Remissions in newly diagnosed Type 1 (insulin-dependent) diabetes: influence of interferon as an adjunct to insulin therapy

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Summary. We studied the effect of interferon as an adjunct to conventional insulin therapy on the early course of Type 1 diabetes in 43 newly diagnosed patients. Compared with conventional therapy, interferon administration slightly delayed the improvement of glucose homeostasis and the rise of high density lipoprotein cholesterol, while C-peptide secretion was unaffected. Independent of the type of therapy, 18 patients (42%) entered partial remission. The remission began 2.0 ± 0.6 months (mean $\pm$ SEM) from the start of therapy and lasted for 4.1 ± 1.1 months. Seven patients (16%) were still in remission 1 year after diagnosis. The patients who entered remission had

higher initial C-peptide secretion, lower glycosylated haemoglobin levels and better initial control than patients without remission. Thus, interferon provided no benefits as an adjunct to conventional insulin therapy in unselected patients with newly diagnosed Type 1 diabetes. An important factor for the development of remission was the presence of C-peptide secretion at the time of diagnosis.

Key words: Type 1 diabetes, interferon, insulin therapy, remission.

Epidemiological data [1–3] and a few case reports [5–7] indicate that a viral infection of β cells may play a role in the development of Type 1 (insulin-dependent) diabetes. Since human leucocyte interferon is a potent antiviral agent [7–9], there is a possibility that it might reduce virus-induced β -cell damage. Indeed, the addition of interferon to mouse β -cell cultures in vitro delayed the β -cell damage induced by encephalomyocarditis (EMC) virus infection [10]. In addition, virus production in β -cell cultures was reduced between five and ten-fold by interferon. Furthermore, recent studies in mice have demonstrated that EMC virus-induced diabetes can be prevented by interferon [11]. Only anecdotal evidence exists in man. In two newly diagnosed diabetic children, the administration of interferon failed to have any therapeutic effect [12].

The present study was undertaken to examine, in a controlled manner, the effect of interferon as an adjunct to conventional insulin treatment on the early course of newly diagnosed Type 1 diabetes. Since in normal man interferon reduces HDL-cholesterol levels [13], serum cholesterol and HDL-cholesterol were also determined.

Subjects and methods

Subjects

Forty-five newly diagnosed Type 1 diabetic patients were invited to participate in the study. Two refused, and 43 patients (23 male and 20 females) were studied. The criteria for inclusion were: random blood glucose > 15 mmol/l, ketonuria (Ketostix + +), no signs of secondary diabetes and no allergy to egg protein. An age limit of 15–25 years was used in order to obtain adult patients, while avoiding the inclusion of Type 2 diabetic subjects. All patients participating in the study gave their informed consent. The study protocol was approved by the Ethical Committees of the participating hospitals and of the National Public Health Institute. Clinical data of the patients are shown in Table 1.

Interferon

Human leucocyte α interferon was produced and purified as described previously [14, 15]. The preparation used was of P-IF A type [14] (sp. act. 1.3×10^6 IU/mg protein). The interferon vials contained 3×10^6 IU interferon and 2.3 mg protein in 0.5 ml of physiological phosphate buffered saline (pH 7.3). The placebo vials contained 2.3 mg human albumin in 0.5 ml phosphate buffered saline.

Table 1. Clinical details of the patients at the time of diagnosis of diabetes

	Age (years)	Weight (kg)	Duration of symptoms (months)	HbA _{1c} (%)
Control group (n=23)	21 ± 1	57 ± 2	2.5 ± 0.5	12.0 ± 0.6
Interferon group (n=20)	20 ± 1	60 ± 3	1.5 ± 0.3	11.4 ± 0.5

Results expressed as mean ± SEM

Fig. 1. Fasting blood glucose levels during the initial 14 days of therapy in control (O-----O) and interferon (x-----x) treatment groups

Experimental design

The patients were admitted to the University Hospitals of Helsinki (20 patients), Turku (13) and Kuopio (10). After admission, insulin and dietary therapy were started and patient education was given in a similar fashion at each centre. Within 3 days of admission, the patients were randomized in a double-blind fashion into two groups: as an adjunct to conventional insulin treatment, one group was injected daily with human leucocyte interferon 3×10^6 IU subcutaneously for 2 weeks ($n=20$), while the other group was administered the same volume of placebo ($n=23$). After 2 weeks of initial therapy, patients were discharged and were seen thereafter at 1, 2, 4, 6, 9 and 12 months. Throughout the study, comparable principles of insulin therapy were followed at each centre.

Measurements

During the 2 week initial admission to hospital, we determined fasting and diurnal blood glucose levels and glycosuria by a glucose oxidase method adapted for Auto-analyzer II (Technicon, Territown, New York, USA). Glycosylated haemoglobin (HbA_{1c}) levels were determined by microcolumn chromatography throughout the 12 month follow-up period [16], serum total and high density lipoprotein (HDL)-cholesterol were determined during the initial admission to hospital and at 1–2 month intervals thereafter [13]. Serum C-peptide measurements in the fasting state and after glucagon stimulation [17] were carried out within 3 days following admission. Fasting or post-prandial blood glucose and HbA_{1c} levels were determined at each visit throughout the 12 month follow-up period. Serum lipids were measured up to 4 months and C-peptide at 6 and 12 months after the discharge. In addition, the patients were asked to measure urinary glucose excretion (Clinitest, Ames, Slough, UK and Diabur test 5000, Boehringer Mannheim, FRG) at least four times a day, 2 days per week, for the adjustment of their insulin dosage.

Definition of remission

Insulin therapy was changed to glibenclamide if the patient had no glycosuria and a fasting blood glucose of ≤ 7 mmol/l, 1 h post-prandial ≤ 9 mmol/l or 2 h post-prandial ≤ 8 mmol/l with insulin dose of 16 U/day or less. If the fasting blood glucose level was ≤ 6 mmol/l with glibenclamide, the drug was discontinued and dietary therapy only continued. The period without insulin therapy was called 'partial remission'. Insulin therapy was re-established if the patient had ketonuria, glycosuria or if two blood glucose measurements were as follows: fasting ≥ 7 mmol/l, 1 h post-prandial ≥ 9 mmol/l or 2 h post-prandial ≥ 8 mmol/l. If fasting blood glucose levels were between 6–7 mmol/l and glycosuria > 10 g/day, glibenclamide therapy was restarted.

Statistical analyses

Comparison between the two groups was made by the unpaired 't-test' and within the group by the paired t-test. The results are given as mean ± SEM.

Results

Recent infections

Prior to the diagnosis of diabetes or within the preceding 6 months, five out of 20 patients in the interferon group and seven out of 23 in the control group had experienced an infection with fever. The aetiological agents of these infections were unknown.

Glycaemic control

Fasting blood glucose levels during the initial 14 days of insulin therapy were comparable between the control and interferon groups (Fig. 1). The mean daily glucose excretion during the initial 14 days was greater in the interferon group (42 ± 8 g) than in the control patients (19 ± 3 g, $p < 0.05$). Initial C-peptide levels were comparable in the two groups (Table 2). During the 12 months of therapy, C-peptide levels rose slightly in both groups at 6 months ($p < 0.05$). However, no significant differences between the two groups were seen either in the basal or stimulated C-peptide levels during the follow-up period (Table 2).

Initial HbA_{1c} levels were similar in the control and interferon groups (Table 3). Throughout the 12 month follow-up period, both HbA_{1c} levels and glycosuria were comparable in both groups, whereas a slight difference in insulin dose was observed after 2 months of therapy (Table 3).

Remission

During the 12 month follow-up, 12 of the 23 (52%) conventionally treated and 6 out of 20 (30%) in the interferon group entered a partial remission and insulin therapy was discontinued. Three out of 12 in the control group and four out of six in the interferon group were given glibenclamide (5–15 mg/day), whereas the others

Table 2. Serum C-peptide levels ($\mu\text{g/l}$) in the basal state and after glucagon stimulation in control and interferon treatment groups and in relation to the presence or absence of remission

	Initial		14 days		6 months		12 months	
	Basal	6 min after stimulation	Basal	6 min after stimulation	Basal	6 min after stimulation	Basal	6 min after stimulation
Control ($n=23$)	0.60 ± 0.07	1.02 ± 0.11	0.66 ± 0.09	1.05 ± 0.14	0.93 ± 0.14^a	1.43 ± 0.21	0.69 ± 0.15	0.99 ± 0.19
Interferon ($n=20$)	0.51 ± 0.06	0.88 ± 0.11	0.57 ± 0.11	0.94 ± 0.19	1.23 ± 0.16^a	1.59 ± 0.25^a	0.98 ± 0.18	1.46 ± 0.29
Remission present ($n=18$)	0.77 ± 0.11^b	1.17 ± 0.14^b	0.72 ± 0.13	1.12 ± 0.16	1.09 ± 0.16	1.65 ± 0.23	0.73 ± 0.10	1.19 ± 0.22
Remission absent ($n=25$)	0.47 ± 0.06	0.80 ± 0.03	0.62 ± 0.10	0.99 ± 0.16	0.99 ± 0.16^a	1.35 ± 0.20^a	0.93 ± 0.19	1.18 ± 0.25

Results expressed as mean $\pm$ SEM^a $p < 0.05$ versus initial; ^b $p < 0.05$ versus patients without remission**Table 3.** Glycaemic control and insulin requirements from 1 to 12 months after the diagnosis of diabetes in control and interferon treatment groups

Groups	Months after diagnosis				
	1	2	4	6	12
HbA _{1c} ^a (%)					
Control	9.1 ± 0.4	7.9 ± 0.4	7.6 ± 0.5	7.9 ± 0.5	9.7 ± 0.7
Interferon	8.9 ± 0.3	7.8 ± 0.4	8.4 ± 0.5	8.1 ± 0.5	8.6 ± 0.6
Glycosuria (g/day)					
Control	13 ± 6	18 ± 7	19 ± 10	7 ± 4	11 ± 5
Interferon	13 ± 5	23 ± 9	45 ± 10	27 ± 12	26 ± 11
Insulin dose (U/day)					
Control	21 ± 2	18 ± 2	18 ± 3	17 ± 3	20 ± 3
Interferon	25 ± 3	27 ± 3^b	25 ± 3	27 ± 5	27 ± 5

Results expressed as mean $\pm$ SEM^a Normal values: 4.1–5.7%; ^b $p < 0.05$ compared with the control group

were not given any medication. The remission frequencies were not significantly different between the two groups. The time from the start of therapy to the beginning of remission was similar in the control (1.8 ± 0.4 months) and interferon groups (2.2 ± 0.8 months) and the duration of remission (4.1 ± 1.1 months) was also identical in both groups. One year from diagnosis, four patients (17%) in the control and three (15%) in the interferon group were still in partial remission without insulin therapy.

When the patients were analyzed according to the presence or absence of remission regardless of the type of initial therapy, the duration of symptoms was similar in patients with (1.9 ± 0.5 months) or without remission (1.9 ± 0.4 months). At the time of diagnosis, patients who later entered remission had 50%–80% higher C-peptide levels both in the basal state and after glucagon stimulus compared with the subjects who failed to have a remission (Fig. 2). During the follow-up period, C-peptide levels rose, but no significant differences between the two groups were observed (Table 2).

Fasting blood glucose levels during the first 14 days of therapy were lower in the patients who later had re-

mission compared with those continuously on insulin therapy (Fig. 3).

Lipids

Serum cholesterol and HDL-cholesterol levels at the time of diagnosis and throughout the 12 months of initial follow-up are shown in Table 4. At diagnosis, the mean levels of total or HDL-cholesterol were within the normal range. During the initial 7 days of insulin therapy, cholesterol levels declined in each group and HDL-cholesterol began to increase in control patients. In patients receiving interferon, HDL-cholesterol levels were below control levels at 2 weeks and rose only after the discontinuation of interferon therapy.

Long-term follow up

Nine control patients and nine patients from the interferon group were re-studied 30–36 months after diagnosis. No significant differences were observed between the control and interferon groups, respectively, in their mean daily insulin requirements (44 ± 4 versus $36 \pm$

Fig. 2. Serum C-peptide levels in the basal state and 6 min after intra-venous injection of 1 mg glucagon. The measurements were carried out within 3 days of hospital admission. Those patients who later entered remission are shown in the left panel, while those who did not enter remission are shown in the right-hand panel. Both basal and stimulated C-peptide levels were higher in those with remission than those without ($p < 0.05$)

Fig. 3. Fasting blood glucose levels during the initial 14 days of insulin therapy in the those who later had (▲—▲) or did not have (□---□) a remission. $\times = p < 0.05$, $\times \times = p < 0.01$

Table 4. Serum total and HDL-cholesterol levels during the first 4 months from diagnosis of diabetes in control and interferon treatment groups

Groups	Time from diagnosis					
	At diagnosis	7 days	14 days	1 month	2 month	4 month
Total cholesterol (nmol/l)						
Control	5.20 ± 0.4	4.40 ± 0.3	4.70 ± 0.3 ^a	4.90 ± 0.3	4.80 ± 0.3	4.70 ± 0.3
Interferon	5.40 ± 0.4	4.50 ± 0.2 ^a	4.40 ± 0.2 ^a	4.60 ± 0.2 ^b	4.70 ± 0.2 ^b	4.80 ± 0.2
HDL-cholesterol (nmol/l)						
Control	1.03 ± 0.05	1.12 ± 0.07 ^a	1.21 ± 0.07 ^b	1.33 ± 0.06 ^b	1.37 ± 0.08 ^b	1.29 ± 0.09 ^b
Interferon	0.94 ± 0.06	0.94 ± 0.08	0.98 ± 0.07 ^c	1.31 ± 0.08 ^b	1.42 ± 0.09 ^b	1.43 ± 0.10 ^b

Results expressed as mean ± SEM

^a $p < 0.05$ versus the value at diagnosis; ^b $p < 0.001$ versus the value at diagnosis; ^c $p < 0.05$ versus control group

4 U), C-peptide secretion (basal 0.3 ± 0.1 versus 0.4 ± 0.2 µg/l, stimulated 0.3 ± 0.2 versus 0.5 ± 0.2 µg/l) or HbA_{1C} levels (9.5 ± 0.7 versus $9.8 \pm 0.6\%$).

Discussion

In the current study, 20 newly diagnosed Type 1 diabetic patients were treated with the anti-viral agent interferon as an adjunct to insulin therapy. They failed to show improved metabolic control, a greater incidence of remission or lower insulin requirements as compared with their 23 counterparts treated with conventional insulin therapy alone.

Several reasons may contribute to the failure of interferon therapy: (1) The patient population was unselected and diabetes may have been caused by factors other than a viral infection [18]. (2) In animal studies, the virus-induced β -cell damage was prevented by interferon when the drug was administered before or at the time of virus inoculation [10, 11]. In our patients, the

mean duration of symptoms before the start of therapy was 1.9 months. Thus, if there was a viral aetiology, irreversible β -cell damage had probably occurred prior to the interferon administration. (3) The virus may have been unresponsive to interferon. (4) The effect of interferon upon the virus may have been reversible. (5) The dose of interferon may not have been sufficient. (6) The human leucocyte α -interferon employed in the present study may not have been the appropriate type. Thus, a variety of factors in unselected patients such as ours could have contributed to the therapeutic failure of interferon. It remains to be seen whether a better effect can be obtained, if patients with diabetes of viral aetiology can be identified and interferon can be administered soon after the infection.

Regarding possible adverse effects of interferon, one of our patients had a slight fever, but we failed to find any allergic, haematological or neurological effects as reported by others [9, 19]. However, when metabolic control was compared between the interferon and control groups, two differences emerged. First, diurnal gly-

cosuria was higher during interferon than conventional therapy. It is theoretically possible that interferon could damage β cells by activating macrophages [20] and natural killer cells [21]. However, C-peptide levels were similar in the interferon and control groups when measured 6, 12 and 30 to 36 months after therapy. Thus, our data fail to demonstrate any β -cell damage caused by interferon, which is in keeping with previous findings in healthy subjects [22]. Second, HDL-cholesterol levels were lower in the interferon than the conventionally treated patients during the fortnight of interferon therapy. Ehnholm et al have demonstrated an HDL-cholesterol lowering effect by interferon in normal man [13]. Our findings confirm this also occurs in diabetic patients.

When all our material was combined, 42% of the patients (18 out of 43) had a partial clinical remission. Compared with patients who failed to have a partial remission, the subjects entering remission were characterized by higher C-peptide and lower HbA_{1C} levels at the time of diagnosis, and lower fasting blood glucose levels during the early course of therapy. However, no differences in C-peptide levels between the two groups were observed during the 12-month follow-up period. These data suggest that initial C-peptide secretion and good metabolic control contribute to the development of remission. Moreover, they raise the possibility that factors other than endogenous insulin secretion, perhaps for example variations in body sensitivity to insulin, may influence the occurrence of partial remission [23].

Taken together, our data indicate that more than 40% of newly diagnosed Type 1 diabetic patients between the ages of 15 and 25 years enter a partial clinical remission during the first months of therapy, and about 15% of them are still in remission 1 year after diagnosis. A primary factor contributing to the development of remission is the presence of endogenous insulin secretion at the time of diagnosis. Interferon, as an adjunct to insulin therapy, offers no benefit in unselected adult patients with newly diagnosed Type 1 diabetes.

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References

- Gamble DR, Kinsley ML, Fitzgerald MG, Bolton R, Taylor KW (1969) Viral antibodies in diabetes mellitus. *Br Med J* 3: 627–630
- Gamble DR, Taylor KW (1969) Seasonal incidence of diabetes mellitus. *Br Med J* 3: 631–633
- Gamble DR, Taylor KW, Cumming H (1973) Coxsackie viruses and diabetes mellitus. *Br Med J* 4: 260–262
- Messaritakis J, Karabula C, Kattamis C (1971) Diabetes following mums in sibs. *Arch Dis Child* 46: 561–562
- Yoon JW, Austin M, Onodera T, Notkins AL (1979) Virus-induced diabetes mellitus: isolation of a virus from the pancreas of a child with ketoacidosis. *N Engl J Med* 300: 1173–1179
- Champsacer K, Dussaix E, Samolyk D, Fabre M, Beach Ch, Assam R (1980) Diabetes and Coxsackie B5 infection. *Lancet* 1: 251
- Friedman RM (1964) Role of interferon in viral interference. *Nature* 201: 848–849
- Cantell K (1979) Why is interferon not in clinical use today? In: Gresser I (ed) *Interferon I*. Academic Press, London pp 1–28
- Stieham ER (1982) Interferon: immunology and clinical significance. *Ann Int Med* 96: 80–93
- Wilson GL, Bellomo SC, Craighead JE (1983) Effect of interferon on encephalomyocarditis virus infection of cultured mouse pancreatic B cells. *Diabetologia* 24: 38–41
- Vialettes B, Baume D, Charpin C, Mayer-Guignard De J, Vaque Ph (1983) Assessment of viral and immune factors in EMC virus induced diabetes: effects of cyclosporin A and interferon. *J Clin Lab Immunol* 10: 35–40
- Rand KH, Rosenbloom AL, Maclaren NK, Silverstein JH, Riley WJ, Butterworth BE, Yoon JW, Rubenstein AH, Merigan TC (1981) Human leucocyte interferon treatment of two children with insulin-dependent diabetes. *Diabetologia* 21: 116–119
- Ehnholm C, Aho K, Huttunen JK, Koistiainen E, Mattila K, Pikkarainen J, Cantell K (1982) Effect of interferon on plasma lipoproteins and on the activity of post-heparin plasma lipases. *Arteriosclerosis* 2: 68–73
- Cantell K, Hirvonen K, Kauppinen H-L, Myllylä G (1981) Production of interferon in human leukocytes from normal donors with the use of Sendai virus. *Meth Enzymol* 78: 29–38
- Cantell K, Hirvonen S, Koistinen V (1981) Partial purification of human leukocyte interferon on a large scale. *Meth Enzymol* 78: 499–505
- Trivelli LA, Rauney HM, Lai H-T (1971) Hemoglobin components in patients with diabetes mellitus. *N Engl J Med* 284: 353–357
- Faber OK, Binder C (1977) C-peptide response to glucagon. A test for the residual β -cell function in diabetes mellitus. *Diabetes* 26: 605–610
- Dippe SE, Miller M, Bennett PH, Maynard JE, Berqvist KR (1975) Lack of association between Coxsackie B4 virus infection and diabetes. *Lancet* 1: 1314–1317
- Smedley H, Katrak M, Sikora K, Wheeler T (1983) Neurological effects of recombinant human interferon. *Br Med J* 286: 262–264
- Stewart WE (1979) Non-antiviral actions of interferons. In: Stewart WE (ed) *The interferon system*. Springer-Verlag, New York, pp 251–252
- Ennis FA, Meager A, Beare AS, Yi-Hua Q, Riley D, Schild GC, Rock AH (1981) Interferon induction and increased natural killer-cell activity in influenza infections in man. *Lancet* 2: 891–893
- Kaupila A, Cantell K, Jänne O (1982) Serum sex steroid and peptide hormone concentrations and endometrial estrogen and progesterin receptor levels during administration of human leukocyte interferon. *Int J Cancer* 29: 291–294
- Yki-Järvinen H, Koivisto VA (1984) Insulin resistance in newly diagnosed Type 1 diabetics: normalization with a 3 month insulin therapy. *J Clin Endocrinol Metab* (in press)

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