Immunogenicity of Long-Term Intraperitoneal Insulin Administration With Implantable Programmable Pumps

Metabolic consequences

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OBJECTIVE — To assess immunogenicity of intraperitoneal insulin infusion via implanted pumps by two methods and to evaluate the possible influence of an increased antibody level on metabolic and clinical parameters.

RESEARCH DESIGN AND METHODS — We studied insulin antibody levels in 17 type I diabetic patients before and until 24 months after implantation of a programmable pump delivering insulin intraperitoneally. Antibody levels were determined by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). They were correlated with HbA_{1c}, insulin requirements, free insulin, and the incidence of hypoglycemia.

RESULTS — Insulin antibodies increased as soon as the 3rd month after implantation. This increase was sustained throughout the study period (month 0, 25.4 ± 16.2%; month 3, 41.2 ± 23.5%; month 12, 45.9 ± 26%; month 24, 48.7 ± 25%). The data was correlated with the two assay methods (RIA and ELISA). Postimplantation level was correlated with preimplantation level, which could indicate a predictive value of the latter. No correlation was observed with any metabolic parameters, particularly the number of hypoglycemic episodes.

CONCLUSIONS — Our results indicate that intraperitoneal insulin administration by implantable programmable pumps leads to an increase of insulin antibodies, which are probably high-affinity antibodies (recognized by both RIA and ELISA). This increase in insulin immunogenicity did not induce significant metabolic consequences, which is reassuring for the future of programmable insulin pumps.

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ANOVA, analysis of variance; DCCT, Diabetes Control and Complications Trial; ELISA, enzyme-linked immunosorbent assay; MI, multiple injections; PEG, polyethylene glycol; RIA, radioimmunoassay.

Several studies have demonstrated the feasibility of using implantable programmable insulin pumps (1–4). Investigations are currently under way to obtain regulatory approval for the use of these systems for intensive insulin therapy. In addition to the hardware itself (pump and catheter), an insulin formula suitable for this mode of delivery has been developed to provide the patient with 1–3 months of autonomy (5). Current pumps use semisynthetic Genapol-stabilized 21 PH insulin designed by Hoechst (Frankfurt, Germany). In most clinical trials, insulin has been administered by the peritoneal route. The main advantage of this route is that it restores the physiological insulin gradient between the portal vein and the peripheral circulation (6,7).

However, some data (8,9) suggest that this type of treatment induces a stronger immune response against insulin than does conventional treatment. The clinical consequences of insulin antibodies have been studied in diabetic patients using the subcutaneous route of insulin administration. The results are controversial. Some authors have reported lipoatrophy, poor diabetic control, insulin resistance, and changes in insulin kinetics, but others have failed to confirm these adverse effects (10,11). Some of these studies are old or are difficult to compare because they have been performed with insulin preparations of very different purities, methods of manufacture, and retarding agents. However, such discrepancies could also be due to antibody heterogeneity and/or differences between assay methods, e.g., radioimmunoassay (RIA) and solid-phase enzyme-linked immunosorbent assay (ELISA) detect antibody-binding properties differently.

To confirm the benefits of peritoneal infusion of insulin with an implantable pump, it is important to evaluate long-term immunogenicity of this type of treatment and to assess the possible metabolic consequences for type I diabetic patients.

RESEARCH DESIGN AND METHODS

— Seventeen C-peptide–negative type I diabetic patients were recruited for clinical testing of implantable programmable insulin pumps. The subjects included eight men and nine women with a mean age of 36 ± 7 years (mean ± SD) and mean duration of diabetes of 20.9 ± 7 years. During the month preceding implantation, all patients underwent intensive subcutaneous insulin therapy using a portable pump (n = 15) or multiple injections (n = 2). Human insulin (Actrapid U100, Novo Nordisk) was used in 4 cases, highly purified porcine insulin (Velosulin U100, Novo Nordisk) in 11 cases, and bovine insulin (Ultratardum U40, Organon) in 2 cases.

Implantation was performed in all patients between 1990 and 1992 after informed written consent was obtained. The pump was implanted subcutaneously in the abdomen, and a silicone-coated polyethylene catheter was inserted into the peritoneal cavity. The two pumps tested were the model 1000 Infusaid pump (n = 5) (Pfizer-Infusaid, Norwood, MA) and the 2001 MIP pump (n = 12) (Minimed, Sylmar, CA). Both pumps use 21 PH insulin (Hoechst), which is a pH-neutral semisynthetic human insulin containing 27.8 μg/ml zinc ions when used at a concentration of 100 U/ml (Infusaid pump) and 111 μg/ml zinc at 400 U/ml (Minimed pump), 2 mg/ml phenol, 16 mg/ml glycerol, and 50 mmol/l Tris. It is stabilized with 10 μg/ml polyethylene-polypropylene-glycol (Genapol, Hoechst). Mean duration of follow-up after implantation was 28.8 months (range: 12–43 months).

Blood samples were collected in the morning from fasting patients before implantation (month 0 [M0]) and 3 (M3), 12 (M12), and 24 (M24) months after implantation. Anti-insulin antibody levels were measured by both RIA and ELISA. A method derived from Palmer et al. (12) and Thivolet et al. (13) was used for the fluid-phase radioassay. The sera were first extracted by charcoal dextran to remove insulin. Then, 100 μl was incubated at 4°C for 24 h with a tracer dose (2 μU) of mono ^{125}I-labeled (Tyr A14) human insulin (Novo Nordisk) (specific activity, 250–400 μCi/μg). Antibody-bound radiolabeled insulin was precipitated with 15% cold polyethylene glycol (PEG) (molecular weight 6,000). After centrifugation (2,000g, 30 min, 4°C), the pellet was washed in 12.5% PEG and centrifuged again. The resulting pellets were counted, and the results were expressed as the percentage of total radioactivity that could be precipitated. Nonspecific binding was ~1.5%.

ELISA of insulin IgG (AI-IgG) was performed as previously described (14). Microplates (Nunc 4–39454, GIBCO, Paisley, U.K.) were coated with 1 μg/75 μl solution of insulin (bovine and human crystallized insulins were a gift from Novo Industri, Copenhagen, Denmark). Standard curves were performed as follows: 0, 10, 20, 40, 60, 80, 90, and 100% of sample containing AI-IgG diluted in antibody-free serum. Each curve sample was diluted with phosphate-buffered saline ($NaH_2PO_4 \cdot 2H_2O$, 0.52 g/l; $K_2HPO_4 \cdot 3H_2O$, 3.67 g/l; NaCl, 8.76 g/l; bovine serum albumin, 2 g/l; and Tween 20, 0.5 ml/l: pH = 7.2) to obtain an optical density between 1.5 and 2.0 U as 100%. Fifty-microliter samples were used; as a blank, instead of serum, buffer was added to one well per row. After a 2-h incubation at 37°C and three successive washes with the buffer, a mouse monoclonal anti-human IgG was added (MH 001, Gamma S.A., Sart Tilman, Liège, Belgium). After a second 2-h incubation at 37°C and three more washes, a rabbit anti-mouse Ig serum conjugated to peroxidase (P260, Dakopatts, Glostrop, Denmark) was allowed to react for 1 h at 37°C. After four washes with tap water, 2.2′-azino-di-(3-ethylbenzthiazolinsulfonate-6) (Boehringer Mannheim, Germany) was added, and the oxidized chromogen was measured by reading absorbance at 405 nm. The results were expressed as the reciprocal dilution.

HbA_{1c} was determined by high-performance liquid chromatography and expressed as a percentage of total hemoglobin (15). Free insulin (μU/ml) was measured after precipitation by PEG and immediate centrifugation (16).

Daily insulin requirements were recorded (U/24 h). The incidence of hypoglycemia was determined by home blood glucose monitoring. The hypoglycemia rate was defined as the number of capillary glycemia determinations per month <60 mg/dl on the basis of four determinations performed every day. Severe hypoglycemic episodes, defined according to the Diabetes Control and Complications Trial (DCCT) (17), were also recorded.

Statistical analysis

Statistical analysis of the evolution of antibodies and metabolic parameters was performed using the analysis of variance (ANOVA) model for repeated measures. Because ANOVA revealed a significant difference, Scheffé's and Fisher's tests were also applied to determine the specific levels at which the differences occurred. Comparisons between groups were made by Wilcoxon's rank-sum test. Correlation coefficients between various parameters were assessed by regression analysis (after logarithmic transformation of antibody-level data).

RESULTS

Anti-insulin antibody levels measured by RIA

According to RIA (Table 1), the mean anti-insulin antibody level at M0 was 25.4 ± 16.2% (mean ± SD). By the 3rd month after implantation, the level had already risen significantly to 41.2 ± 23.5% (P < 0.001), and this increase was sustained throughout the study (M12: 45.9 ± 26%, M24: 48.7 ± 25%). These findings were independent of type of pump, insulin concentration, and sex of the patient. A close correlation was noted between anti-insulin antibody levels before and after implantation (M0 vs. M3, r = 0.78; M0 vs. M12, r = 0.79; P < 0.01).

Table 1—*Clinical characteristics of subjects and evolution of insulin antibody levels measured by RIA*

Subject	Treatment before implantation: Mode	Treatment before implantation: Type of insulin	Insulin antibody level (%): M0	M3	M12	M24
Infusaid U100						
1	MI	1	28.3	80.4	70.0	64.3
2	CSII	2	26.8	27	25.8	36.6
3	CSII	3	38.0	71.8	67.2	61.0
4	CSII	2	37.8	41.4	55.5	57.0
5	CSII	3	9.0	41.2	30.3	53.9
Mean ± SD			27.9 ± 11.8	52.3 ± 22.6*	49.7 ± 20.6*	54.5 ± 10.7*
Minimed U400						
6	CSII	2	33.2	57.6	65.2	59.0
7	CSII	2	19.3	61.5	76.8	62.7
8	CSII	2	43.1	60.3	53.1	70.7
9	CSII	2	17.7	43.1	48.1	43.1
10	CSII	2	16.2	34.7	37.0	48.0
11	CSII	2	6.4	8.8	3.0	6.0
12	CSII	2	1.5	4.3	2.1	2.5
13	CSII	3	47.2	63.2	73.9	81.2
14	CSII	1	44.1	54.6	60.0	87.2
15	MI	2	49.4	22.1	53.2	44.2
16	CSII	3	1.8	4.4	4.3	3.1
17	CSII	2	12.9	24.4	35.5	48.2
Mean ± SD			24.5 ± 18	36.5 ± 23.2*	44.3 ± 28.6*	46.3 ± 29.1*
Whole group mean ± SD			25.4 ± 16.2	41.2 ± 23.5*	45.9 ± 26*	48.7 ± 25*

Type of insulin: 1, highly purified porcine + bovine insulin (U40); 2, highly purified porcine insulin (U100); 3, hemisynthetic human insulin (U100). CSII, continuous subcutaneous insulin infusion. MI, multiple injections. * Significant difference at 95% vs. M0.

Anti-insulin antibody levels measured by ELISA

According to ELISA (Table 2), the mean anti-insulin antibody level at M0 was 124.1 ± 9.4 (mean ± SD). After implantation, this level increased significantly and continuously (M3: 293.7 ± 289.6, M12: 403 ± 584.7, $P < 0.01$). There was a strong correlation between antibody levels before and after implantation (M0 vs. M3, $r = 0.94$; M0 vs. M12, $r = 0.90$; $P < 0.001$).

Statistical analysis showed a close correlation between data obtained by RIA and ELISA at all times studied (M0, $r = 0.65$; M3, $r = 0.82$; M12, $r = 0.82$; $P < 0.01$).

Metabolic and clinical parameters

Changes in metabolic and clinical parameters are shown in Table 3. They were analyzed with insulin antibody levels measured by RIA. The same results were observed when antibody levels were measured by ELISA (data not shown).

Diabetes control. HbA_{1c} levels improved after implantation. The difference between M0 and M12 was significant. There was no correlation between antibody levels and HbA_{1c} at any time (M0, $r = 0.08$; M3, $r = 0.08$; M12, $r = 0.17$).

Insulin requirements. Daily insulin requirements increased moderately. The difference between M0 and M12 was significant. However, no correlation was observed between antibody levels and insulin requirements, whether analyzed by plotting all the data obtained throughout the study ($r = 0.1$) or at each time of intraperitoneal delivery only (for example, at M12, $r = 0.1$).

Fasting free insulin. Fasting free insulin exhibited a moderate but nonsignificant decrease. There was no significant correlation between anti-insulin antibody lev-

Table 2—*Evolution of insulin antibody levels measured by ELISA*

	Insulin antibody levels: M0	M3	M12
Whole group	124 ± 91	293 ± 289	403 ± 584*

Data are means ± SD (reciprocal dilution for OD = 0.3. * Significant difference at 95% vs. M0.

Table 3—*Evolution of clinical and metabolic parameters after implantation*

	M0	M3	M12	M24
HbA_{1c} (%)	7.1 ± 0.8	6.4 ± 0.7*	6.6 ± 0.8*	7 ± 1.1
Plasma free insulin (μU/ml)	17.8 ± 10.5	15.8 ± 6.2	13.3 ± 6.3	12.9 ± 6.9
Insulin requirement (U/day)	38.3 ± 10.1	40.9 ± 8.6	42.1 ± 9.5*	43.7 ± 10.8*
Hypoglycemia (episodes/month)	11 ± 8.5	14.5 ± 10.1	15.1 ± 11.4	15.6 ± 12.1

Data are means ± SD. * Significant difference at 95% vs. M0.

els and fasting free insulin, either for the whole study ($r = 0.2$) or for any month of intraperitoneal treatment (for example, at M12, $r = 0.2$).

Hypoglycemia rate. The number of hypoglycemic episodes per month did not change significantly after implantation. There was no correlation between anti-insulin antibody levels and hypoglycemia rate at any time during follow-up ($r = 0.2$). No severe hypoglycemia was observed, but one patient had to decrease her nocturnal basal rate of insulin to 20% to avoid hypoglycemia.

CONCLUSIONS — Our present study demonstrates that the use of the two implantable programmable pumps tested to deliver insulin into the peritoneal cavity results in an increase in the immunogenicity of insulin. This finding is in agreement with other data obtained by RIA (8,9) and previous results using a similar type of insulin (18). Compared with these studies, our results show some differences: preimplantation levels are higher and the relative increase is lower. These differences could be explained by the fact that most of our patients were treated before implantation by external pumps using a highly purified porcine insulin, and species variations could account for a relatively higher basal level of insulin antibody. However, it is also possible that this could be due to the intensive management of our patients before implantation, because our results are strictly comparable to those obtained by Dahl-Jorgensen et al. (19) in type I diabetic patients treated by continuous subcutaneous insulin infusion or multiple injections.

The rise in antibody levels was continuous. A clear-cut increase was obvious as early as the 3rd month after implantation and persisted at 12 and 24 months. Because most of the patients had been treated previously with highly purified porcine insulin, it was reasonable to expect that switching to semisynthetic human insulin would have lowered antibody levels (20). The increase in anti-insulin antibodies was independent of sex, pump model, or insulin concentration. However, antibody level before pump implantation was predictive of the postimplantation level, suggesting an individual susceptibility.

Several putative causes can be proposed to explain this increase in anti-insulin antibodies. The first is the formula of 21 PH insulin, which contains glycerol and Genapol as stabilizers. It has been shown that the immune reaction after subcutaneous administration is stronger if the insulin used contains protamine, zinc, or other contaminants. Another factor that could contribute to the stronger immune reaction when an implantable programmable pump is used is the peritoneal route itself. The peritoneum is a macrophage-rich region and has been used to stimulate antibody production in animals. A third possibility is that the pump-catheter system could induce formation of more immunogenic aggregates. There is no current evidence to confirm or rule out any of these possibilities.

In all cases, RIA and ELISA data were correlated. The fact that both assay techniques documented enhancement of immune response suggests that insulin treatment with an implantable programmable pump stimulates binding capacity without changing antibody affinity (11).

The consequences of high anti-insulin levels in type I diabetic patients are controversial. Several authors have speculated that high antibody levels change insulin kinetics by delaying the insulin peak after a subcutaneous bolus of fast insulin (20,21), increasing the half-life of insulin and postponing the return to baseline insulin level (21,22). Although they have not been confirmed by all authors (23,24), these effects could have two metabolic consequences: postprandial hyperglycemia, which could lead to a gradual deterioration in metabolic control, and/or late hypoglycemia (21). In our study, the increase in anti-insulin antibody levels was not associated with a deterioration of glycemic control. In fact, an improvement in mean HbA_{1c} was observed. In contrast with the preliminary study by Boivin et al. (9), our data showed no individual correlation between antibody levels and HbA_{1c}. No evidence that the presence of antibodies affects long-term glycemic control has been published.

It has often been suggested that untimely release of insulin by antibodies could result in hypoglycemia. An autoimmune hypoglycemia syndrome caused by insulin antibodies has been described in nondiabetic patients (25). However, in type I patients, only brief and mostly old studies have associated hypoglycemia with antibody levels (26–28). In their study, Charles and colleagues (8) reported that 16% of patients treated with implanted pumps had high antibody levels associated with nighttime or early morning hypoglycemia. However, some of these patients had high antibody levels without hypoglycemia. In our study, a decrease of nighttime insulin requirement was observed in 1 (6%) of the 17 patients; this subject had a high level of antibodies. The decrease in nighttime insulin requirement was transient, although antibody level remained high. Therefore, we cannot conclude that antibodies play a direct role. Bolli et al. (29) have shown that

the recovery from hypoglycemia is delayed in diabetic patients with high free insulin levels and elevated anti-insulin antibody levels. In our study, we did not observe a significant increase in the incidence of hypoglycemia (despite better metabolic control, which according to the DCCT [17] increases the risk of severe hypoglycemia). Furthermore, no individual correlation was seen between insulin antibody levels (determined by either assay technique) and either free insulin or the incidence of hypoglycemia. This finding is reassuring for the future of insulin treatment using implantable pumps for the majority of patients, even if we cannot exclude, for rare patients, a link between very high insulin antibody titers and nocturnal hypoglycemia, as has been described by Charles and colleagues (8). One explanation for the absence of a hypoglycemic effect in our patients would be that the antibodies induced by insulin pump treatment form a relatively homogeneous population of medium or high affinity. This hypothesis is supported by the correlation between the antibody levels observed with the two assay techniques. It should be remembered that RIA recognizes low-affinity antibodies poorly. If low-affinity antibodies had developed, the correlation between the two assay techniques would not have been as good. High-affinity antibodies do not release insulin easily and, thus, should not be associated with a hypoglycemia syndrome.

We also observed a moderate but significant increase of 8% in the daily insulin dose required after implantation. This finding, which was not mentioned in previous studies (1,2), could be related to elevated levels of high-affinity antibodies, as described in diabetic patients who undergo conventional treatment (30). However, we found no individual correlation between insulin requirements and antibody levels. It is more likely that technical problems associated with long-term use of an implanted pump account for the increase in insulin requirement. Indeed, after 1 year, insulin deposits forming in the reservoir and/or in the catheter led to a discrepancy between the amount indicated by the device and the dose actually delivered, and consequently, a readjustment of the daily insulin dose was required to obtain the same degree of glycemic control (3).

In conclusion, treatment of diabetic patients using an implantable programmable pump to deliver 21 PH insulin into the peritoneal cavity leads to a greater immunogenicity of insulin than does conventional therapy. Additional studies are necessary to explain this finding. However, it should be emphasized that this increase had no effect on long-term glycemic control or on the incidence of hypoglycemia for the majority of patients.

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