Continuous intraperitoneal insulin infusion does not increase the risk of organ-specific autoimmune disease in type 1 diabetic patients: results of a multicentric, comparative study

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Objectives: The purpose of the national multicenter prospective study by the French EVADIAC group was to investigate the possibility that continuous intraperitoneal insulin infusion using an implanted pump (CIPII) increases the risk of autoimmune disease in type 1 diabetic patients as it increased anti-insulin immunogenicity.

Methods: Prevalence of clinical (Hashimoto’s disease, hyperthyroidism, gastric atrophic disease and vitiligo) and subclinical (presence of anti-thyroperoxidase antibodies, anti-intrinsic factor antibodies, abnormal TSH levels) autoimmune diseases was estimated by comparing two groups of patients already treated by either CIPII (n=154) or external pump (CSII) (n=121) for an average of 6 years. Incidence of autoimmune disease was determined by comparing the same measurements one year after inclusion.

Results: No significant difference was observed for the total prevalence of clinical and subclinical auto-immune thyroid and gastric diseases (35.6% and 3.2% respectively in the CIPII group versus 40.4% and 2.6% in the CSII group). No significant difference for the incidence of clinical and subclinical auto-immune diseases was observed: 7.2% and 0% in CIPII and 7.3% and 1.7% in CSII.

Conclusion: As previously shown AIA (anti-insulin antibodies) levels were higher in CIPII than in CSII (32.9% vs 20.2%, P<0.0001) but no correlation was observed with either clinical or subclinical autoimmune disease. This large-scale study eliminates the possibility that CIPII increases the risk of autoimmune disease.

Key-words: Type 1 diabetes · Implanted pump · Thyroid autoimmunity · Gastric autoimmunity · Anti-insulin antibodies.

RÉSUMÉ
Le traitement par pompe implantée avec perfusion intra-péritonéale d’insuline n’augmente pas le risque de maladie auto-immune d’organe chez les diabétiques de type 1 : résultats d’une étude multicentrique comparative

Objectifs: Le but de cette étude française multicentrique conduite par le groupe EVADIAC était d’évaluer si le traitement par pompe à insuline implantée augmentait le risque de maladies auto-immunes, comme il augmente l’immunité anti-insuline.

Méthodes: La prévalence des atteintes auto-immunes cliniques (hypo-et hyperthyroïdie, atrophie gastrique et vitiligo) et subcliniques (présence d’anticorps antithyroperoxydase, antifacteur intrinsèque, anomalies de concentrations de TSH) a été évaluée en comparant 2 groupes de patients déjà traités soit par pompe implantée (n = 154) soit par pompe externe (n = 121) depuis 6 ans. L’incidence annuelle a été déterminée en réévaluant les mêmes paramètres un an après l’inclusion.

Résultats: Aucune différence significative n’a été trouvée dans la prévalence totale des maladies auto-immunes cliniques et infracliniques thyroïdiennes et gastriques (respectivement 35,6 % et 3,2 % pour le groupe pompe implantée vs 40,4 % et 2,6 % pour le groupe pompe externe), ni dans l’incidence annuelle des maladies auto-immunes cliniques ou infracliniques (7,2 % et 0 % dans le groupe pompe implantée vs 7,3 % et 1,7 % dans le groupe pompe externe).

Conclusion: Cette étude nationale multicentrique élimine la possibilité d’une augmentation du risque de maladies auto-immunes lié au traitement par pompe implantée.

Mots-clés: Pompe à insuline implantée · Diabète de type 1 · Maladies auto-immunes · Thyroïdite.
Introduction

The Diabetic Control and Complications Trial (DCCT) [1] demonstrated a correlation between the quality of glycemic control and occurrence of complications in patients with type 1 diabetes. Based on this finding, intensified subcutaneous insulin therapy with multiple injections or external pumps was proposed to improve glycemic control. Despite such intensified treatment, metabolic control remained unstable in some patients partially due to variations in insulin absorption by subcutaneous tissue. Continuous intraperitoneal insulin infusion (CIPII) using an implanted programmable pump has proven effective in achieving stable glycemic control [2,3] and in reducing hypoglycemic episodes [4], probably by lessening variations in bioavailability of insulin [5,6]. Beside well known technical problems, adverse effects [3,7,8] include an increase in anti-insulin antibodies (AIA) [9,10,11] with little impact on glycemic control in most patients [12]. The report of 5 cases of hyperthyroidism in one series of 62 patients [13] treated by CIPII suggested that this treatment could trigger more generalized immune system reactivity in type 1 diabetic patients who are already known to be prone to autoimmune disease. However another study with a total of 83 patient-years [14] did not confirm these findings. But as stated by Charles [15] statistical limitations may prevent any definitive conclusion on the basis of these two contradictory single-center studies. So the EVADIAC group decided to design a prospective multicenter comparative study to determine whether implanted pumps enhance the frequency of autoimmune disease. Then prevalence and incidence of organ-specific autoimmune diseases were compared in a large cohort of type 1 diabetic patients treated using either CIPII or CSII.

Patients and methods

Patients

Any male or female patient between the age of 18 and 70 years already treated either by CIPII or by CSII for C-peptide negative type 1 diabetes at the 14 EVADIAC centers was eligible for enrollment in this study. The CIPII group included all patients undergoing treatment using implanted pumps in France. Patients of the CSII group were matched in each center with patients of the CIPII group according to age, sex and duration of diabetes. The implanted pump models used in these patients were either the MIP 2001 or MIP 2007 pump (Minimed Technologies, Sylmar, CA, USA) infusing intraperitoneally semi-synthetic HOE21PH (Aventis) insulin at a concentration of 400 U/ml. Pump refilling was performed at intervals of 6 to 8 weeks. Treatment in the CSII group was performed using either Minimed (c/supra) or HTRON (Disetronic Medical Systems, Solzbach, Switzerland) external pumps with either lispro (Lilly) or regular (Velosuline® NovoNordisk) insulin.

Protocol

Prevalence of autoimmune disease was determined by evaluation of clinical and laboratory findings at the time of inclusion (T0), representing an average of 6 years treatment by CIPII or CSII. Annual incidence was determined on the same findings reevaluated one year (±3 months) (T1) after the date of inclusion (T0). Clinical data at both T0 and T1 were recorded by a designated coordinator at each center using standardized forms. Auto-immune hypothyroidism was defined by L Thyroxine (LT4) treatment and presence of anti-thyroid peroxidase antibodies (anti-TPOab). Graves’ disease was diagnosed by an history of treatment for hyperthyroidism and the presence of anti TSH binding inhibitor antibody (anti-TBI) or anti-TPOab, auto-immune gastric atrophy by biopsy and presence of anti-intrinsic factor antibodies (antiIF-ab), vitiligo by clinical signs and Addison’s disease by treatment for primary adrenocortical insufficiency. Subclinical diseases were defined by the presence of anti-TPOab, with normal free T3 and T4 for thyroiditis and IFab with normal biopsy for gastric atrophy. Testing was centralized in the same two University laboratories in Marseille, the INSERM U 38 for AIA, and U 555 for all the other antibodies.

Laboratory methods

Specimens collected at T0 and T1 were used for the following determinations: TSH, anti TPOAb, anti IFAb and anti insulin antibodies (AIA) levels. At each time two 5 ml samples were collected in dry tubes from each patient. Serum was collected, centrifuged for 5 min at 5000 rpm then stored frozen at -20°C until and during transport to Marseille.

Measurement of TSH in human serum was achieved by immunoluminometric assay (LUMI test TSH, BRAHMS, Berlin, Germany). Diagnostic reference values were as follows: normal thyroid function: 0.4 to 4 mU/L, preclinical hyperthyroidism: 0.1 to 0.4 mU/L, hyperthyroidism: <0.1 mU/L, subclinical hypothyroidism: 4 to 20 mU/L, and hypothyroidism: >20 mU/L. Measurement of TPOAb level in human serum was carried out by competitive immunoluminometric assay (LUMI test anti-TPO, BRAHMS, Germany). The cutoff value for positivity was 60 U/ml. Determination of TPOAb was chosen rather than determination of anti-thyroglobulin antibodies because of greater specificity and sensitivity in the presence of autoimmune thyroid disease. Measurement of AIA level in human serum was achieved by radioimmunoassay of free and total AIA using a technique adapted from that of Palmer as previously described [16].
Statistical analysis

The Chi-square test was used for comparison of qualitative data and the Student t test with unequal variance for comparison of means. Results for both tests were considered as significant if P<0.05.

RESULTS

At the end of the study period, a total of 275 patients with type 1 diabetes had been included at the 14 EVADIAC centers, i.e. 154 in the CIPII group and 121 in the CSII group. However due to a problem involving specimen transportation, matching could not be entirely realized so that this study must be considered as a comparison of two groups. There was no significant difference between the two groups (CIPII vs CSII) with regard to sex (M/F: 79/75 vs. 63/58, age: 47±10.2 years vs. 46.3±11.2 years) or duration of diabetes (24.8±10.2 years vs. 24.8±10.2 years). Mean duration of pump treatment was 6.1 years in the two groups.

Prevalence of autoimmune disease according to treatment mode (CIPII vs. CSII)

Clinical diseases

Census performed at the time of inclusion that is after a mean of 6 years treatment by CIPII or CSII, demonstrated 13 cases of Hashimoto’s disease, 2 cases of Grave’s disease, 3 cases of gastric atrophic disease and 2 cases of vitiligo in the CIPII group. In the CSII group there were 9 cases of Hashimoto’s disease, 3 cases of Grave’s disease, one case of gastric atrophic disease and 5 cases of vitiligo. No case of Addison’s disease was observed in either group. The prevalence of autoimmune disease was not significantly different between the CIPII and CSII group. Results are summarized in table I.

Table I
Number (n) of cases and prevalence of autoimmune disease observed at the time of inclusion in the two groups of patients with type 1 diabetes treated by continuous intraperitoneal insulin infusion (CIPII) using an implanted pump or continuous subcutaneous insulin infusion (CSII) using an external pump.

<table>
<thead>
<tr>
<th>CIPII (n=154)</th>
<th>CSII (n=121)</th>
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<tr>
<td>n</td>
<td>Prevalence</td>
</tr>
<tr>
<td>Hasimoto’s disease</td>
<td>13</td>
</tr>
<tr>
<td>Grave’s disease</td>
<td>2</td>
</tr>
<tr>
<td>Gastric atrophic disease</td>
<td>3</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>2</td>
</tr>
<tr>
<td>Addison’s disease</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
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</table>

Subclinical diseases

To analyze the prevalence of subclinical thyroid disease, patients presenting clinical thyroid autoimmune disease at the time of inclusion were excluded, leaving 139 patients in the CIPII group and 108 patients in the CSII group. Similarly the prevalence of anti-IF antibodies was studied in the 151 and 120 patients remaining in the CIPII and CSII groups respectively after exclusion of patients with clinical gastric atrophic disease. Table II lists the number and percentage of patients presenting positive TPOAb (>60 U/mL) and positive IFAb. The prevalence of subclinical thyroid and gastric autoimmune disease was not significantly different, i.e., 25.9% and 1.3% respectively in the CIPII group versus 30.6% and 1.6% respectively in the CSII group. Again there was no significant difference between the CIPII and CSII groups with regard to the total prevalence, i.e. clinical and subclinical, of thyroid and gastric autoimmune disease: 33.6% and 3.2% respectively in the CIPII group versus 40.4% and 2.0% respectively in the CSII group.

Based on these findings it appears that implanted pump treatment does not increase the prevalence of clinical and subclinical autoimmune disease in comparison with external pump treatment.

Incidence of autoimmune disease according to treatment mode (CIPII vs. CSII)

Clinical examination and laboratory determination of freeT3 and T4, TSH, TPOAb, and IFAb levels could be performed one year after inclusion in 103 patients in the CIPII group and 58 patients in the CSII group. A number of patients in the CSII group were lost to follow-up due to a change in the mode of surveillance in France allowing CSII patients to see private instead of hospital practitioners. In the CIPII group some patients discontinued implanted pump treatment and a few samples were lost. All lost to follow-up patients (CSII and CIPII) were analyzed. This group does not differ in term of prevalence of autoimmune diseases from remaining patients.

Table II
Number (n) of cases and prevalence of subclinical autoimmune disease in the two groups of patients with type 1 diabetes treated by continuous intraperitoneal insulin infusion (CIPII) using an implanted pump or continuous subcutaneous insulin infusion (CSII) using an external pump.

<table>
<thead>
<tr>
<th>CIPII</th>
<th>CSII</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>Prevalence</td>
</tr>
<tr>
<td>TPO&gt;60 mL/U</td>
<td>36/139</td>
</tr>
<tr>
<td>Positive IFAb</td>
<td>2/151</td>
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</table>
No new case of clinical autoimmune disease was observed in either the CIPII or CSII group. In the CIPII group, 5 of the 69 patients who were negative at T0 developed TPOAbs. No patient in the CIPII group developed IFAb. In the CSII group, 3 of the 41 patients who were negative at T0 developed TPOAbs, 1 of the 58 patients who were negative developed IFAb. There was no significant difference between the CIPII and CSII group with regard to the incidence of clinical and subclinical thyroid and gastric autoimmune disease (including clinical and subclinical): 7.2% and 0% respectively in the CIPII group versus 7.3% and 1.7% respectively in the CSII group.

Anti-insulin antibodies (AIA)

Mean AIA level was significantly higher (P<0.0001) in the CIPII than CSII group at T0 (32.9±24.5% versus 20.2±15.9%) and T1 (33.1±25.6% versus 22.9±16.8%). Patients in the CIPII or CSII group had comparable AIA levels whether or not they presented autoimmune disease (CIPII: 37.8±25.2% vs. 30.1±23.3%; CSII: 19.7±15.4% vs. 20.3±16.2%). When CIPII patients were subdivided into 3 groups according to AIA levels (high >70%, n=17, intermediate 30-70%, n=54 and low <30%, n=83), the respective prevalences of autoimmune disease were 47.1%, 40.7% and 30.1%. Despite a tendency, the differences were not significant (P=0.55 between high and low groups).

Prevalence and incidence of autoimmune disease regardless of treatment mode

This multicenter study also provided an opportunity to evaluate the prevalence and incidence of autoimmune disease in a large cohort of patients with type 1 diabetes by combining the CIPII and CSII groups.

In the whole study population, the prevalence of thyroid autoimmune disease was 9.8% for clinical disease and 28% for subclinical disease. The total prevalence of gastric autoimmune disease including clinical and subclinical forms was 2.9%. The total prevalence of all clinical and subclinical autoimmune disease in the combined study population was 31%.

No new case of autoimmune disease was recorded one year after inclusion. The overall incidence of subclinical forms of thyroid autoimmune disease in the whole study population of type 1 diabetic patients was 7.3% and that of clinical and subclinical forms of gastric autoimmune disease was 0.6%. The overall incidence of subclinical forms of thyroid and gastric autoimmune disease was 8%. Analysis of data also showed that the prevalence of autoimmune disease was not correlated with age, duration of diabetes, or duration of external or implanted pump treatment. Conversely a significant correlation was observed with sex since the prevalence of thyroid autoimmune disease was 41.5% in women versus 20.5% in men (P<0.05).

Discussion

This study shows that if patients affected by type 1 diabetes and treated for a long time by CIPII have higher level of insulin antibodies than comparable group treated by CSII, they have a comparable prevalence and incidence of autoimmune disease either clinical or subclinical. In other term CIPII treatment does not trigger organ specific autoimmunity. Intraperitoneal insulin therapy via a programmable implanted pump has been shown to reduce the frequency of severe hypoglycaemia together with an improvement of glycaemic control [2,3,4], however adverse events related to this technique have been described in the literature including several articles by the EVADIAC group [3,7,8]. Occurrence rates are relatively low for electronic failures (2.5% patients-years), local complications (8% patients-years) and catheter occlusion (10.2% patients-years). The frequency of pump slowdown fell after introduction of a new type of HOE insulin [17,18].

A potentially serious problem associated with CIPII using an implanted pump is enhanced immunoreactivity against insulin in comparison with subcutaneous treatment. As early as 1994 Olsen et al. described a series of 25 patients in which 44% presented with elevated AIA levels [9]. This effect has been confirmed by several authors [10,11]. However if very high AIA levels may have metabolic consequences, i.e. moderate postprandial glycaemia, hyper and occasional “low morning syndrome” characterized by hypoglycaemia at the end of the night, moderate levels of insulin antibody do not hamper metabolic benefits of CIPII treatment. A recent study showed that insulin immunogenicity was not directly due to HOE insulin stabilized with Genapol [19]. Conformational alterations due to interactions with the pump mecanism leading to aggregates may induce specific immune reactions. Moreover this reaction may be enhanced by the peritoneal route of administration. The present study confirms this enhanced anti-insulin immune response in CIPII group compared to CSII despite comparable age, gender, diabetes duration and duration of pump treatment. Observation of five cases of hyperthyroidism in a series of 62 implanted patients [13] raised the possibility that the immune reaction against exogenous insulin could trigger a cross reaction against auto antigen, inducing then autoimmune diseases. Another group did not confirm these results [14] However, as pointed out by Charles [15], a larger study was warranted since association with organ-specific autoimmune disease would raise serious questions about the benefit-to-risk ratio of implanted pump treatment.

This prospective comparative multicenter study conducted by the EVADIAC group provides sound evidence against the possible implication of implanted pump treatment in development of organ-specific autoimmune disease in patients with type 1 diabetes. Indeed results showed that the overall prevalence of organ-specific autoimmune disease was identical in patients treated using an implanted...
pump and patients treated using an external pump after an average of 6 years. Finally no correlation was found between AIA levels and autoimmune disease in any group.

Several comments are necessary regarding the methodology used in this study. Although initially planned, matching of patients in the CIPII and CSII group at each center according to age, sex and duration of diabetes was not entirely performed because of a problem involving specimen transportation. However the clinical characteristics of patients in the 2 groups were similar and comparison of the two groups therefore is valid. Due to a change in follow-up modalities for patients with external pumps in France, the population used to determine incidence was smaller than the one used to determine prevalence. Indeed many patients initially treated in public hospitals were subsequently monitored by private practitioners and could not be reexamined.

The findings of this study are in contradiction with the observations reported in the first small series [13]. In fact patients with type 1 diabetes are known to present a higher prevalence of organ-specific autoimmune disease involving both endocrine tissue (thyroid and adrenal gland) and non-endocrine tissue (stomach and intestinal mucosa) [20]. More recent studies have confirmed these findings and shown that prevalence of high titers antiTPO antibodies was between 25% and 30% in patients with type 1 diabetes ,with a clear-cut female predisposition [21-25]. Although various methodologies have been used in these different studies, our results are comparable.

Comparatively to control subjects, patients with type 1 diabetes show a higher prevalence of gastric atrophic disease i.e. 1% in women [20] which is often associated with thyroid disease [22,26]. Results concerning gastric atrophy disease in our overall population are consistent with those previously reported in the literature, especially with regard to high prevalence of thyroid autoimmune disease and female predisposition to this type of disease [20,22,26].

In conclusion this study confirms increased insulin immunogenicity when administered through CIPII but allows to exclude any stimulation of organ specific autoimmune disease. The latter finding is reassuring for the future of implanted pumps that are now being tested in closed loop systems.

References