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Basal and stimulated calcitonin levels in patients with type 2 diabetes did not change during 1 year of Liraglutide treatment

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ABSTRACT

Background and aims. The administration of Liraglutide, a long-acting GLP-1 receptor (GLP-1R) agonist, is associated with C-cell adenomas and carcinomas in rats. In humans, GLP-1R is highly expressed in C-cells hyperplasia (CCH) and in medullary thyroid cancer (MTC), though no changes in basal serum calcitonin (bCT) levels were recorded in type 2 diabetic (T2DM) patients treated with Liraglutide. To diagnose the possible development of CCH during Liraglutide treatment, we evaluated CT levels stimulated by calcium test (sCT).

Materials and methods. bCT and sCT and metabolic and anthropometric parameters were evaluated in 26 T2DM patients at baseline and at 1, 3, 6 and 12 months of treatment.

Results. In all patients, bCT remained within the normal range during the entire study period. In females and males, the higher sCT values were reached after 3 months and 1 month, respectively, with a progressive reduction at 6–12 months. The greater decrease of HbA1c values was reached at 3 months, while body weight and waist circumference decreased over the first 4 weeks of therapy. Lipase levels significantly increased, with a peak value at 1 month.

Conclusion. The chronic administration of Liraglutide did not lead to statistically significant variations in both bCT and sCT. Stimulated CT levels increased, though always below the normal range, during the first 1–3 months of treatment, and progressively decreased to baseline levels. This finding is consistent with the effects recorded at the glycometabolic level, and suggests the possible induction of a drug tolerance involving also the C cells and thus preventing CCH.

Abbreviations: GLP-1, Glucagon-like peptide 1; GLP-1R, GLP-1 receptor; CT, Calcitonin; bCT, basal Calcitonin; sCT, stimulated Calcitonin; T2DM, Type 2 diabetes mellitus; CCH, C-cells hyperplasia; MTC, Medullary thyroid cancer; OAD, Oral antidiabetics; FAT, Fat mass; FFM, Free fat mass; BMI, Body mass index; WC, Waist circumference; HbA1c, Glycosylated hemoglobin; FPG, Fasting plasma glucose; HDL, High density lipoprotein; LDL, Low density lipoprotein; TSH, Thyroid-stimulating hormone; FT4, Free triiodothyronine; FT3, Free thyroxine; AbTg, Antithyroglobulin antibody; AbTPO, Antithyroid peroxidase antibody; PTH, Parathyroid hormone; Ca, Calcium; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; HOMA-IR, Homeostatic model assessment-insulin resistance index; n, Number; SD, Standard deviation; ANOVA, One-way analysis of variance.

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1. Introduction

Among the novel drugs for type 2 diabetes mellitus (T2DM) treatment, the long-acting GLP-1 receptor agonist Liraglutide is widely used [1]. Glucagon-like peptide-1 (GLP-1) is an incretin hormone, secreted after meals by intestinal mucosal endocrine cells (L cells) [2]. It acts in a pleiotropic way that leads to a reduction of blood glucose concentration and caloric intake. The actions of GLP-1 are mediated by G-protein coupled receptors, highly expressed in the pancreas, intestine, stomach and nervous system, though receptors are also present in other tissues [3,4]. In pre-clinical trials, the expression of GLP-1 receptors (GLP-1R) has been also documented in rodents' thyroid glands [5], and GLP-1 receptor agonists were found to elicit calcitonin (CT) gene expression and CT release by parafollicular C cells in a dose-dependent manner. Liraglutide administration was found to associate in rats with the onset of C-cell adenomas and carcinomas, whereas in monkeys neither C cells hyperplasia (CCH) nor C cells tumors were observed [5]. In TT cells, derived from human medullary thyroid cancer (MTC), GLP-1 receptor agonists were found not to stimulate CT release. Moreover, the expression of GLP-1 receptors was very low with mRNA transcripts 14- to 21-fold lower than those recorded in the rat C-cells [5]. On the contrary, GLP-1R has been recently found to be highly expressed in human CCH and MTC [6]. In particular, GLP-1R immunoreactivity was detected in 91.6% of analyzed MTCs, and in the majority of them a high proportion (>70%) of neoplastic cells were stained for GLP-1R. GLP-1R immunoreactivity was always documented in CCH, either reactive or associated with *RET* germline mutations, while it was found only in a minority of normal C cells and papillary thyroid carcinoma cells, indicating that it is almost limited to diseased C-cells in humans, likely as a consequence of dysregulated cell cycle control, and may be aberrantly expressed in follicular cells [6].

To evaluate the possible effect of Liraglutide on the C cell compartment in humans and the related risk of CCH and MTC, basal serum CT (bCT) levels were previously monitored in T2DM patients and non-diabetic obese subjects treated for over 2 years [7], and no significant changes with any dose of the drug were observed. Nevertheless, since the best way to diagnose either CCH, which is a preneoplastic lesion, or microMTC is to evaluate stimulated CT (sCT), we performed the sequential evaluation of both bCT and sCT during Liraglutide treatment. The stimulation of CT has been obtained by means of the high-dose calcium test, which has been validated as the best test available to date [8].

2. Patients and methods

2.1. Study population

We enrolled 26 patients with type 2 diabetes (T2DM). Inclusion criteria were: (1) presence of T2DM, treated with oral antidiabetics (OAD)/insulin therapy; (2) age between 18 and 80 years; (3) HbA1c levels ranging 6.5–10.0% (48–86 mmol/mol, previous OAD monotherapy ≥3 months) or HbA1c ranging 6.5–9.0% (48–76 mmol/mol, previous combination therapy ≥3 months); (4) capability to perform self-blood glucose monitoring; and (5) no personal/familiar history of MTC or elevated bCT values.

At baseline all patients started therapy with once daily dose of Liraglutide (1.2 or 1.8 mg/day; Novo Nordisk A/S, Bagsvaerd, Denmark) injected subcutaneously. Treatment was given in combination with metformin. Metformin was maintained throughout the study, but could be added sulfonylurea (Gliclazide) if unacceptable glucose control.

Withdrawal criteria included: (1) insufficient glycemic control, defined as HbA1c levels ≥9% after 1 month of treatment; (2) severe hypoglycemia; and (3) gastro-intestinal intolerance and adverse effects, like acute pancreatitis and allergic reactions.

Each patient was evaluated at baseline and after 1, 3, 6, and 12 months of therapy with Liraglutide. All patients gave their informed consent to be enrolled in this study, which has been previously approved by the ethical committees of the institution involved (E.C.: 144/2013-788/2013).

2.2. Clinical parameters

Body weight and height were measured with scale and stadiometer whereas body mass index (BMI) was calculated as patient's weight (in kg) divided by patient height (in meters) squared. Waist circumference was taken at the umbilicus. Blood pressure was measured with a mercury sphygmomanometer after the patient had been lying supine for at least 5 min. Fat mass (FAT) and fat free mass (FFM) were measured using impedance method (Akern Quantum/S®).

2.3. Biochemical parameters and procedure

All patients were evaluated at any time for fasting plasma glucose (FPG), triglycerides, and total and HDL cholesterol, with calculation of low-density lipoprotein (LDL) cholesterol by the FriedWald formula, aspartate transaminase, alanine transaminase, γ-glutamyl transferase, total bilirubin, serum creatinine, urea, amylase and lipase values, PTH, serum calcium and phosphate, thyroid-stimulating hormone (TSH), free triiodothyronine (FT4), free thyroxine (FT3), antithyroglobulin antibody (AbTg) and antithyroid peroxidase antibody (AbTPO) levels. Hemoglobin A1c (HbA1c) was assessed by the use of a high performance liquid chromatography, National Glycohemoglobin Standardisation Program-certified and Diabetes Control and Complications Trial-standardized method. Patients were also evaluated for insulin and C-peptide levels. Insulin was analyzed by immunoenzymetric one-step assay (Medgenics Diagnostics, Belgium) and electrochemiluminescence immunoassay (Roche Diagnostics, Germany). Insulin resistance was then estimated by the homeostatic model assessment-insulin resistance (HOMA-IR) index (http://www.dtu.ox.ac.uk/homacalculator/index.php). CT was measured using a two-site automated chemiluminescent immunometric assay (Immulite 2000; Siemens Diagnostics, Deerfield, IL) in blood samples obtained before and at 2, 5, and 15 min from the end of the infusion of Ca by in-dwelling IV cannula. The assay has an analytical sensitivity of 2 pg/ml. Ca gluconate was administered IV at the dose of 25 mg/kg at 10 ml/min (2.3 mg or 0.12 mEq of elemental Ca), being adjusted body weight calculated (www.manuelsweb.com/IBW.htm for ideal body weight and adjusted body weight calculator) for each patient to avoid an over-dosage in obese. Thyroid ultrasound