Trial Outline – Protocol 300A

Trial ID: NCT02256189

The Effect of Sitagliptin on Glucagon Dynamics and Incretin Hormones During Mild Hypoglycemia in Elderly Patients with Metformin-Treated Type 2 Diabetes (SITACLAMP)

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1. Table of Contacts

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2. Introduction

Incretin hormones are important for normal pancreatic islet function and glucose homeostasis. While glucagon-like peptide 1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) both enhance glucose-dependent insulin secretion, GLP-1 also suppresses glucagon secretion under hyperglycemic conditions, thereby contributing to glucose regulation which is impaired in type 2 diabetes (T2DM) (1). Sitagliptin enhances the endogenous levels of both GLP-1 and GIP by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4), which is responsible for their inactivation (2). Multiple clinical studies with sitagliptin have demonstrated reductions in HbA1c in both monotherapy and add-on combination therapy, with durable maintenance of efficacy over 52 weeks associated with improvements in islet function (2). Furthermore, sitagliptin has been shown to have good tolerability and safety in both monotherapy

and combination therapy. A special characteristic of sitagliptin is the low risk for hypoglycemia.

In patients with T2DM there are several defects in α-cell function, including relative glucagon hypersecretion at normal and elevated glucose levels, and often an impaired response to hypoglycemia (for review see 3). Most of the abnormalities of α-cell function seen in T2DM may be considered to reflect an impairment of glucose sensing. It has previously been shown that sitagliptin reduces glucagon secretion both during an oral glucose tolerance test (4) and after a mixed meal (5). What is importance, however, is that sitagliptin should not only reduce glucagon in the context of hyperglycemia but also sustain glucagon secretion under condition of hypoglycemia, to avoid hypoglycemia. This is important because glucose levels below normal are associated with increased cardiovascular risk due to recurrent local release of catecholamines in the heart every time glucose levels are reduced below 3.3-3.5 mmol/l (6,7). Preservation of α-cell response to severe hypoglycemia (2.5 mmol/l) has previously been documented for another DPP-4 inhibitor, vildagliptin (8), but the glucagon dynamics at higher, but still low, glucose during DPP-4 inhibition is not known. Therefore, this study aims at inducing graded hypoglycemia in patients treated with sitagliptin in combination with metformin to examine the glucagon dynamics as well as incretin hormones (GLP-1 and GIP) and counter-regulatory catecholamine responses and PP (pancreatic polypeptide), a marker of the autonomic nervous system, and cortisol. The hypothesis is that sitagliptin, in comparison with placebo, will protect the glucagon dynamics and counter-regulatory mechanisms at moderate hypoglycemia in metformin-treated subjects with T2DM, which will be a mechanism underlying a potential protection of moderate hypoglycemia by sitagliptin.

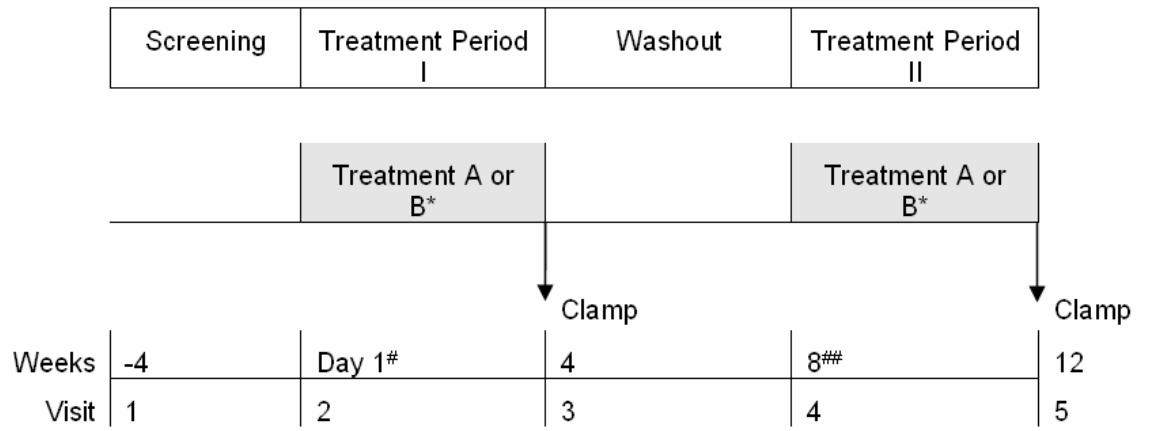
3. Objective of the study

To assess if sitagliptin can improve the glucagon secretory response to mild hypoglycemia in elderly patients with metformin-treated T2DM.

4. Study design

The study is carried out according to this protocol and in compliance with the Declaration of Helsinki and applicable Swedish laws and regulations. The study is a single-center, double-blind, placebo controlled, cross-over study. Twenty-eight patients with T2DM who are treated with metformin (stable dose $\geq$ 0.5 g during the preceeding three months) and HbA1c 6.0-8.5% (43-67 mmol/mol) will be randomized. Each patient will attend one screening visit (Week -4) where the inclusion/exclusion criteria will be assessed. Eligible patients will then be randomized at visit 2 (Day 1) and complete two treatment periods, receiving a different blinded study medication during each period (sitagliptin 100 mg QD and placebo, in random order) in addition to their continued metformin regimen. A baseline visit will be performed during each of the two treatment periods and the blinded study medication will be dispensed for 4 weeks of outpatient treatment. After 4 weeks treatment, a combined meal/hyperinsulinemic hypoglycemic clamp (3.5 and 3.0 mmol/l)/meal re-challenge test will be performed. The meal preceding the clamp is given to ensure a release of incretin hormones and the meal after the clamp to allow glucose to return to baseline in a standardized manner. The blinded study medication will then be discontinued and a 4-week washout period will occur before the next treatment period is started. Also this treatment is 4 week and end with a combined meal/hyperinsulinemic hypoglycemic clamp. The blinded study medication will then be discontinued and the subjects continue the regular treatment with metformin alone.

Figure of Study Design



* patients will receive two treatments in a randomized, *balanced Latin square design*:

A = sitagliptin 100 mg QD; B = placebo;

first day of study medication treatment period I;

first day of study medication treatment period II

5. Study population

The study population will consist of male and female patients with metformin-treated T2DM and HbA1c 6.0-8.5% (inclusive) (43-67 mmol/mol; inclusive), aged ≥65 years. It is planned to screen approximately 56 patients in order to randomize 28.

5a. Inclusion criteria

1. Written consent has been given.

2. Patients with metformin treated T2DM (metformin dose ≥0.5/day and stable during the preceeding 3 months)

3. Age ≥65 years.

4. HbA1c 6.0-8.5% (43-67 mmol/mol; inclusive) at visit 1.

5. Ability to complete the study

5b. Exclusion criteria:

1. Use of other glucose-lowering therapy than metformin within three months prior to visit 1.
2. A history of any secondary forms of diabetes, e.g., Cushing's syndrome and acromegaly.
3. Type 1 diabetes, positive GAD antibodies
4. eGFR $\leq$60 ml/min
5. Acute infections which may affect blood glucose control within 4 weeks prior to visit 1
6. Any history of recent (<2 weeks) recurrent or severe hypoglycemic episodes.
7. Any history of acute pancreatitis
8. Any history of anaphylaxis, angioedema, and exfoliative skin conditions including Stevens-Johnson syndrome.
9. Liver disease such as cirrhosis or chronic active hepatitis
10. History of coronary heart disease or heart failure class III or IV
11. Donation of one unit (500 ml) or more of blood, significant blood loss equaling to at least one unit of blood within the past 2 weeks or a blood transfusion within the past 8 weeks.
12. Treatment with growth hormone of chronic oral or parenteral corticosteroid treatment (> 7 consecutive days of treatment) within 8 weeks prior to visit 1.
13. Use of other investigational drugs at visit 1 or within 30 days of visit 1, unsuitable for the study
14. Hypersensitivity to sitagliptin or any compound in the tablet core (microcrystalline cellulose (E460), calcium hydrogen phosphate, anhydrous (E341), croscarmellose sodium (E468), magnesium stearate (E470b) or sodium stearyl fumarate) or in the film coating (poly(vinyl alcohol), macrogol (E3350), talc (E553b), titanium dioxide (E171), red iron oxide (E172) or yellow iron oxide (E172))

6. Study medication

6a. Study drug

The study drug sitagliptin is a DPP-4 inhibitor meaning that it inhibits the enzyme DPP-4. This enzyme is responsible for the inactivation of the incretin hormones (mainly GLP-1) and therefore the study drugs prevent the inactivation of these hormones. This in turn results in increased plasma levels of the active form of the incretin hormones which through effects on islet hormone secretion result in reduced fasting and postprandial glucose and therefore HbA1c in subjects with T2DM. Sitagliptin is a small molecule which is taken orally once or twice daily. In terms of adverse events, it has been shown to be safe in studies undertaken so far. The study drug was approved by EMA in 2007 and is a well-established glucose-lowering therapy in T2DM world-wide, including in Sweden.

6b. Handling of study medication.

Sitagliptin and placebo are distributed to the Clinical Research Department from the Pharmacy at the University Hospital. Patients will be given a box with 35 tablets (1 tablet à 100 mg daily) which covers the 4 week treatment period at the Clinical Research Department by the Study Nurse. At randomization, subject will receive a written dosage card explaining the dosage of the study medication, i.e., 1 tablet daily. After end of treatment period, remaining study medication will be destroyed by the hospital pharmacy.

7. Study flow and methods

7a. Study flow

Visits are scheduled at Week -4, Day 1, Weeks 4, 8 and 12. On visit days patients should have fasted overnight (i.e. no food or drinks, except water, after 10 pm on the day prior to the scheduled visit). Study visits should occur before 10 am. Medication (sitagliptin/placebo and metformin) should not be taken the morning of study visits.

Table 1 lists all of the assessments and indicates with an "X" the visits when they are performed.

Table 1 Assessment schedule

Visit	1	2	3	4	5
Week	-4	Day 1	4	8	12
Informed Consent	X				
Inclusion/exclusion criteria	X	X			
Record concomitant medications	X	X	X	X	X
Height	X				
Anti GAD/fasting C-peptide	X				
Demography, history of diabetes, medical history	X				
Hyperinsulinemic hypoglycemic clamp (see below)			X		X
Physical examination	X				X
Vital signs, body weight	X	X	X	X	X
ECG,	X				X
Hematology, Liver enzymes, creatinine	X	X	X	X	X
HbA1c, fasting glucose	X	X	X	X	X
Adverse events including hypoglycemia			X	X	X

7b. Methods

Hyperinsulinemic hypoglycemic clamps will be performed at Week 4 and Week 12 following a standardized mixed meal and followed by a standardized meal re-challenge. Patients will arrive at the study site in the morning after an overnight fast (from 10 pm).

Patients should be instructed to bring their study medication to these visits. Blinded study medication (1 tablet sitagliptin or placebo) and the usual morning dose of metformin will be taken 15 minutes before the start of the standardized mixed breakfast. The start of the meal will be designated time 0 (t = 0 min).

The standardized mixed breakfast meal must be consumed in 15 minutes, and will consist of 524 kcal as rye and wheat bread (67% carbohydrate; 60g), 40% enriched margarine (10g), smoked ham from pork with 3% fat (15g), cheese with 17% fat (15g), juice (285g), green paprika (40g), light sour milk with 0.5% fat (200g) and mix-muesli cereal with fruit (40g) with water and non-sweetened coffee or tea. This breakfast consists of 524 kcal with 60% from carbohydrate, 20% from fat and 20% from protein.

After an interval period of 2 hours, in which the patients will rest, the hyperinsulinemic hypoglycemic clamp will start. Patients will receive a fixed-rate infusion of insulin. As necessary, glucose will be infused at a rate that results in the desired plasma glucose concentration. Plasma glucose concentrations will be allowed to decrease to 3.5 mmol/l during the initial 30 min and will be maintained at this target concentration for 30 min. Then, plasma glucose concentrations will be allowed to decrease further to 3.0 mmol/l during the subsequent 30 min and will be maintained at this target concentration for another 30 min. Then, insulin infusion will then be stopped, and a standardized mixed lunch meal (780 kcal) will be served, consisting of meatballs, mashed potatoes, peas, beef Bouillon cream sauce, cranberry, one tomato, juice (200g), whitebeard (15g) and margarine (3g) (70%) with water and non-sweetened coffee or tea. This meal consists of 780 kcal 40% from carbohydrate, 45% from fat and 20% from protein.

Sampling of plasma glucagon, C-peptide, intact GLP-1, intact GIP, PP, cortisol, adrenaline and noradrenaline will be performed at fixed time points as described in Table 2. Blood

glucose concentrations will be determined at the same time points as glucagon until the start of the clamp test (test time -20 min to < 120 min), at 5 min intervals during the hypoglycemic clamp step, as well as at 15 min intervals during the recovery period (see Table 2).

Table 2. Hyperinsulinemic, hypoglycemic clamp schedule

Time (min)	Procedures	Period	Glucagon, GLP-1, GIP, PP and C-peptide	Cortisol, adrenaline, noradrenalilne
-20			X	
-15	Drug intake			
-5			X	X
0	Meal served			
15			X	
30			X	
45			X	
60			X	
90			X	
120	Begin insulin/ glucose infusion	Clamp 1; 3.5 mmol/l	X	X
150			X	X
165			X	X
180		Clamp 2; 3.0 mmol/l	X	X
210			X	X
225			X	X
240	End of infusion/ Meal given		X	X
255			X	
270			X	

300			X	
330		End	X	X

For the hyperinsulinemic, hypoglycemic clamp, patients receive a primed, continuous infusion of insulin with glucose infused at a variable rate to achieve 3.5 mmol/l. At the second hypoglycemic period, glucose infusion rate will be reduced to allow glucose to fall to 3.0 mmol/l. The insulin priming is as follows If initial glucose is below 7.5 mmol/l: 90 ml/h min 0-4, 75 ml/min min 4-7, 60 ml/h min 7-10 and 45 ml/h therafter and If initial glucose is 7.5 mmol/l or above: 150 ml/h min 0-4, 125 ml/min min 4-7, 100 ml/h min 7-10 and 75 ml/h therafter; insulin concentration 400 pmol/m^2).

8. Randomization

Randomization list will be prepared by statistician at Region Skåne and study medication will be distributed to the patient according to the randomization. The medication will be packed in 1 box per patient with 35 tablets in each box.

9. Data management and safety registration

Registration. The data are recorded in case and visit notes (hospital records) and entered in the CRF containing demographic and medical information, laboratory data, electrocardiograms, and the specific study-related results of any other tests or assessments. Specific worksheets will be the source documents for the variables derived from the hyperinsulinemic hypoglycemic clamps. These will be stored in the CRF. A certified study monitor will regularly inspect the CRFs to ensure accuracy with source data.

Safety. At first visit, study subjects are informed of the characteristic symptoms of acute pancreatitis (persistent and abdominal pain). Adverse events are acute pancreatitis and hypoglycemia and all other symptoms reflecting a change in the health situation

reported by the participating subjects. Adverse events are sought by non-directive questioning of the patient at each visit during the study, except directive questioning of symptoms of acute pancreatitis and hypoglycemia. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments. All adverse events, including hypoglycemias, are recorded in the clinical case note and CRF and classified as of mild, moderate or severe degree, and a judgment whether it is related or unrelated to study medication. Hypoglycemia is defined as either a)symptomatic hypoglycemia, b)confirmed hypoglycemia (blood glucose $\leq$3.1 mmol/l) or c)hypoglycemia episode that require assistance for control.

The SmPC will be used for Reference Safety Information (RSI) for expected Serious Adverse Events (SAE). These are defined as any untoward medical occurrence that

- Results in death
- Is life threatening (which refers to an event/reaction in which the patient was at risk of death at the time of the event/reaction; it does not refer to an event/reaction which hypothetically might have caused death if it were more severe),
- Requires inpatient hospitalization or results in prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a medically important event or reaction.

Medical and scientific judgment should be exercised in deciding whether other situations should be considered serious, such as important medical events that might not be immediately life-threatening or result in death or hospitalization, but might jeopardize the patient or might require intervention to prevent one of the other outcomes listed in the definition above.

The investigator informs the sponsor on an SAE within 24 hours and an AE within 7 days and the sponsor reports SAE to the sponsor and to MSD (through fax 08-578 13 900) within 24 hours.

Access to the randomization code during the study. The code must be broken when knowledge of the study treatment is essential for patient´s management or if required for a regulatory purpose or in case of Serious Unexpected Suspected Adverse Reaction (SUSAR). In such cases, the investigator or responsible representative contact the hospital pharmacy who has 24h access to a decode envelope. SUSAR will also be reported to the health authorities (through the EudraVigilance database) and unblinded by the sponsor. Each treatment will be supplied to the local pharmacy with a decoding material that contains the name of the treatment. It is the responsibility of the pharmacist to ensure that these decoding materials are stored safely, but are readily available to relevant staff. If the blind is broken, the investigator will document the date, the time of the day, and the reason for code breaking.

10. Withdrawal criteria

A subject may be withdrawn from the trial at the discretion of the Sponsor if judged non-compliant with trial procedures or due to a safety concern.

A subject must be withdrawn if the following applies:

1. Withdrawal of consent: The subject may withdraw at will at any time.
2. Protocol deviation: If a protocol violation or concurrent illness occurs, which, in the clinical judgement of the Sponsor, may invalidate the trial, the subject will be withdrawn by the Sponsor.
3. Change in medication: If a subject changes the dose of their existing medications during the trial, which, in the clinical judgement the Sponsor, may invalidate the trial, the subject will be withdrawn by the Sponsor.
4. If a subject reports symptoms, which are considered unacceptable by the subject or the Sponsor, such as acute pancreatitis, the subject will be withdrawn from the trial.

11.End-points

Primary end-point is the effect of sitagliptin on glucagon counter-regulation to hypoglycemia measured as Δglucagon (min 180-150) and Δglucagon (min 240-210)

Secondary end-points are

the effect of sitagliptin compared to placebo on the counter-regulatory hormones cortisol, adrenaline and noradrenaline at the hypoglycemic clamp steps, determined as Δ for these variables min 180-150 and 240-210, respectively,

the effect of sitagliptin compared to placebo on the insulin secretory rate estimated from the C-peptide responses and GLP-1 and GIP levels at the hypoglycemic clamp steps determined as Δ for these variables min 180-150 and 240-210, respectively.

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12.Sample size calculation

The sample size calculation was based on detecting a difference between sitagliptin and placebo in the mean glucagon level of the last 30 min of a hypoglycemic clamp step, after 4 weeks of treatment. In our previous study on the effect of the DPP-4 inhibitor vildagliptin on the glucagon counter-regulation to hypoglycaemia (8) we found that the 30 min glucagon response to steady-state hypoglycaemia during a clamp was 34±7 ng/l. Based on these data and expecting similar variation in the data in the elderly population studied here, a power analysis aiming at showing a 30% difference in glucagon levels by sitagliptin with 90% sensitivity results in requirement of 24 subjects when doing paired analysis. To allow for even more safe statistics and allow for discontinuation, we will recruit four additional subjects; hence we aim at including 28 patients in the study.

13.Statistical evaluation

The variables (glucagon, C-peptide, GLP-1, GIP, PP, cortisol, adrenaline and notradrenaline responses to hypoglycemia) will be analyzed using a 2-sided test for superiority of sitagliptin versus placebo. The Completers population alone will be used.

14.Monitoring, data handling and archiving

The study will be monitored by a certified monitor who is not involved in the study. Study documentation, including informed consent, CRF and laboratory test results, will during the proceeding of the study be kept at the Clinical Research Unit. Sponsor and study nurse will have access to these documents. For statistical analysis, anonymous data file will be created at the Biomedical Center, Lund University. Sponsor will have access to this file. After completion of the study, all documents will be archived at the Biomedical Center at Lund University for15 years.

15.Risk-benefit analysis

This is a study aiming at understanding the glucagon counter-regulation to hypoglycemia after treatment with the DPP-4 inhibitor sitagliptin in patients with metformin-treated type 2 diabetes. Since the study is a mechanistic study to understand whether the low risk of hypoglycemia seen during treatment with sitagliptin is mediated by enhanced counter-regulation, the study is of no immediate benefit for the participating patients. The results of the study will, however, be beneficial for the patient group treated with this therapy since it will provide rationale for future treatment by allowing the most efficacious therapy to prevent hypoglycemia to be prescribed to elderly subjects with T2DM. The risk for the patients participating in the study is minimal since sitagliptin has been shown to be safe with low risk of adverse events, even in long-term studies, and the risk with hyperinsulinemic hypoglycemic clamp is minimal in view of the extensive experience with this test at the Clinical Research Department.

16.Insurance

Insurance is covered by the regular healthy authorities.

17. Study timeline:

- Q4/2014: Obtaining all permissions and planning of the logistics
- Q1/2015: Screening of patients
- Q1-Q4/2015: Performing the study with FPFV planned for January 2015 and LPFV planned for October 2015
- Q1/2016: Analyses

18. Publication

Results will be presented at international meetings and published in international scientific journal.

19. References

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Bo Ahrén, sponsor