Comparison of Antigenicity of Hoechst 21PH Insulin Using Either Implantable Intraperitoneal Pump or Subcutaneous External Pump Infusion in Type 1 Diabetic Patients

Nathalie Jeandidier, MD, PhD
Sylvie Boullu, MD
Marie-Sophie Busch-Brafin
Gerard Chabrier, MD
Remy Sapin
Francoise Gasser
Michel Pinget, MD, PhD

OBJECTIVE — To assess the antigenicity of the insulin Hoechst 21PH (Hoe21PH) using continuous subcutaneous insulin infusion (CSII) and to compare the antigenicity of this insulin when administered intraperitoneally or subcutaneously.

RESEARCH DESIGN AND METHODS — Peritoneal administration of Hoe21PH (Hoechst-Roussel, Somerville, NJ) insulin using implantable devices (continuous peritoneal insulin infusion [CPII]) increases anti-insulin antibody (AIA) levels in type 1 diabetic patients. Intraperitoneal administration, addition of a stabilizer (polyethylene polypropylene glycol), or insulin modifications due to storage in the pump may be involved in this antigenicity. In this nonrandomized study, 24 type 1 diabetic patients were treated with either CSII (n = 11, group 1) or CPII (n = 13, group 2). AIA levels were measured by radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA) before starting patients on Hoe21PH and again after 3 and 6 months.

RESULTS — Patients were comparable in the two groups. AIA levels (RIA) remained stable (24.3 ± 8.5% [month 0] to 24.9 ± 8.5% [month 6]) in group 1 and increased (21.8 ± 6.7% [month 0] to 41.8 ± 6.9% [month 6]) in group 2 (P = 0.005, Wilcoxon’s rank-sum test). Using ELISA, AIA remained stable in the patients in group 1 (n = 9; 3.8 ± 0.8 units/ml [month 0] and 4.1 ± 1.0 units/ml [month 6]) and tended to increase in the patients in group 2 (n = 12; 4.1 ± 0.7 units/ml [month 0] to 17.5 ± 6.0 units/ml [month 6]) (P = 0.07). Comparison of the evolution of AIA formation between the two groups, using RIA at months 0, 3, and 6 showed a significant difference (analysis of variance, P = 0.009).

CONCLUSIONS — No increase in AIA levels was demonstrated when Hoe21PH insulin was administered subcutaneously as assessed by two different assays. CPII is proven to be more antigenic than CSII, and this is not related to a specific antigenicity of Hoe21PH insulin. The intraperitoneal route of administration or insulin modifications due to insulin storage in implantable devices might explain this antigenicity.

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Intraperitoneal (IP) insulin absorption has been shown to be rapid and reproducible (1–3). The rationale for this mode of administration is essentially based on the fact that 99% of the insulin administered intraperitoneally is absorbed via the portal route and reaches the liver directly, improving hepatic protein synthesis and the lipid profile (4).

Long-term continuous peritoneal insulin infusion (CPII) requires the use of implantable devices to avoid risk of infection (5). Clinical trials have shown the feasibility of such a therapy as well as its efficacy in allowing good glycemic control while reducing severe hypoglycemia (6).

IP insulin therapy via implantable devices has been shown to dramatically increase (P < 0.001) anti-insulin antibody (AIA) levels in ~40% of type 1 diabetic patients during long-term follow-up (>3 years) (7–9). In these patients, significantly higher occurrence of postprandial hyperglycemia was observed (P < 0.05). Some rare cases of clinically relevant insulin resistance and significant glycemic decreases during nighttime were reported (7,8). Long-term complications linked to high levels of AIA remain to be determined.

Low antigenicity had been expected in these clinical trials, because the insulin used was Hoechst 21PH (Hoe21PH; Hoechst-Roussel, Somerville, NJ), humanized, neutral, and regular (10,11). It is important to assess whether this antigenicity is also observed in continuous subcutaneous insulin infusion (CSII), because this insulin is commonly used in external insulin pumps. The causes of this high antigenicity during CPII must be assessed. Different factors may be involved; polyethylene/polypropylene glycol, the surfactant stabilizing Hoe21PH, may act as a carrier and boost antibody production.

From the 1Department of Endocrinology and Diabetes, University Hospital, Strasbourg, France, and the 2Institute of Physics and Biology, University Hospital, Strasbourg, France.

Address correspondence and reprint requests to Nathalie Jeandidier, Department of Endocrinology and Diabetes, Hôpital Civil, 1 Place de l’Hôtel, Strasbourg Cedex 67091, France. E-mail: nathalie.jeandidier@chru-strasbourg.fr.

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Abbreviations: AIA, anti-insulin antibody; ANOVA, analysis of variance; CSII, continuous subcutaneous insulin infusion; CPII, continuous peritoneal insulin infusion; ELISA, enzyme-linked immunosorbent assay; IP, intraperitoneal; RIA, radioimmunoassay.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.
Peritoneal administration The presence of macrophages may facilitate this antigenic reaction with peritoneal administration (12), because the peritoneum is a macrophage-rich region, which contains particular B-cells that are very sensitive and more reactive to immune stimulation than other lymphocytes in other tissues. This explains that, in mice, higher quantities of AIA are produced in the serum after an IP insulin injection than after a subcutaneous injection (13). In addition, no important production of insulin antibodies was reported during continuous intravenous infusion of insulin in a small group of type 1 diabetic patients (14) equipped with implantable pumps.

Insulin modifications or degradation products The increased AIA formation may also be caused by insulin modification or degradation products occurring specifically during storage in the implantable pump reservoir, as reported during clinical trials (15,16).

Insulin stability has always been one of the main issues concerning the use of an implantable pump (17). With these devices, all conditions are gathered to favor the aggregation of insulin molecules: thermal exposure, long-term contact with metallic and synthetic surfaces, mechanical stress in the pump itself, and stirring, all of which are known to induce fibrillation of insulin in neutral solution. In 1989, with the finalization of the Hoe21PH insulin, the problem seemed to be solved and large clinical trials began. In 1992, uncontrolled decreases in pump insulin rates were reported, and the frequency of this incident increased over time. Insulin aggregates were observed in the system pulling the insulin out of the Minimed MIP 2001 reservoir (Minimed Technologies, Sylmar, CA) as well as in insulin sampled from the devices during clinical trials. Further inquiries demonstrated that the decrease in insulin stability was due to minor changes in insulin preparation performed to fulfill new requirements of European Pharmacopoeia (18,19).

Because high antigenicity of the Hoe21PH insulin was shown during CPII, this study aimed to assess the antigenicity of Hoe21PH insulin during CSII and to compare the evolution of AIA levels during infusion of Hoe21PH insulin using CPII or CSII in two groups of comparable type 1 diabetic patients. The effect of polyethylene polypropylene glycol and the influence of the peritoneal route of administration could then be evaluated.

RESEARCH DESIGN AND METHODS

Patients In a nonrandomized study, 11 patients were enrolled in the CSII group (group 1) and 13 patients were enrolled in the CPII group (group 2). In one patient in group 2, “low morning syndrome” developed, with high AIA levels after 3 months of therapy. Intravenous boluses of steroids were administered to correct this nocturnal hypoglycemia, and thus, this patient was excluded from the study. The remaining 24 patients all had type 1, C-peptide–negative diabetes with a disease duration of >1 year; none had received any animal insulin during the last 3 years. No patients had ever received Hoe21PH insulin before entering the study, and none were given any medications that would possibly modify antibody generation either at enrollment or during the course of the study.

In all patients in the study, previous insulin treatment comprised either daily multi-injections of regular and intermediate insulin or CSII. The types of insulin used were regular or lente human insulin and/or Humalog (Eli Lilly, Indianapolis, IN). The main characteristics of the patients were comparable between the two groups. Mean age was $3.1 \pm 3.4$ and 36.8 $\pm 1.7$ years, and duration of diabetes was 24.4 $\pm 3.8$ and 19.2 $\pm 2.3$ years in the patients in groups 1 and 2, respectively ($P > 0.05$, Mann-Whitney $U$ test). There were six men in group 1 and six men in group 2 ($P > 0.05$, $X^2$ test).

Insulin Hoe21PH, a regular, neutral, semisynthetic polyethylene polypropylene glycol–stabilized insulin, was used in all implantable pumps trials (19) at a concentration of either 100 or 400 units/ml, depending on the type of implantable device: Insufasad Model 1000 (Shiley Insufasad, Norwood, MA), Siemens Promedos ID3 (Siemens Elema, Solna, Sweden), or Minimed MIP 2001 (Minimed Technologies, Sylmar, CA). Polyethylene polypropylene glycol, at a concentration of 10 $\mu$g/ml, acts as a surfactant avoiding the deformation of insulin molecules, which may lead to insulin degradation (loss of biological activity and formation of fibrillar aggregates) (17).

In this study, insulin concentration was 400 units/ml for the implantable devices (Minimed MIP 2001) and 100 units/ml (Insuman Infusat; Aventis Pharma, Frankfurt, Germany) for external H-tron infusion devices (Disetronic Medical Systems, Sulzbach, Switzerland). The characteristics of implantable and external devices have been described in several articles (4). Implantable devices used in this study have a negative pressure in the reservoir; therefore, the insulin must be degassed before refilling to avoid formation of air bubbles. Refilling is performed every 6 weeks after the remaining insulin has been removed. Insulin is stored in the pump reservoir at body temperature and subject to agitation during 6 weeks.

In the external pump, a cartridge containing 3 ml insulin is changed every week, on average, and the catheter is changed every 3 days. During infusion, insulin is stored at room temperature because the pump is carried most of time in a pouch secured around the patient’s waist.

AIA assays In all patients, sera was tested for human insulin antibodies using a radioimmunoassay (RIA) kit from Biorad, Marnes la Coquette, France. Sera containing AIA were incubated for 24 h with human A14 $^{125}$I-labeled insulin. The $^{125}$I-insulin bound to the AIA fraction was separated from the free $^{125}$I-insulin by precipitation with polyethylene glycol. After centrifugation, radioactivity counting of the pellets (bound fraction) allowed calculation of the binding percentage of labeled insulin. The binding percentage of a negative control serum was subtracted from each result, and 2.5% was fixed as the positive threshold for humans.

A total of 9 patients in group 1 and 12 patients in group 2 could be tested with a new enzyme-linked immunosorbent assay (ELISA) anti-insulin antibody (Synelisa insulin antibodies; Pharmacia & Upjohn, Friburg, Germany). For technical reasons, only 8 patients in group 1 and 10 patients in group 2 could be tested at months 0, 3, and 6. Biotin-labeled insulin antigen binds to insulin antibodies in standards (0–100 units/ml), control subjects, and patient samples in a fluid phase. The suggested normal range is <12 units/ml.
Insulin antibody levels were assessed at the beginning of the insulin Hoe21PH administration (month 0), and then after 3 and 6 months during routine clinical blood collection.

Statistical analysis
Statistical analysis was performed using the BMDP statistical software (Los Angeles, CA). All data are presented as the mean ± SEM unless otherwise stated. All AIA data were transformed to logarithms (after the addition of 1 to each number) for each antibody level to normalize the distribution of values before statistical evaluations. Log-transformed AIA levels were compared at months 0 and 6 in each group using a Wilcoxon’s rank-sum test because of the small number of values (<30 patients in each group). The evolution of AIA formation between groups 1 and 2 was performed using an analysis of variance (ANOVA). Statistical significance was defined for P values <0.05.

RESULTS— The evolution of mean AIA levels from month 0–6 was assessed for each group. RIA showed that AIA levels remained stable in patients in group 1 but increased significantly (P = 0.005) in patients in group 2. ELISA revealed that AIA levels also remained stable in patients in group 1 (n = 9) but tended to increase (P = 0.07) in patients in group 2 (n = 12).

When comparing the evolution of AIA formation during the course of the study between the two groups, AIA levels measured by RIA were statistically different between the two groups (ANOVA, P = 0.009). In group 2, a higher concentration of AIA caused by continuous increased formation during the course of the study was observed (Fig. 1). When comparing the evolution of AIA levels measured with ELISA between the two groups, the evolution was not statistically different (ANOVA, P = 0.24) (Fig. 2).

CONCLUSIONS— As reported previously (7–9), during IP infusion of Hoe21PH insulin using implantable devices, mean AIA levels increased significantly when assessed using RIA and increased when assessed using ELISA. This confirmed the first observations reported regarding the high antigenicity of this mode of infusion in type 1 diabetic patients, although this antigenicity was not observed in type 2 diabetic patients treated with CPII (20). One patient in group 2 had “low morning syndrome” after 3 months of CPII, thus confirming the particular clinical effect of this antigenicity in some patients.

The evolution of mean AIA levels was very different in a comparable group of type 1 diabetic patients when the subcutaneous route was used, because mean AIA levels remained stable according to two different assays (RIA and ELISA). Therefore, our data ruled out a possible specific antigenicity of the Hoe21PH related to the presence of polyethylene polypropylene glycol. Based on the results observed with IP, some authors (21) suggested a deleterious effect of this insulin and pleaded for the withdrawal of Insumin in CSII, but our data did not support this hypothesis.

One could consider 6 months to be too brief to exclude a late increase of AIA levels in group 1. The choice of 6 months was based on the data of previous IP studies reporting a significant increase of AIA levels after 3–6 months (7–9). Nevertheless, we assessed the AIA levels of the patients in our study 6 months after the end of the study (month 12). In group 1, six patients remained on CSII and Hoe21PH; their AIA levels remained stable compared with baseline: 28.4 ± 10.3% (RIA) and 3.9 ± 0.6 units/ml (ELISA). In patients in group 2 (n = 13), AIA levels remained elevated compared with month 0: 39.6 ± 8.3% (RIA, P = 0.03) and 14.8 ± 5.2 units/ml (ELISA, P = 0.01). These results confirm the 6-month data, and statistical significance obtained after 12 months with ELISA could be explained by a slower increase of the AIA levels measured by this assay (9).
Measurement of AIA using two different assays showed the same tendency. The lack of statistical significance of the increase of AIA levels measured with ELISA in group 2 may be explained in part by heterogeneous variables with a high SEM (two patients respond with AIA levels >50 units/ml at 6 months) or/and by the small number of values. Such heterogeneous responses have been reported by other authors (9). As seen before, the SEM was lower at month 12 and explained the significance observed. Another explanation is that RIA and ELISA do not recognize the same antibodies among the polyclonal population of AIA. RIA recognizes mainly high-affinity, rapidly binding IgG or IgM antibodies, whereas ELISA tends to recognize mostly low- to medium-affinity IgG antibodies (22). The patient in whom “low morning syndrome” developed during the course of the study had very high levels of AIA measured by RIA at month 0 without any symptoms; the nocturnal hypoglycemia occurred after 3 months, while AIA levels measured by ELISA had increased from 10 to 48 units/ml. AIA levels with a low affinity measured by ELISA could be better correlated with “low morning syndrome,” based on a nocturnal lower insulin concentration, allowing separation of insulin from the antibodies. This must be verified because, until now, no ELISA AIA measurements have been performed during the symptoms of “low morning syndrome” (7,8).

In the literature, the difference in insulin concentration, 100 vs. 400 units/ml, was not involved in the different degree of antigenicity; it has been shown that both insulin concentrations induced a similar antigenic response during CPII (7–9).

Our study was not randomized, which is one of its main limitations. Nevertheless, the main characteristics of the patients were comparable, especially concerning the factors that may be involved in AIA formation. Classical factors known to be related to high AIA formation (23,24), such as advanced age, long duration of diabetes, and high baseline AIA levels (10) were found in group 1 patients and would explain a high AIA response in group 1. Initial levels of AIA (RIA) were comparable between the two groups of the study and to the levels of AIA observed in a group of type 1 diabetic patients treated with subcutaneous insulin (patients seen in our clinic). We compared our study data with the AIA levels (RIA, 26.2 ± 4.3%) of the first 35 type 1 diabetic patients who routinely attended our clinic in 2001 (ANOVA between the three groups, P = 0.51).

The significant difference in the evolution of AIA between the two groups confirmed that IP proved more antigenic than subcutaneous infusion. Either the route of administration or some insulin modifications occurring in the pump reservoir during the 6 weeks of storage could be responsible for the increased antigenicity observed. Studies in rats have shown that IP insulin injections of Hoe21PH sampled directly from the vial were significantly less antigenic than the insulin sampled from the pump reservoir after 6 weeks of storage during a clinical trial (25). This would favor the hypothesis of insulin degradation in the antigenicity observed, although such extrapolation from animals to humans should be regarded with caution.

The mean insulin storage in the pump cartridge was ~5 days, and insulin was exposed to ambient temperature rather than body temperature in external pumps. These conditions might explain the lower antigenicity observed because of a lower degradation of insulin.

Fibrillar aggregates have been found in the reservoir of an implantable pump and in the ejection system; this accumulation caused the pump rate slow-down reported in clinical studies (16). Fibrillar insulin aggregates proved to be antigenic because of the formation of new epitopes (26), which form a poorly soluble polymer, likely to be engulfed by macrophages, that is a potent stimulus to antigenicity (27). A chemically or physically degraded protein such as fibrillar insulin aggregate might trigger an important immune response with increased IgG antibody concentration, and the AIA observed in the clinical trials were IgG (7). In immunization experiments in rabbits, partially fibrillized insulin samples proved to be capable of increasing the formation of IgG antibodies in comparison with a reference sample of native insulin and the concentration of aggregates was correlated to the quantity of AIA (28).

Other insulin factors related to modifications in the implantable pump reservoir could also be involved in the high antigenicity observed. In particular, silicone oil was found in the insulin sampled from pump reservoirs in concentrations ranging from 0.3 to 1.4 µg/ml (mean 0.65 µg/ml; n = 89 pumps) (B. Van Antwerp, unpublished data). Silicone oil is commonly used to lubricate the syringes used to refill the pump reservoir and probably accumulates. Silicone has been reported to be a classical immunologic adjuvant when mixed with a foreign protein (29).

Considering these data, the modifications due to the insulin storage in implantable devices reservoir could be responsible for the increased antigenicity, and the contamination by silicone oil more likely could play the role of an antigen adjuvant.

This study showed that during CSII treatment using the Hoe21PH insulin in type 1 diabetic patients, AIA remained stable during 6 months, thus ruling out a specific antigenicity of the polyethylene glycol-stabilized insulin and a deleterious clinical effect of this mode of therapy. Using IP insulin infusion via implantable devices, the significant increase in AIA levels was confirmed, pointing out the possible role of either the route of administration or the deleterious effects of insulin storage in reservoirs of implantable devices, such as fibrillar aggregates, and/or silicone oil contamination. Aggregates would be good candidates, because they are known to increase insulin antigenicity and have been found in significant amount in the implantable pump reservoirs. Additional animal studies using calibrated aggregates concentrations and/or different concentrations of silicone oil in the same insulin are needed.

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