Disappearance rate of insulin antibodies after discontinuing insulin treatment in 42 Type 2 (non-insulin-dependent) diabetic patients

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Summary. The disappearance rate of insulin antibodies was studied after cessation of insulin treatment which had been given for 3 months to 6 years in 42 Type 2 (non-insulin-dependent) diabetic patients. Insulin antibodies were measured before and 15 days after interruption of insulin treatment, and every 30 days until the disappearance of insulin antibodies. The mean $\pm$ SD value of insulin binding in the entire group before the interruption of insulin treatment was $32 \pm 14\%$. There was no relationship between the antibody level at that time and the duration of insulin treatment. However, the insulin antibody level was significantly higher in 17 diabetic patients on an insulin dose of > 20 U/day ($p < 0.02$) than in 25 on an insulin dose of < 20 U/day ($39 \pm 13\%$ versus $28 \pm 12\%$). A

positive correlation was found between initial insulin binding and the time required for it to fall below 10% ($r = 0.74$). Antibodies were absent 60 days after discontinuing insulin treatment in eight of ten subjects presenting with initial binding of $< 20\%$. In contrast, in only two of 12 patients with an initial binding of $> 40\%$ were insulin antibodies detectable 150 days after discontinuation of insulin therapy. Disappearance of insulin antibodies sometimes took up to 1 year and occasionally even more than 2 years.

Key words: Insulin antibody, insulin treatment, insulin immunogenicity, immunoglobulins, Type 2 diabetes.

Although it is 25 years since Berson and Yalow [1] demonstrated the presence of insulin antibodies in the sera of patients treated with exogenous insulin, their clinical significance is still disputed. In addition to their well-recognized adverse effects, the presence of insulin antibodies has often been considered to have some beneficial effects, at least under certain circumstances [2, 3]. Regardless of the clinical significance of circulating insulin antibodies, assessment of their kinetics is of considerable interest [1, 4–8]. Fairly precise data are available concerning the dynamics of the appearance of antibodies in the circulation, but with regard to their disappearance our knowledge is limited. Here, we report a study of circulating insulin antibodies after discontinuation of insulin treatment in 42 Type 2 (non-insulin-dependent) diabetic patients.

Subjects and methods

Subjects

The study included 42 Type 2 diabetic patients (25 males and 17 females, aged 46–72 years) who had been treated with insulin for periods from 3 months to 6 years and gave informed consent for discontinuation of insulin therapy (Table 1). Of the 42 patients, 38 were on

conventional insulin preparations: 32 on long-acting insulin (28 on Novo-Lente and eight on Hoechst Long) and three on rapid-acting insulin (bovine crystalline insulin, Biofarm, Bucharest, Romania). A further four patients received highly purified insulins (two patients biphasic bovine/porcine Rapitard-MC, Novo, Copenhagen, Denmark; and two patients MC-Lente, Novo). The duration of insulin therapy was 3 months to 6 years; in 18 patients it was less than 1 year. After discontinuation of insulin therapy, treatment was by sulphonylureas in 24, biguanides in six and sulphonylureas + biguanides in 12. In three patients insulin treatment had to be re-introduced.

Methods

Insulin antibodies were assessed in the sera by a radioimmunological method as described previously in detail [9] using polyethylene-glycol (PEG) separation techniques [10]. Serum was separated within 6 h after sampling, and aliquots were stored at -20°C until assayed. The assay mixture contained 100 μl of serum, about 60 fmol of tracer (^{125}I -insulin with a sp. act. of approximately 10 000 MBq/mg, as specified by the producer, Central Nuclear Institute, East Berlin, DDR) and the total incubation volume was 500 μl , using as incubation medium phosphate buffer (0.04 mol/l, pH 7.4). After 24 h at 4°C , the bound radioactivity was separated using 20% PEG. The non-specific binding of $4.15 \pm 1.54\%$ (mean $\pm$ 2 SD) was subtracted from the binding of the unknown samples. Intra-batch precision and inter-batch reproducibility was estimated using pooled control sera. Measurements were carried out in duplicate and the results are expressed as percentage binding of insulin. The inter-batch coefficient of variation was 9%

Table 1. Course of insulin antibodies after discontinuation of insulin treatment in 42 Type 2 diabetic patients

Patient	Sex	Age (years)	Insulin treatment (U/day)	Duration of insulin treatment	Insulin antibodies (% bound)	Interval after discontinuation of insulin treatment (days)													
						Initial	15	30	60	90	120	150	180	210	240	270	300	330	360
1	F	47	32 Lente-Novo	6 years	69	66	46	29	–	16	12	9	7	3					
2	M	65	20 Lente-Novo	2 years	32	27	32	42	38	42	37	–	32	–	34	17	7		
3	F	70	20 Lente-Novo	1 year	50	21	28	21	17	12	7	5	6						
4	F	66	36 Lente-Novo	3 years	47	38	33	32	36	31	28	–	–		21	17	–	7 ^b	
5	F	46	28 Insuline Crystalline ^a	6 months	38	37	38	27	19	16	17	10	7	5	3				
6	M	49	16 Lente-Novo	2 years	14	16	10	5	–	4									
7	F	56	36 Lente-Novo	1 year	35	35	36	35	25	21	17	8	5						
8	F	53	16 Lente-Novo	6 months	32	33	28	24	18	12	–	9	6	4	3				
9	F	70	26 Insuline Crystalline ^a	3 months	15	11	7	5	3										
10	M	63	16 Lente-Novo	2 years	33	28	16	13	12	10	5	4	3						
11	F	56	20 Lente-Novo	1 year	19	16	14	12	7	–	4								
12	M	76	24 Insuline Crystalline ^a	9 months	30	32	25	18	11	6	–	5	5						
13	M	73	16 Lente-Novo	5 years	61	62	51	47	52	–	42	46	26	–	24	–	17	13 ^c	
14	M	52	12 Lente-Novo	10 months	47	46	46	40	30	28	27	20	18	17	13	–	7	5	
15	M	72	24 Long-Hoechst	2 years	31	28	15	15	10	7	5	6	4						
16	F	59	28 Lente-Novo	18 months	36	35	32	30	30	30	24	22	16	13	7	4	3		
17	F	69	24 Lente-Novo	8 months	46	40	37	32	19	13	7	5	4						
18	M	52	16 Long-Hoechst	9 months	36	35	28	17	13	10	7	5	4						
19	M	62	12 Lente-Novo	1 year	33	30	28	24	16	–	12	7	5						
20	F	54	24 Long-Hoechst	9 months	34	28	30	25	17	12	7	–	3	4					
21	M	71	20 Lente-Novo	1 year	28	31	26	–	20	–	10	–	5						
22	M	55	16 Long-Hoechst	7 months	16	12	10	8	3										
23	M	56	16 Long-Hoechst	14 months	38	33	28	24	–	17	8	5	3						
24	F	49	24 Lente-Novo	2 years	43	36	28	21	16	12	6	5	7	5	4				
25	F	67	16 Lente-Novo	16 months	18	13	8	6	4										
26	M	59	24 Lente-Novo	7 months	51	49	43	43	39	–	25	21	21	17	12	16	12	9 ^d	
27	M	52	16 Lente-Novo	15 months	37	32	28	29	24	22	17	18	13	8	7	7	5		
28	F	65	28 Lente-Novo	7 months	29	31	29	25	20	24	16	12	9	5	3				
29	M	57	20 Long-Hoechst	11 months	24	20	17	16	13	8	7	4							
30	F	62	32 Lente-Novo	3 years	42	39	36	29	25	31	24	16	–	8	3				
31	M	52	16 Rapitard MC-Novo	6 months	12	7	5												
32	M	71	24 Lente-Novo	28 months	17	20	21	16	7	8	7	5							
33	F	63	28 Lente-Novo	9 months	49	46	38	29	31	25	–	24	18	14	7	5	3		
34	M	54	16 Long-Hoechst	6 months	24	16	12	16	–	7	5	4							
35	M	62	20 Lente-Novo	12 months	33	29	23	18	12		7	5							
36	F	67	16 Lente MC-Novo	7 months	28	32	27	24	17	8	6	5		4					
37	M	57	12 Lente-Novo	13 months	12	9	5	3											
38	F	63	32 Lente-Novo	8 months	47	39	35	29	25	–	16	12	8	7	5	4			
39	M	66	16 Long-Hoechst	2 years	25	30	25	20	16	12	7	–	6	7	5				
40	M	58	12 Lente-Novo	5 months	13	9	6	4											
41	M	62	16 Rapitard MC-Novo	8 months	16	9	8	6	4										
42	M	72	20 Lente MC-Novo	4 months	21	27	16	13	9	6	5								

^a Biofarm, Bucharest, Romania; ^b 3% at 390 days; ^c 12% at 420 days; 10% at 480 days; 8% at 720 days and 3% at 830 days; ^d 7% at 390 days; 4% at 410 days

and the corresponding intra-batch coefficient was 4%. Specific binding (presence of insulin antibodies) was considered to be present for values of >10%. Blood samples were drawn before and after discontinuation of insulin treatment, at 15 days, and every 30 days until the disappearance of the antibodies.

Statistical analysis

Results are presented as mean $\pm$ SD and statistical analysis was performed using Student's t-test.

Results

The mean $\pm$ SD of insulin binding in the entire group before the interruption of insulin treatment was $32 \pm 14\%$. Individual data are given in Table 1. There was no

relationship between the duration of insulin treatment and the percentage of insulin binding. Initially, insulin binding was significantly greater in the 17 diabetic patients on an insulin dose of >20 U/day ($39 \pm 13\%$, $p < 0.02$) than in the 25 patients on <20 U/day ($28 \pm 12\%$). The mean insulin binding of the 38 patients under treatment with conventional insulin preparations was significantly greater than that in the four patients receiving highly purified insulin preparations (22 ± 7 versus $17 \pm 2\%$, $p < 0.01$); however, the insulin dose used in the latter group was only 17 ± 3 versus 22 ± 4 U/day in the former.

The disappearance rate of insulin binding after discontinuation of insulin therapy in the 42 patients studied is given in Table 1. In 15 patients, circulating anti-

Fig. 1. Correlation between the initial antibody level and the time required for their decrease to less than 10% ($r = 0.74$, $p < 0.01$). Patients 4, 13 and 26 have been omitted as insulin antibodies were present for more than 400 days after discontinuation of insulin treatment

bodies required more than 6 months to disappear. In the patients 4 and 6, insulin binding fell below 10% after 360 days and in patient 13 only after 720 days from discontinuation of insulin treatment.

A positive correlation was found between the initial level of insulin binding and the time required for the disappearance of insulin binding from the circulation ($r = 0.74$; Fig. 1). Thus, antibodies were absent 60 days after discontinuation of insulin in eight of 10 patients with initial binding of $< 20\%$. In contrast, only two out of 12 patients with initial binding of $> 40\%$ were free of antibodies by 150 days after discontinuation of insulin therapy. All three of the diabetic patients in whom antibodies were still detectable at 360 days had presented with high initial levels: patient 4: 47%, patient 26: 52% and patient 13: 61%. However, patient 3, with comparably high initial insulin binding, had lost the antibody by 150 days.

The insulin antibody disappearance curve took on one of the following patterns: slight but constant decrease ($n = 19$), rapid fall during the first 6 months, then considerably slower decrease rates ($n = 8$); slow decrease in the first 6 months, then more rapid disappearance of insulin binding ($n = 9$); progressive decrease of binding but sometimes with marked fluctuations of antibody levels ($n = 6$).

Discussion

Circulating insulin antibodies might substantially modify the pharmacokinetics and the bioavailability of exogenous insulin. Thus, the half-life of insulin may increase from approximately 6 min in the absence of circulating insulin antibodies to 15 min in their presence [11]. It is well known that large amounts of insulin, of the order of 20–100 U/l, may circulate in the extracellular fluid in the presence of insulin binding antibodies.

Vaughan et al. [3] have shown that in non-insulin-resistant diabetic patients, the insulin-binding capacity of serum (in general 10 U/l) is compatible with the maintenance of free insulin in the physiological range (5–80 mU/l). If insulin clearance occurs entirely via free insulin, antibodies in such titres will act as a simple binding protein without influencing insulin requirements in the long run [7].

The marked differences in the rate of decrease of the concentration of circulating antibodies may be explained by their heterogenous affinity for insulin. At high insulin antibody levels the large size of insulin-antibody complexes might impede their access to and clearance by the reticulo-endothelial system [7]. Furthermore, increased antibody affinity implies a slow dissociation of the insulin-antibody complex and therefore a lower clearance rate. In contrast, a low antibody affinity implies a rapid dissociation of the insulin-antibody complex, so that the antibodies will be more rapidly accessible to the catabolizing cells [7, 12–14].

Despite the overall relationship between the initial antibody level and the disappearance time (Fig. 1), individual disappearance times from comparable initial levels may vary considerably, exceeding 1 year in three of the 42 patients studied and 720 days in patient 13.

Little is known about the mechanism of insulin antibodies catabolism; but presumably it does not differ much from that of the IgG class of immunoglobulins to which these antibodies generally belong. It has been calculated that the catabolism rate of IgG is 32 mg/kg body weight per day when equal to the rate of synthesis under conditions of constant plasma antibody concentrations [15]. If the catabolic rate corresponds to the 22.5 day half-life assumed for IgG, and if antibody synthesis ceases with the withdrawal of insulin therapy, then insulin antibodies ought to disappear within less than 100 days. However, in only 24% of the 42 patients studied did the antibodies disappear within this interval, and then only when the initial level was low ($< 20\%$ binding). In most cases antibodies persisted for longer intervals, sometimes for more than one year, suggesting continued synthesis of these antibodies after discontinuation of insulin therapy, progressive decrease of their catabolism, or both.

Some authors have suggested that circulating insulin antibodies in low-to-moderate amounts may be beneficial, rather than harmful, at least for some types of insulin treatment strategies [3, 5, 16]. In this context, it is of note that persistence of insulin antibodies after discontinuation of insulin therapy did not appear to prevent the achievement of satisfactory control in the patients of this study.

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