DIABETICMedicine

DOI: 10.1111/dme.13437

Case Report

No calcitonin change in a person taking dulaglutide diagnosed with pre-existing medullary thyroid cancer

S. I. Sherman[1], R. T. Kloos[2], R. M. Tuttle[3], A. Pontecorvi[4], H. Völzke[5], K. Harper[6], C. Vance[7], J. T. Alston[6], A. L. Usborne[6], K. W. Sloop[6] and M. Lakshmanan[6]

[1]Department of Endocrine Neoplasia and Hormonal Disorders, Division of Internal Medicine, University of Texas MD Anderson Cancer Center, Houston, TX, [2]Department of Medical Affairs, Veracyte, Inc., South San Francisco, CA, [3]Endocrinology Service, Memorial Sloan Kettering Cancer Center, New York, NY, [4]Department of Internal Medicine, Catholic University, Rome, Italy, [5]Institute for Community Medicine, University Medicine, Greifswald, Germany, [6]Eli Lilly and Company, Indianapolis, IN, and [7]Rocky Mountain Diabetes and Osteoporosis Center, PA, Idaho Falls, ID, USA

Accepted 25 July 2017

Abstract

Background Glucagon-like peptide-1 receptor agonists, such as dulaglutide, exenatide and liraglutide, are approved to treat Type 2 diabetes mellitus. Although these drugs provide substantial glycaemic control, studies in rodents have prompted concerns about the development of medullary thyroid carcinoma. These data are reflected in the US package insert, with boxed warnings and product labelling noting the occurrence of these tumours after clinically relevant exposures in rodents, and contraindicating glucagon-like peptide-1 receptor agonist use in people with a personal or family history of medullary thyroid carcinoma, or in people with multiple endocrine neoplasia type 2. However, there are substantial differences between rodent and human responses to glucagon-like peptide-1 receptor agonists. This report presents the case of a woman with pre-existing medullary thyroid carcinoma who exhibited no significant changes in serum calcitonin levels despite treatment with dulaglutide 2.0 mg for 6 months in the Assessment of Weekly AdministRation of LY2189265 [dulaglutide] in Diabetes-5 clinical study (NCT00734474).

Case report Elevated serum calcitonin was noted in a 56-year-old woman with Type 2 diabetes mellitus at the 6-month discontinuation visit in a study of long-term dulaglutide therapy. Retroactive assessment of serum collected before study treatment yielded an elevated calcitonin level. At 3 months post-study, calcitonin level remained elevated; ultrasonography revealed multiple bilateral thyroid nodules. Eventually, medullary thyroid carcinoma was diagnosed; the woman was heterozygous positive for a germline *RET* proto-oncogene mutation.

Conclusion The tumour was not considered stimulated by dulaglutide therapy because calcitonin remained stable throughout.

Diabet. Med. 35, 381–385 (2018)

Introduction

Glucagon-like peptide-1 receptor agonists (GLP-1RAs), such as dulaglutide [1], exenatide extended-release [2] and liraglutide [3], are approved for improvement of glycaemic control in people with Type 2 diabetes mellitus. Although efficacious, concerns about the development of medullary thyroid carcinoma (MTC) have been raised based on studies in rodents [1–4], hence, a boxed warning is included in the US package insert and GLP-1RA product labelling. Medullary thyroid carcinoma accounts for 1–2% of primary thyroid malignancies and originates from parafollicular cells, also called C cells, which represent ~1% of cells in the human thyroid [5]. Between 1983 and 2012, the mean annual age-adjusted incidence of MTC rose significantly, from 0.14 to 0.21 per 100 000 people [6]. Cases of MTC are mostly sporadic (80%), but ~20% may occur in hereditary form, typically associated with a mutation in the RET proto-oncogene [5].

In rodents, activation of glucagon-like peptide-1 receptors (GLP-1Rs) increases cyclic adenosine monophosphate in thyroid C cells, initiates the release of calcitonin, and eventually promotes C-cell proliferation and tumours [7–11]. Although calcitonin serves as an important biomarker for the presence of MTC [4,12,13], the GLP-1RA-mediated calcitonin increases noted in rodents have not been observed in studies in non-human primates [11,12] or in humans with Type 2 diabetes [14,15].

Correspondence to: M. Lakshmanan. E-mail mcl@lilly.com

What's new?

- A woman with an unrecognized pre-existing medullary thyroid carcinoma (MTC) who received a glucagon-like peptide-1 receptor agonist (GLP-1RA) in a clinical trial provides a possibly unique case in the GLP-1RA drug development literature.
- A lack of both serum calcitonin stimulation and functional glucagon-like peptide-1 receptor in this MTC was observed.

Dulaglutide is approved at once-weekly doses of 0.75 and 1.5 mg (by subcutaneous injection) for treatment of Type 2 diabetes [1]. Assessment of Weekly AdministRation of LY2189265 [dulaglutide] in Diabetes-5 (AWARD-5) was a phase II/III efficacy and safety study of dulaglutide compared with sitagliptin in people with Type 2 diabetes on metformin; participants were initially treated with dulaglutide 0.25, 0.50, 0.75, 1.00, 1.50, 2.00 or 3.00 mg during the dose-finding portion of the study [16].

The present report describes an AWARD-5 participant with pre-existing MTC who exhibited no significant changes in serum calcitonin levels despite treatment with dulaglutide 2.0 mg for 6 months.

Case report

The participant was a 56-year-old white woman with no personal or family history of endocrine neoplasms. Relevant medical history included Type 2 diabetes, hypertension, oesophageal reflux, and obesity (BMI 30.6 kg/m^2); she was a non-smoker with no reported alcohol use.

She received once-weekly dulaglutide 2.0 mg for Type 2 diabetes. Regular calcitonin monitoring was initiated after reports during the AWARD-5 study of the potential effect of long-acting GLP-1RAs in animals [17]. Table 1 shows a timeline noting calcitonin measurements and other events. At the 6-month discontinuation visit, the woman's fasting calcitonin level was elevated [61.7 pg/ml (18.05 pmol/l); reference range 0.0–11.5 pg/ml (0.0–3.36 pmol/l); Table 1].

The woman's baseline calcitonin level was measured using stored serum and was elevated [91.5 pg/ml (26.77 pmol/l); Table 1]. She was not taking GLP-1RA or dipeptidyl peptidase-4 inhibitors at baseline. She was taking omeprazole, which may increase serum calcitonin, although not typically above 20 pg/ml (5.85 pmol/l) [18]. Thyroid ultrasonography showed multiple small bilateral nodules, the largest 1.1 × 0.7 × 0.8 cm in the left lobe. Twelve weeks after dulaglutide was discontinued (week 23), serum calcitonin remained elevated and unchanged (Table 1). A fine-needle aspiration of the large left lobe nodule was consistent with a follicular neoplasm.

Approximately 1 year after initiation of the study drug and 6 months after discontinuation, she underwent left hemithyroidectomy, and pathological examination confirmed MTC.

Table 1 Serum calcitonin by weeks following randomization into the AWARD-5 study

	AWARD-5 treatment day −1*	AWARD-5 discontinuation	Ultrasonography	Left hemithyroidectomy	Postoperative follow-up visits				Completion thyroidectomy	Ultrasonography
Week	0	23	35	50	51	54	62	65	76	278
Date	17 December 2008	4 June 2009	27 August 2009	11 December 2009	17 December 2009	7 January 2010	5 March 2010	25 March 2010	9 June 2010	28 April 2014
Calcitonin, pg/ml	91.5	61.7	82.8	-	12.0	14.0	8.0	16.2	-	<2.0

AWARD-5, Assessment of Weekly AdministRation of LY2189265 [dulaglutide] in Diabetes Assessment-5.
*As per the AWARD-5 protocol, the collection of a stored sample was drawn the day of randomization. Note: data presented in standard units. Système International (SI) units are pmol/l (conversion factor×0.2926).

Calcitonin level was reduced partially in the 3 months after surgery but remained elevated (Table 1). Approximately 5 months after surgery, ultrasonography of the right thyroid lobe nodules remained unchanged from presurgical assessments. A completion thyroidectomy was performed ~6 months after the first surgery. MTC was noted in the pathological assessment, 0.3 cm in greatest dimension, negative for lymphatic and vascular invasion, with two benign lymph nodes. Resection margins were free from malignancy. The last serum calcitonin level obtained, ~44 months after this second procedure, was <2 pg/ml (0.59 pmol/l). An assay of excised normal thyroid tissue indicated that the woman was heterozygous positive for the RET V804M mutation, a common variant associated with hereditary MTC [5,19,20]. Subsequently, immunohistochemistry staining was performed to assess the presence of GLP-1R in her tumour tissue.

Details of the methodology are provided in the Supporting Information, File S1. Results (Fig. 1) showed positive cytoplasmic staining for GLP-1R in a few scattered cells in the MTC sample, while membranous GLP-1R staining was not seen. Pancreas tissues from mice that express the human GLP-1R from the murine *Glp-1r* promoter and endogenous upstream regulatory elements [21] were used to confirm the ability of the antibody to detect membranous GLP-1R (consistent with a functional receptor). Further, HEK293 cells expressing the GLP-1R showed positive signal, whereas none was observed in the parent HEK293 cells or Glp-1r knockout tissues (data not shown).

Discussion

Given a retrospective baseline serum value for calcitonin nearly nine times the upper limit of normal, and evidence of a germline RET proto-oncogene mutation, MTC in this woman was considered to be pre-existing to the short-term GLP-1RA exposure that began in December 2008. Beginning in April 2009, people with Type 2 diabetes in clinical trials involving long-acting GLP-1RAs were required to be screened for serum calcitonin to rule out C-cell disease [17].

The membranous GLP-1R immunohistochemistry signal in the control knock-in mouse pancreas is consistent with published results [22,23]. Results from GLP-1R immunohistochemistry of the woman's MTC sample showed a lack of cell surface GLP-1R staining.

Broad conclusions are limited based on this report in one woman with MTC with a RET mutation (V804M) [5]. This mutation is associated with a less aggressive course of MTC; therefore, generalization to others with MTC may not be appropriate. Additionally, the duration of GLP-1RA treatment was only 23 weeks, and although no obvious change in serum calcitonin was observed, it may not be possible to conclude that longer-term treatment would not have influenced tumour progression or serum calcitonin level.

The lack of calcitonin stimulation and absence of plasma membrane GLP-1R in this case of MTC are reassuring; however, as screening for MTC is not routine before initiating GLP-1RA treatment [4], this woman's experience may be similar to that in clinical practice. Whereas the

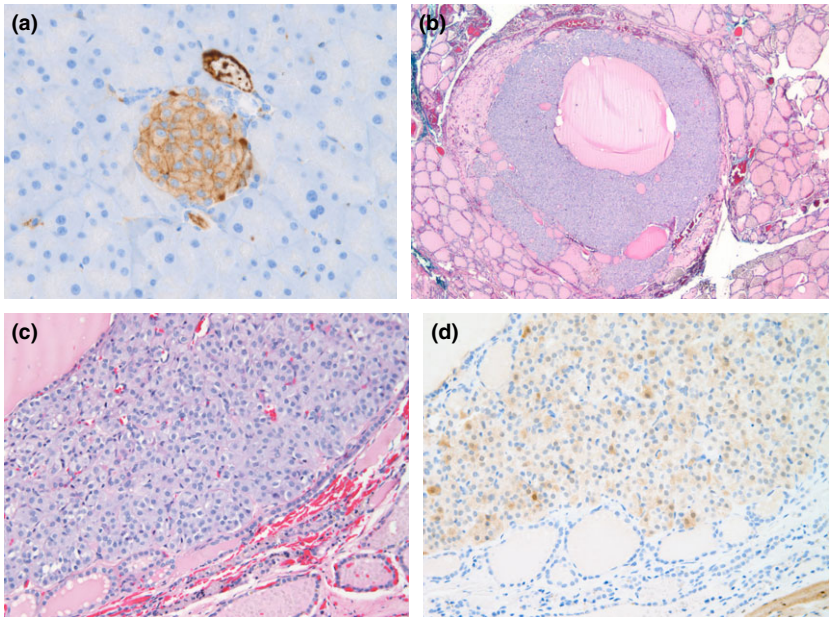

FIGURE 1 Immunohistochemistry of human glucagon-like peptide-1 receptor (GLP-1R) knock-in mouse pancreas (a) at control (40×) magnification, demonstrating membranous GLP-1R detection in the central cells of the islet (consistent with β-cell labelling). Haematoxylin and eosin-stained sections of the patient medullary thyroid cancer (MTC) (b) at low (2.5×) magnification, demonstrating entire tumour nodule with compression of surrounding normal thyroid follicles and (c) and (d) at 20× illustrating both haematoxylin and eosin and immunohistochemistry.

incidence of clinical MTC is very low [5], the incidence of occult MTC is higher [24,25], therefore, others with occult MTC are likely to be exposed to long-acting GLP-1RAs. Future investigations are needed to evaluate the role of GLP-1RAs on the initiation and, perhaps more importantly, the natural history of C-cell neoplasia in humans [26]. The MTC Registry, established in 2010 to provide a source of data for the incidence and prevalence of MTC and GLP-1RA exposure, may prove to be an investigational resource for subsequent research as additional such cohorts are identified [26].

Funding sources

Funding was received from Eli Lilly and Company.

Competing interests

S.I.S. reports personal fees from Novo Nordisk, personal fees from Veracyte, grants and personal fees from Genzyme, personal fees from Eisai, personal fees from Bristol-Myers Squibb, personal fees from LOXO Oncology, personal fees from Rosetta Genomics, and personal fees from Onyx Pharmaceuticals outside the submitted work. R.T.K. is an employee and equity owner of Veracyte, Inc. and received personal fees from Novo Nordisk. R.M.T. serves on the data monitoring board for Novo Nordisk. A.P. was an advisor to Eli Lilly and Company in 2011 and 2012. H.V. received personal fees from Eli Lilly and Company during the conduct of the study. K.H. is an employee of Eli Lilly and Company and a stock owner. C.V. is a principal investigator for Eli Lilly and Company and for Novo Nordisk. J.T.A. has nothing to disclose. A.L.U. is an employee of Eli Lilly and Company and a stock owner. K.W.S. is an employee of Eli Lilly and Company and a stock owner. M.L. is an employee of Eli Lilly and Company and a stock owner.

Acknowledgements

The authors would like to thank Jeffrey Cohn, PhD of inVentiv Health Clinical for his writing and editorial contributions. We are grateful to Rebecca Jaye Threlkeld and Julia Ann Shell of Eli Lilly and Company for their assistance in obtaining the participant's data on conclusion of the AWARD-5 study when the MTC was diagnosed and treated, as well as obtaining the MTC tissue used in testing for the GLP-1R. We would also like to acknowledge Novo Nordisk Inc. for their kind gift of the validated antibody to the GLP-1R.

References

1 Eli Lilly and Company. Trulicity (dulaglutide) injection, for subcutaneous use [prescribing information], 2015. Indianapolis, IN, USA.
2 Astra Zeneca. Bydureon (exenatide) extended-release for injectable suspension [prescribing information], 2015. Wilmington, DE, USA.
3 Novo Nordisk Inc. Victoza (liraglutide [rDNA origin] injection), solution for subcutaneous use [prescribing information], 2015. Princeton, NJ, USA.
4 Parks M, Rosebraugh C. Weighing risks and benefits of liraglutide–the FDA's review of a new antidiabetic therapy. *N Engl J Med* 2010; **362**: 774–777.
5 Wells SA, Jr, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF *et al.* Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid* 2015; **25**: 567–610.
6 Randle RW, Balentine CJ, Leverson GE, Havlena JA, Sippel RS, Schneider DF *et al.* Trends in the presentation, treatment, and survival of patients with medullary thyroid cancer over the past 30 years. *Surgery* 2017; **161**: 137–146.
7 Nauck MA, Friedrich N. Do GLP-1-based therapies increase cancer risk? *Diabetes Care* 2013; **36**(Suppl. 2): S245–S252.
8 Crespel A, De Boisvilliers F, Gros L, Kervran A. Effects of glucagon and glucagon-like peptide-1-(7-36) amide on C cells from rat thyroid and medullary thyroid carcinoma CA-77 cell line. *Endocrinology* 1996; **137**: 3674–3680.
9 Lamari Y, Boissard C, Moukhtar MS, Jullienne A, Rosselin G, Garel JM. Expression of glucagon-like peptide 1 receptor in a murine C cell line: regulation of calcitonin gene by glucagon-like peptide 1. *FEBS Lett* 1996; **393**: 248–252.
10 Bjerre Knudsen L, Madsen LW, Andersen S, Almholt K, de Boer AS, Drucker DJ *et al.* Glucagon-like peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. *Endocrinology* 2010; **151**: 1473–1486.
11 Byrd RA, Sorden SD, Ryan T, Pienkowski T, LaRock R, Quander R *et al.* Chronic toxicity and carcinogenicity studies of the long-acting GLP-1 receptor agonist dulaglutide in rodents. *Endocrinology* 2015; **156**: 2417–2428.
12 Hegedüs L, Moses AC, Zdravkovic M, Le Thi T, Daniels GH. GLP-1 and calcitonin concentration in humans: lack of evidence of calcitonin release from sequential screening in over 5000 subjects with type 2 diabetes or nondiabetic obese subjects treated with the human GLP-1 analog, liraglutide. *J Clin Endocrinol Metab* 2011; **96**: 853–860.
13 Niafar M, Dabiri S, Bozorgi F, Niafar F, Gholami N. Metastatic medullary thyroid carcinoma: a case report. *J Res Med Sci* 2011; **16**: 568–573.
14 Nauck M, Weinstock RS, Umpierrez GE, Guerci B, Skrivanek Z, Milicevic Z. Efficacy and safety of dulaglutide versus sitagliptin after 52 weeks in type 2 diabetes in a randomized controlled trial (AWARD-5). *Diabetes Care* 2014; **37**: 2149–2158.
15 Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JF, Nauck MA *et al.* Liraglutide and cardiovascular outcomes in Type 2 diabetes. *N Engl J Med* 2016; **375**: 311–322.
16 Weinstock RS, Guerci B, Umpierrez G, Nauck MA, Skrivanek Z, Millicevic Z. Safety and efficacy of once-weekly dulaglutide versus sitagliptin after 2 years in metformin-treated patients with type 2 diabetes (AWARD-5): a randomized, phase III study. *Diabetes Obes Metab* 2015; **17**: 849–858.
17 US Food and Drug Administration Endocrine and Metabolic Drug Advisory Committee. 2 April 2009. Novo Nordisk's Liraglutide (injection) for the treatment of patients with type 2 diabetes, NDA 22-341, briefing document. 4-2-2009. Available at https://www.fda.gov/ohrms/dockets/ac/09/briefing/2009-4422b2-01-FDA.pdf Last accessed 3 August 2017.
18 Erdogan MF, Gursoy A, Kulaksizoglu M. Long-term effects of elevated gastrin levels on calcitonin secretion. *J Endocrinol Invest* 2006; **29**: 771–775.
19 Rich TA, Feng L, Busaidy N, Cote GJ, Gagel RF, Hu M *et al.* Prevalence by age and predictors of medullary thyroid cancer in

patients with lower risk germline *RET* proto-oncogene mutations. *Thyroid* 2014; **24**: 1096–1106.
20 Moline J, Eng C. Multiple endocrine neoplasia type 2: an overview. *Genet Med* 2011; **13**: 755–764.
21 Jun LS, Showalter AD, Ali N, Dai F, Ma W, Coskun T *et al*. A novel humanized GLP-1 receptor model enables both affinity purification and Cre-LoxP deletion of the receptor. *PLoS One* 2014; **9**: e93746.
22 Waser B, Blank A, Karamitopoulou E, Perren A, Reubi JC. Glucagon-like-peptide-1 receptor expression in normal and diseased human thyroid and pancreas. *Mod Pathol* 2015; **28**: 391–402.
23 Pyke C, Heller RS, Kirk RK, Ørskov C, Reedtz-Runge S, Kaastrup P *et al*. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology* 2014; **155**: 1280–1290.
24 Ahmed SR, Ball DW. Clinical review: incidentally discovered medullary thyroid cancer: diagnostic strategies and treatment. *J Clin Endocrinol Metab* 2011; **96**: 1237–1245.
25 Valle LA, Kloos RT. The prevalence of occult medullary thyroid carcinoma at autopsy. *J Clin Endocrinol Metab* 2011; **96**: E109–E113.
26 Koro CE, Hale PM, Ali AK, Qiao Q, Tuttle RM. The rationale, objectives, design and status of the medullary thyroid carcinoma (MTC) surveillance study: a case-series registry. *Thyroid* 2016; **26** (S1):A-125 [Abstract No. 357].

Supporting Information

Additional Supporting Information may be found in the online version of this article:

File S1. Methodology.