CHAPTER 9

EFFECTS OF 6 WEEKS TREATMENT WITH A DIPEPTIDYLPEPTIDASE 4 INHIBITOR ON COUNTERREGULATORY AND INCRETIN HORMONES DURING ACUTE HYPOGLYCAEMIA IN PATIENTS WITH TYPE 1 DIABETES:
A RANDOMIZED DOUBLE BLIND PLACEBO-CONTROLLED CROSS-OVER STUDY

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ABSTRACT

Aims/hypothesis
Within a few years of onset of type 1 diabetes the glucagon response to hypoglycaemia is severely diminished. Inhibitors of the enzyme dipeptidyl peptidase 4 (DPP-4), which under normal circumstances inactivates the incretin hormones (glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1)), enhance glucagon secretion during hypoglycaemia in patients with type 2 diabetes. The aim of this study was to assess whether the DPP-4 inhibitor sitagliptin affects glucagon and/or catecholamine counterregulatory responses to hypoglycaemia in patients with type 1 diabetes.

Methods
We conducted a single-centre, randomized, double-blind, placebo-controlled, three-period cross-over study. We studied 16 male patients with type 1 diabetes aged 18-52 years, with diabetes duration of 5-20 years and intact hypoglycaemia awareness. Participants received sitagliptin (100 mg/day) or placebo as outpatients for 6 weeks and attended the hospital for three acute hypoglycaemia studies (at baseline, after sitagliptin treatment and after placebo).

Results
Sitagliptin treatment significantly increased active levels of both GIP and GLP-1. No significant differences were observed for glucagon or catecholamine counterregulatory responses during the 3 hypoglycaemia studies. Growth hormone concentration at 40 minutes after occurrence of autonomic reaction was significantly lower after sitagliptin treatment [23 (0.2-211.0) mEq/l] compared to placebo [90 (8.8-180) mEq/l] (p=0.008).

Conclusions
Sitagliptin does not affect glucagon or catecholamine counterregulatory responses in patients with type 1 diabetes.
INTRODUCTION

Hypoglycaemia is a well-known complication of insulin therapy in patients with type 1 diabetes. It remains a major barrier in maintaining glycaemic control. The counterregulatory response to hypoglycaemia, with glucagon as the most important mediator, is diminished within a few years of the onset of type 1 diabetes and subsequently lost, aggravating the increased risk for hypoglycaemia associated with exogenous insulin. No clear explanation is currently available for the loss of glucagon response to hypoglycaemia in type 1 diabetes. A reduction in α-cell mass, autonomic neuropathy and the loss of paracrine inhibition by insulin have been suspected to contribute to the impaired hypoglycaemia-induced glucagon response in type 1 diabetes. The secretory response of the pancreatic α-cells to other stimuli seems to remain intact: glucagon is still secreted in response to exercise and arginine infusion. This indicates that the reduced glucagon response to hypoglycaemia in type 1 diabetes probably reflects impaired intrinsic signalling rather than a structural defect in the α-cells of the pancreas.

Dipeptidyl peptidase 4 (DPP-4) inhibitors are a new class of drugs for the management of type 2 diabetes. DPP-4 inhibitors exploit the glucose-lowering properties of the gut incretin hormones glucose-dependent insulino tropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). These hormones augment glucose-induced insulin secretion and GLP-1 also exerts glucose-dependent (during hyperglycaemia) suppression of circulating glucagon. Interestingly, both GLP-1 and GIP have been shown to increase glucagon responses during hypoglycaemia in healthy individuals. In line with these findings a recent cross-over study in 25 patients with type 2 diabetes showed that 28 days of DPP-4 inhibitor treatment significantly suppressed glucagon secretion during hyperglycaemia and augmented the glucagon response to insulin-induced hypoglycaemia compared to placebo. Another study in patients with type 2 diabetes showed that hypoglycaemia was less frequent and less severe when vildagliptin was given as an add-on to insulin, again suggesting that DPP-4 inhibition may exert a protective effect against hypoglycaemia in patients with type 2 diabetes.

The present study was undertaken to examine whether the glucagon response to hypoglycaemia is augmented after 6 weeks of DPP-4 inhibitor (sitagliptin) therapy in patients with type 1 diabetes.

METHODS

Study design
This was a single-centre, double-blind, randomized, placebo-controlled, crossover study of 6 weeks treatment with sitagliptin (100 mg/day). Participants came to the Academic Medical
Center in Amsterdam for a screening visit to determine eligibility for the study. The study enrolled Caucasian male patients with type 1 diabetes of 5 to 20 years duration, aged between 18 and 55 years, who were C-peptide negative and/or GAD65 positive. Patients were excluded if they were found to have impaired awareness of hypoglycaemia (assessed with the Gold \(^{25}\) and Clarke \(^{26}\) methods \(^{27}\)), had a history of seizures, cardiac arrhythmia (or used \(\beta\)-adrenoreceptor blockers) and/or acute infection in the 12 weeks before the study, or if evidence of severe diabetes complications (autonomic neuropathy, nephropathy or proliferative retinopathy) was present.

Eligible subjects attended the centre for 3 hypoglycaemia study days separated by approximately 6 weeks. All participants were expected to complete two treatment periods, receiving different blinded study medication during each period (sequence A: sitagliptin 100 mg/day followed by placebo; sequence B: placebo followed by sitagliptin 100 mg/day). Patients were randomized following the first hypoglycaemia study day (baseline). To balance group sizes blocked randomization was used.

At each hypoglycaemia study day patients came to the hospital in fasting state, having taking their long-acting insulin the night before, but omitting their morning dose of short acting insulin. When on continuous subcutaneous insulin infusion (CSII), patients stopped their pump 1 hour before each hypoglycaemic experiment. All subjects were asked to monitor their blood glucose with care for the preceding 48 hours, including bedtime testing. The study was postponed if any capillary blood glucose value of <3.5 mmol/L was registered in the 48 hours prior to the experiment. A cannula was inserted in an antecubital vein for the administration of human insulin (Actrapid®; Novo Nordisk, Bagsvaerd, Denmark) and 20% glucose. Another cannula was placed retrogradely into a vein on the dorsal portion of the contra lateral hand or wrist for blood sampling. The hand was placed in a heated box to ensure arterialisation of venous blood \(^{28}\). Infusion of insulin was started at \(2 \text{ mU} \times \text{kg}^{-1} \times \text{min}^{-1}\) and 20% glucose solution was infused at a variable rate to achieve the desired blood glucose concentrations. In the initialisation phase plasma glucose was stabilized for 30 minutes at 5.0 mmol/L. Thereafter glucose infusion was discontinued until the patient developed objective evidence of an autonomic reaction (see below) or until blood glucose fell below 2.0 mmol/L. An increment of 20% or more in heart rate and/or systolic blood pressure was used to identify autonomic reaction \(^{29}\). Following autonomic reaction or when blood glucose fell below 2.0 mmol/L the insulin infusion was terminated. After sampling of arterialised venous blood for measurement of counterregulatory hormones and incretin hormones a bolus of 20 ml 20% glucose was given. Thereafter an infusion of 20% glucose was commenced at a rate of 50 ml·h\(^{-1}\) to normalise blood glucose. The rate of glucose infusion was increased if the blood glucose remained below 2.0 mmol/L.
Blood was sampled at predefined time intervals for measurement of counterregulatory hormones and incretin hormones. These time points were at the middle of the initialisation phase, during autonomic reaction, and 10, 20 and 40 minutes after the onset of autonomic reaction. Incretin hormones were additionally measured twice in the initialisation phase. At occurrence of the autonomic reaction, time was reset to zero. The timing of samples from autonomic reaction allowed for individual variation in the time necessary for the blood glucose to fall to the threshold level at which autonomic reaction occurred.

The symptomatic responses to hypoglycaemia were assessed using a standard validated symptom questionnaire adapted for experimental hypoglycaemia. A 7-point scale (1=symptom absent; 7=symptom experienced with great intensity) was used to score presence and intensity of autonomic and neuroglycopenic symptoms of hypoglycaemia. Symptom scores were obtained during the initialisation phase, at occurrence of autonomic reaction and again 30 minutes later.

The protocol was approved by the Ethics Committee of the Academic Medical Center and was conducted in accordance with the latest Declaration of Helsinki. Oral and written informed consent was obtained from all participants. The study was registered at ClinicalTrials.gov (registration no. NCT01272583).

**Adverse events**
Spontaneously reported adverse events during blinded study medication were documented by the investigator.

**Biochemical assays**
Plasma glucose was measured bedside using a glucose oxidase method (2300 Stat plus; Yellow Springs Instruments, Yellow Springs, OH, USA). Plasma concentrations of glucagon, intact and total GIP and GLP-1 were measured by specific radioimmunoassays, as previously described. Norepinephrine and epinephrine levels were determined with an in-house high-performance liquid chromatography method. Growth hormone plasma concentrations were determined by a solid phase two-site fluoroimmunometric assay with a detection limit of 0.1 mU/L (Perkin Elmer, Waltham, MA, USA). Cortisol in plasma was determined with a chemiluminescent immunoassay (Immuloite 2000; Diagnostic Products Corp., Los Angeles, CA, USA).

**Calculations and statistical analyses**
Sample size calculation (assuming a difference in means of 5 pmol/L in glucagon secretion during hypoglycaemia with sitagliptin treatment compared to placebo and a standard
Figure 1: Flow chart of participant enrolment, randomisation and treatment sequence

Table 1: Patient characteristics at baseline

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Sequence A (sitagliptin \rightarrow placebo)</th>
<th>Sequence B (placebo \rightarrow sitagliptin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>16</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Age (year)</td>
<td>31.5 [27.0-40.0]</td>
<td>30.5 [26.3-39.0]</td>
<td>33.5 [27.8-40.8]</td>
</tr>
<tr>
<td>Males</td>
<td>16</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Of Caucasian origin</td>
<td>16</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.3 [21.0-25.3]</td>
<td>24.1 [21.2-25.3]</td>
<td>24.6 [20.9-25.3]</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>66 [61-71]</td>
<td>67 [62-81]</td>
<td>65 [57-71]</td>
</tr>
<tr>
<td>Diabetes duration (year)</td>
<td>10.5 [6.0-14.5]</td>
<td>8.0 [5.0-11.8]</td>
<td>12.0 [10.0-15.8]</td>
</tr>
<tr>
<td>Severe hypoglycaemia events in preceding year (number)</td>
<td>0 [0-0]</td>
<td>0 [0-0]</td>
<td>0 [0-0]</td>
</tr>
<tr>
<td>Total insulin dose (IU)</td>
<td>47.5 [39.2-76.0]</td>
<td>55.0 [40.5-79.8]</td>
<td>43.0 [38.5-75.0]</td>
</tr>
<tr>
<td>Number [%] on basal bolus therapy</td>
<td>9 [56]</td>
<td>4 [50]</td>
<td>5 [62.5]</td>
</tr>
<tr>
<td>Number [%] with continuous subcutaneous insulin infusion</td>
<td>7 [44]</td>
<td>4 [50]</td>
<td>3 [37.5]</td>
</tr>
</tbody>
</table>

Data are expressed as median with interquartile range in square brackets, or number and percent in square brackets. BMI: body mass index; HbA1c: haemoglobin A1c; IU: international units.
deviation of differences of 6 pmol/L) showed that a total sample size of 14 was necessary to reach statistical significance with a statistical power of 80%. For glucagon, epinephrine, norepinephrine, growth hormone, cortisol, and intact and total GLP-1 and GIP the change in concentration from the initialisation phase to 40 minutes after occurrence of the autonomic reaction was calculated. AUC values were calculated by the trapezoid method. The Friedman Test was used for ANOVA. Post hoc testing was performed with individual Wilcoxon signed rank tests using Bonferroni adjusted p values. Values are presented as median and interquartile range (IQR). p values <0.05 were considered statistically significant.

RESULTS
Patients studied
All 16 patients completed the study (Figure 1). The baseline characteristics are shown in Table 1.

Hypoglycaemic experiments
Table 2 shows median blood glucose concentrations (with IQR in square brackets), physiological responses and symptoms of hypoglycaemia at the initialisation phase, during onset of autonomic reaction and 30 minutes later. No significant differences were apparent between baseline, sitagliptin treatment or placebo administration. The time to onset of the autonomic reaction was 21 [15-25] minutes at the baseline hypoglycaemic experiment, 20 [16-22] minutes after sitagliptin treatment and 21 [21-25] minutes after placebo (p=0.70).

As expected, sitagliptin treatment significantly increased active levels of both GLP-1 and GIP (Figure 2). No significant changes in plasma concentrations or the AUCs from the onset of the autonomic reaction to 40 minutes after onset were observed for glucagon, epinephrine, norepinephrine, cortisol, total or intact GIP or total or intact GLP-1 between the three hypoglycaemia study days. Neither differences between individual time points were observed.

As illustrated in Figure 2, there was a significant difference in the change of growth hormone concentration from the initialisation phase to 40 minutes after onset of autonomic reaction between the three hypoglycaemia study days (37.1 [7.5-95.7] (baseline), 23.0 [1.5-73.4] (sitagliptin), 82.6 [35.3-137.5] mEq/L (placebo), p<0.001). Post hoc pairwise comparisons indicated that the change in growth hormone concentration after sitagliptin treatment was significantly lower than after placebo (p=0.001). Also comparison of the growth hormone concentration 40 minutes after onset of autonomic reaction revealed significant differences between the three hypoglycaemia experiments (64 [4.0-205] (baseline), 23 [0.2-211] (sitagliptin), 90 [9-180] (placebo) mEq/L, p=0.004) with post hoc analysis showing significant difference after the placebo compared to the sitagliptin period (p=0.008). Finally, the AUC
Table 2 - Glucose concentrations, physiological responses and hypoglycaemic symptom profile

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Sitagliptin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IP</td>
<td>R</td>
<td>R +30</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>4.9 [4.8-5.0]</td>
<td>2.1 [2.0-2.2]**</td>
<td>3.4 [2.8-3.7]**</td>
</tr>
<tr>
<td>Physiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (beats/min) (n = 9)</td>
<td>76 [68-80]</td>
<td>84 [77-90]***</td>
<td>-</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg) (n = 9)</td>
<td>70 [61-77]</td>
<td>75 [61-77]</td>
<td>-</td>
</tr>
<tr>
<td>Symptom profile</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autonomic symptoms score</td>
<td>1.0 [1.0-1.3]</td>
<td>1.7 [1.7-2.9]***</td>
<td>1.0 [1.0-1.8]</td>
</tr>
<tr>
<td>Neurologic symptoms score</td>
<td>1.2 [1.1-1.8]</td>
<td>2.1 [1.7-3.4]***</td>
<td>1.5 [1.3-2.2]</td>
</tr>
<tr>
<td>Cognitive dysfunction score</td>
<td>1.0 [1.0-1.3]</td>
<td>1.7 [2.0-2.8]***</td>
<td>1.2 [1.0-1.7]</td>
</tr>
<tr>
<td>Neuroglycopenia symptoms score</td>
<td>1.5 [1.3-2.4]</td>
<td>2.8 [1.9-4.2]***</td>
<td>2.2 [1.8-3.0]</td>
</tr>
</tbody>
</table>

Glucose concentrations, physiological responses and hypoglycaemic symptom profile at initialisation phase (IP), onset of autonomic reaction (R) and 30 minutes after onset of the autonomic reaction. The symptom profile is based on groupings of the Edinburgh Hypoglycaemia Scale during experimentally induced hypoglycaemia. *p<0.05 **p<0.01 ***p<0.001 from IP. Data are expressed as median with interquartile range in square brackets.
for growth hormone (from the onset of the autonomic reaction to 40 minutes after onset) was significantly different between the three hypoglycaemia study days (1,299 [314-2,560] (baseline), 262 [47-1,499] (sitagliptin), 1,406 [781-2,626] mEq/L×min (placebo), \( p = 0.01 \)), but post hoc pairwise comparisons showed no significant differences.

**Safety**

One patient reported dysuria and fever for one week during sitagliptin treatment. When he reported this to the investigator his complaints had already resolved spontaneously and investigation of the urine sediment showed no abnormalities. A report was sent to the The Netherlands Pharmacovigilance Centre (Lareb).

**DISCUSSION**

The present study found no differences in the magnitude of glucagon or catecholamine counterregulatory responses to acute hypoglycaemia in patients with type 1 diabetes after treatment with the DPP-4 inhibitor sitagliptin compared to placebo treatment or baseline measures. Also, physiological and symptomatic responses were similar. The only counterregulatory change was a reduced growth hormone response during treatment with sitagliptin when compared to placebo.

In the present study a continuous intravenous insulin infusion technique was used to resemble the onset of hypoglycaemia similar to that experienced in everyday life. This method leads to a gradual fall in blood glucose leading to acute activation of the autonomic nervous system and the simultaneous onset of hypoglycaemic symptoms. It entails a maximal hypoglycaemic stimulus for the release of counterregulatory hormones, because the threshold for this secretion is situated at a higher blood glucose level than that required to induce symptoms of hypoglycaemia.

The negative findings with regard to glucagon counterregulatory responses of the current study are consistent with a recent publication by Farngren and colleagues who found no difference in the glucagon counterregulatory response in subjects with type 1 diabetes during a hyperinsulinaemic hypoglycaemic clamp at 2.5 mmol/L following treatment with the DPP-4 inhibitor vildagliptin vs. placebo. As mentioned enhanced glucagon response during hypoglycaemia after vildagliptin treatment has been observed in patients with type 2 diabetes patients. An explanation for the difference between type 1 and type 2 diabetes patients may be found in the concept that a decrease in intra-islet insulin concentration in combination with a decrease in alpha-cell glucose is essential for the secretion of glucagon.
Figure 2 - Glucose levels and counterregulatory hormonal responses to hypoglycaemia
Mean glucose and counterregulatory hormonal responses to hypoglycaemia at baseline (clear circles), after treatment with sitagliptin (grey squares) and placebo (black triangles). AUC values from time of onset of autonomic reaction (R) to time R+40 for all hormones are shown separately. IP, initialisation phase; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1. Error bars represent SDs. ** p<0.01, *** p<0.001
DPP-4 inhibition and glucagon response in type 1 diabetes

during hypoglycaemia. This paracrine effect of insulin on the alpha-cell is eventually lost in patients with type 1 diabetes when there is no residual insulin production left.

Interestingly, we observed an attenuated growth hormone response during hypoglycaemia following sitagliptin treatment in our patients with type 1 diabetes. A similar finding was observed in healthy volunteers who received infusion of GLP-1 during insulin-induced hypoglycaemia. This may suggest that intact GLP-1 is a mediator of the reduced growth hormone response after DPP-4 inhibitor treatment in the present study. The mechanism is unclear, but a role for somatostatin is likely. Somatostatin is released in the paraventricular nucleus of the hypothalamus and inhibits growth hormone release from the pituitary. A rich expression of GLP-1 receptors in the paraventricular nucleus has been found in rodents. It is well known that GLP-1 stimulates somatostatin secretion via GLP-1 receptors on delta cells in the pancreas. A similar effect of GLP-1 receptor stimulation on somatostatin release may take place in the paraventricular nucleus and consequently inhibit growth hormone secretion by the pituitary.

In conclusion, this study shows that sitagliptin does not increase the α-cell response to acute hypoglycaemia in subjects with type 1 diabetes and that growth hormone release during hypoglycaemia is attenuated after sitagliptin treatment.

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