Italy), and basal C peptide concentration was evaluated after euglycaemia was achieved before entry into the trial, and at 3-monthly intervals for 1 year thereafter. C peptide concentra-

Table 2. Metabolic outcomes during follow-up

	Oral insulin	Placebo
Number of patients	44	36
Insulin dose (U/kg) ± SD		
3 months	0.44 ± 0.3	0.37 ± 0.2
6 months	0.48 ± 0.3	0.43 ± 0.2
9 months	0.54 ± 0.3	0.52 ± 0.3
12 months	0.61 ± 0.2	0.58 ± 0.3
Glycated haemoglobin (%) ± SD		
3 months	6.2 ± 1.8	5.8 ± 1.5
6 months	6.5 ± 1.5	6.3 ± 1.5
9 months	7.1 ± 1.6	7.1 ± 1.5
12 months	7.6 ± 1.3	7.1 ± 1.5
Basal C peptide (nmol/l) ± SD		
3 months	0.30 ± 0.2	0.30 ± 0.2
6 months	0.30 ± 0.2	0.30 ± 0.2
9 months	0.20 ± 0.2	0.25 ± 0.2
12 months	0.17 ± 0.2	0.22 ± 0.2

Values between the two groups are not statistically different for insulin dose, HbA_{1c}, C peptide concentration

tion was measured by radioimmunoassay, using a commercially available kit (Bio-Rad). The normal range of fasting C peptide established in 150 control subjects, 71 females and 79 males, aged 5–40 years, median 18 years, with no family history of Type I diabetes was 0.35–1 nmol/l with intracoefficients and intercoefficients varying between 10% and 15%, respectively.

Insulin antibodies. Insulin antibodies (IA), expressed as a concentration of units/5 μl serum, were measured in serum samples drawn from 27 patients treated with oral insulin and 18 receiving placebo at the beginning of the trial and after 3, 6 and 12 months of treatment and stored at –20 °C. A modification of the micro-radio-binding assay of Williams [11] was used as described previously [12]. The threshold and 99th centile of 97 control subjects, 51 females and 46 males, aged 2–48 years, median 21 years, with no family history of Type I diabetes, was calculated at greater than 4.4 insulin antibody units.

Evaluation of response to therapy. Response to therapy was monitored throughout the study by investigating the occurrence of clinical (complete) remission defined, according to the recommendations of the International Diabetes Immunotherapy Group (IDIG), as restoration of normal fasting and postprandial blood glucose concentration without any insulin treatment for more than 2 weeks [6]. Moreover, metabolic control (C peptide, HbA_{1c} and insulin dose) was evaluated at 3-monthly intervals.

Sample size and statistical analysis. The number of patients to be included in the study was calculated from an analysis of results of trials published in the past (courtesy of IDIG Registry). Setting alpha (probability of a type I error) equal to 0.05 and beta (probability of a type II error) equal to 90%, the required sample size was 74 patients for a two-sided test. To ensure the appropriate sample size, 82 patients were recruited to allow for drop outs.

Results obtained in the different treatment groups were analysed blind by a team of statisticians. Differences in clinical remission proportions between patient groups were evaluated by the one-sided Fisher's exact probability test. For the analysis of the integrated measures of metabolic control (C peptide, HbA_{1c} and insulin dose), an analysis of variance was done; for measuring differences between groups at different time intervals, the Mann-Whitney U test was used.

For the analysis of antibody results, median antibody values in the two groups at each time point were compared using the Mann-Whitney U test, whereas proportions of patients with IA at each time point were compared using Fisher's exact probability test.

Results

Recruitment lasted 1 year. There were no significant differences between the two groups of patients in baseline clinical characteristics and metabolic control at the time of enrollment (Table 1). None of the patients suffered from any other autoimmune disease.

Dropouts. Only two patients withdrew from the study and this was because of poor compliance.

Metabolic data. Clinical remission was observed in one patient (lasting 3 months) in the oral insulin group and one patient (lasting 8 months) in the placebo group. Insulin requirement was significantly reduced in all patients after 3 months of treatment compared with the beginning of the trial but the patients treated with oral insulin and placebo did not differ in this respect (Table 2). The subcutaneous insulin dose required to obtain optimal metabolic control was similar in the two groups at 6, 9, and 12 months. Basal C-peptide secretion over 1 year of follow-up had a similar pattern in both groups of patients, with an initial increase (compared with diagnosis), followed by a steady decrease which was slightly more pronounced in the oral insulin group. When age at diagnosis was taken into account, insulin dose, HBA_{1c} values and C-peptide concentrations in patients older than 15 years ($n = 28$) were not different at the beginning of the trial between oral insulin ($n = 16$) and placebo-treated ($n = 12$) patients and did not change thereafter. In patients younger than 15 years ($n = 52$), C-peptide concentrations after 9 and 12 months tended to decline more in the oral insulin ($n = 28$) than in the placebo group ($n = 24$), although the difference between the two groups did not reach statistical significance (Fig. 1). Good metabolic control was achieved by all patients, as shown by the rapid decline in HbA_{1c} values after diagnosis, which persisted until the end of the study. Finally, no adverse effects were noted in patients receiving either oral insulin or placebo.

Insulin antibodies. Insulin antibodies were detectable at disease onset in 17 out of 27 (63%) patients receiving oral insulin and 8 out of 18 (44%) receiving placebo; the humoral response against insulin increased during the study in 23 of the patients receiving oral insulin and 15 of those treated with placebo. No dif-

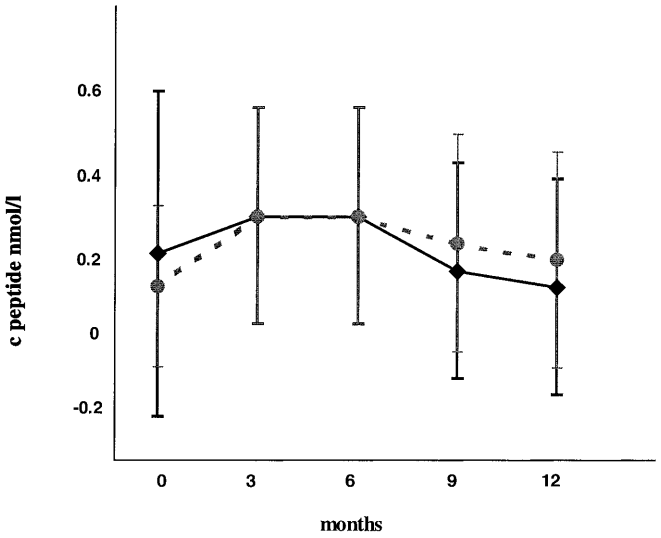

Fig. 1. Basal C-peptide concentration (mean ± SD) in patients younger than 15 years treated with oral insulin ($n = 28$, —◆—) and placebo ($n = 24$, —●—). Values were lower at 9 and 12 months in the patients treated with oral insulin, however they were not statistically different from those patients treated with placebo

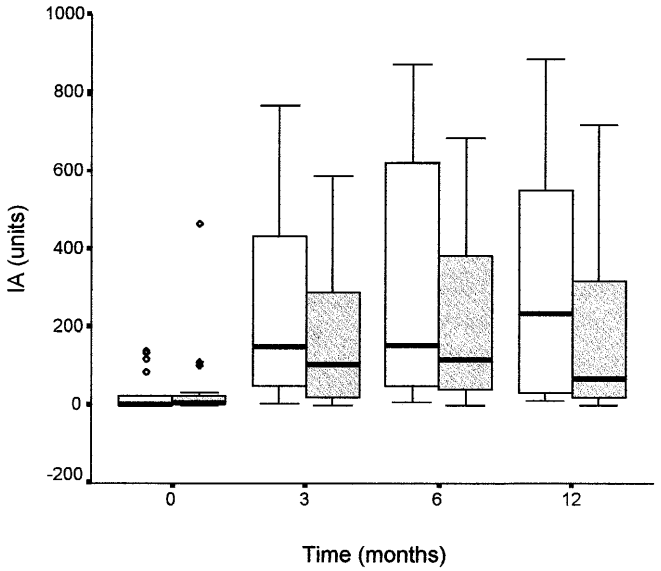

Fig. 2. Box-whisker plot of insulin antibodies measured at various time points in patients treated with oral insulin (filled boxes) or placebo (open boxes). Values between the two groups were not statistically different at any time point

ferences were observed between the two groups in the proportion of patients with IA or in the median insulin antibody concentrations at all time points, although a trend towards lower antibody concentrations was evident in the group treated with oral insulin after 3, 6 and 12 months of treatment (Fig. 2). When antibody results were analysed according to age at diagnosis (15 years), levels of IA after 3, 6 and 12 months were higher in younger patients ($n = 32$) than in older subjects ($n = 13$) ($p < 0.02$ at all time points) but no significant differences were observed between patients treated with oral insulin and those treated with placebo when subdivided by age (data not shown).

Discussion

This double-blind trial with oral insulin in patients with recent-onset Type I diabetes was designed to assess whether the addition of oral insulin at the time of clinical diagnosis could maintain or even improve the residual beta-cell function which is usually detectable in these patients. Oral insulin had no effect on residual beta-cell function, as assessed by C-peptide secretion. Furthermore, patients treated with oral insulin who were younger than 15 years at diagnosis showed a tendency for a more pronounced decline of basal C-peptide concentrations 9 and 12 months after diagnosis compared with patients matched with them for age but treated with placebo, although this difference was not statistically significant. We did not measure stimulated C peptide but limited the investigation to baseline C peptide concentrations. These were measured under strict and controlled conditions of fasting blood glucose less than 180 mg/kg at the time of sampling (if blood glucose concentration was higher sampling for C peptide was postponed). The night before the test patients were also advised to have a light meal and avoid any unnecessary stress. In the light of the results of baseline C-peptide concentrations it is doubtful that oral insulin had an effect on those of stimulated C-peptide. Older patients also did not benefit from the addition of oral insulin: thus, this antigen-based therapy, which is supposed to induce tolerance to a key antigen (e. g. insulin) in Type I diabetes, seems to be ineffective (at least at the doses used in this trial) in protecting residual beta-cell function in patients with recently diagnosed disease.

There are a number of possibilities to explain these findings. One is that the oral insulin daily dose used in this trial (5 mg) was not sufficient. Several experimental data have indicated that the dose of antigen is a critical factor for tolerance induction in autoimmune diseases [13]. A similar trial in France, in which two doses of oral insulin (2.5 mg and 7.5 mg) were used and preliminary data presented in abstract form did not show any effect [14]. As the addition of oral insulin does not influence metabolic control, higher doses of oral insulin should possibly be tested. The use of an adjuvant carrier to increase the tolerogen capacity of insulin is also worth consideration for tolerance induction, with implications for clinical use [15].

Another possibility is that at the time of clinical diagnosis of Type I diabetes residual beta-cell mass is so small that the efficacy of this treatment cannot be de-

tected. This might be different in pre-Type I diabetes, in which beta-cell mass is almost unaffected and the spreading of the autoimmune response to a number of other antigens, which generally amplifies the rate of beta-cell destruction, is still limited. In such a case tolerance induction might still be possible with insulin. It is, however, of concern that in our trial treatment with oral insulin seemed to accelerate the decline of beta-cell function, at least in the very young subjects, because insulin is considered to be the major target of the autoimmune attack against beta cells, especially in young patients. In other autoimmune condition(s) the addition of oral antigens has induced an accelerating effect on disease progression [16]. A reasonable concern is that if oral insulin has no or negative effects on the natural course of beta-cell destruction in the first year after diagnosis it might have similar effects when given before the onset of overt hyperglycaemia. The trend towards lower IA responses observed in patients treated with oral insulin might reflect a modulation of the response induced against exogenous insulin. The reduction was, however, not statistically significant, so it is difficult to draw any definitive conclusion on the effect of oral insulin given at disease onset in terms of modulation of antigen-specific immune reactivity.

All these concerns apply to the prevention trials designed to test whether intervention during the prodromal period of Type I diabetes can delay its clinical onset. Specifically, the objective of the DPT-1 is to determine whether antigen-based therapies (e. g. insulin) in non-diabetic relatives of patients with Type I diabetes can delay the development of overt clinical disease [1]. Based on the results of our trial attention should be paid to the subjects in the oral treatment group of DPT-1 trial to find out whether oral insulin affects C-peptide secretion or insulin antibody concentrations.

In conclusion, the addition of 5 mg daily of oral insulin to regular subcutaneous insulin therapy has no effect on residual beta-cell function in patients with recent-onset Type I diabetes and does not modify the humoral immune response against the hormone. These results have important implications for current thoughts in designing strategies for preventing Type I diabetes.

Acknowledgements. The work was supported by grants from The Italian Office for University and Scientific and Technological Research (MURST) and The International Centre for the Study of Diabetes. Insulin for oral treatment was kindly donated by Eli Lilly. The group in Palermo was supported by a grant from the Italian Society of Diabetes (C. Giordano). We also acknowledge Dr E. Bonifacio and Prof E. Bosi for their comments and Miss C. Devlin for helping to prepare the final version of this paper.

References

1. Steffes MW (1995) The diabetes prevention trial-type 1 diabetes (DPT-1). Implementation of screening and staging of relatives. DPT-1 Study Group. Transplant Proc 27: 3377–3383
2. Weiner HL (1996) Oral tolerance. In: Palmer JP (ed) Prediction, Prevention and Genetic Counseling in IDDM. Wiley, Chichester, pp 292–315
3. Gladstome P, Nepon GT (1995) The prevention of IDDM: Injecting insulin into the cytokine network. Diabetes 44: 859–862
4. Zhang JA, Davidson L, Eisenbarth G, Weiner HL (1991) Suppression of diabetes in NOD mice by oral administration of porcine insulin. Proc Natl Acad Sci USA 88: 10252–10256
5. Hancock WW, Polanski M, Zhang J, Blogg N, Weiner HL (1995) Suppression of insulitis in non obese diabetic (NOD) mice by oral insulin administration is associated with selective expression if interleukin –4 and –10, transforming growth factor-beta and prostaglandin E. Am J Pathol 147: 1193–1199
6. Kolb H, Bach JF, Eisenbarth GS et al. (1989) Criteria for immune trials in Type I diabetes. Lancet ii: 686
7. Kolb H, Pozzilli P (1999) Cow's Milk and Type I diabetes: the gut immune system deserves attention. Immunology Today 20: 108–110
8. Shah SC, Malone LL, Simpson NE (1989) A randomized trial of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. N Eng J Med 320: 550–554
9. Pozzilli P, Visalli N, Signore A et al. (1995) Double blind trial of nicotinamide in recent onset insulin dependent diabetes mellitus. Diabetologia 38: 848–852
10. Visalli N, Cavallo MG, Signore A et al. (1999) A multi-centre randomised trial of two different doses of nicotinamide in patients with recent-onset Type I diabetes (The IMDI-AB VI). Diabetes Metab Rev 15: 181–185
11. Williams AJ, Bingley PJ, Bonifacio E, Palmer JP, Gale EA (1997) A novel micro-assay for insulin-autoantibodies. J Autoimmun 10: 473–478
12. Naserke H, Dozio N, Ziegler AG, Bonifacio E (1998) Comparison of a novel micro-assay for insulin autoantibodies with the conventional radiobinding assay. Diabetologia 41: 681–683
13. Weiner HL (1997) Oral tolerance immune mechanisms and treatment of autoimmune disease. Immunol Today 18: 335–543
14. Chaillous L, Carel JC, Thivolet C, et al. (1999) Lack of effect of one-year oral insulin therapy in recent onset Type I diabetes: results of a multicenter randomised controlled trial. Diabetologia 42: A62 (Abstract)
15. Bergerot I, Ploix C, Peterson J et al. (1997) A cholera toxoid-insulin conjugate as an oral vaccine against spontaneous autoimmune diabetes. Proc Natl Acad Sci USA 94: 4610–4614
16. Blanas E, Heath WR (1999) Oral administration of antigen can lead to the onset of autoimmune disease. Int Rev Immunol 18: 217–228