Trial Outline – Protocol 350A

Trial ID: NCT 02475070 (EudraCT 2015-001334-21)

Study of the Effect of Vildagliptin versus Dapagliflozin on Glucagon Response to Mixed Meal in Metformin-treated Subjects with Type 2 Diabetes

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1. Table of Contacts

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2. Introduction

Both dipeptidyl peptidase-4 (DPP-4) inhibition and sodium glucose-cotransporter-2 inhibition (SGLT2-) are glucose-lowering strategies in type 2 diabetes, although with different mechanisms of action. Thus, DPP-4 inhibition works mainly on the islets to stimulate insulin secretion and inhibit glucagon secretion (1) whereas SGLT-2 inhibitors work mainly on the kidney to facilitate glucose excretion (2). A main indication of these two treatment strategies is as add-on to metformin in subjects with inadequate glycemic control when given metformin in monotherapy.

A potentially important differentiation between the therapies is their different actions on alpha-cell function. Thus, DPP-4 inhibition, as we have shown with vildagliptin, is

known to inhibit glucagon response to mixed meal ingestion and this inhibition is associated with the improved glycemia (3). In contrast, treatment with SGLT-2 inhibition has been shown to increase glucagon secretion which increases liver glucose production (4). This effect of SGLT-2 inhibition may be an adaptative response to the increased glucose excretion which may counteract a glucose-lowering action. It thus seems that the two approached of reducing glucose have opposite effects on a key factor underlying type 2 diabetes, the augmented glucagon response. However, it is not known to what degree glucagon secretion is differently regulated by DPP-4 inhibition versus SGLT-2 inhibition in the clinical context as add-on to metformin and it is also not known to what degree a differentiation on glucagon secretion affects glucose homeostasis. One reason for this lack of knowledge in that this has not been tested in head-to-head-studies. Such information is important when designing the most appropriate pathophysiological treatment of the disease.

Therefore, in this study, the DPP-4 inhibitor vildagliptin or the SGLT-2 inhibitor dapaglifozin will be added to metformin in subjects treated with metformin as monotherapy and their respective effects on meal-induced glucagon secretion will be evaluated.

3. Objective of the study

To assess if vildagliptin and dapaglifolizin have dissociated effects on glucagon secretion after a mixed meal ingestion in metformin-treated subjects with type 2 diabetes and whether this is associated with effects on glucose homeostas

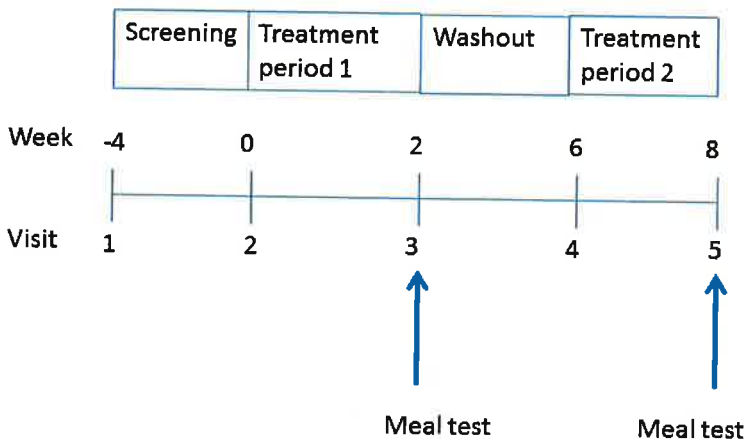
4. Study design

The study will be carried out according to this protocol and in compliance with the Declaration of Helsinki and applicable Swedish laws and regulations. This is an open label cross-over study. Twenty-eight patients with metformin-treated type 2 diabetes and HbA1c 43-72 mmol/mol (6.0-9.0%) 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 study medication during each period (vildagliptin 50 mg BID and dapaglizlozin 10 mg QD, in random order) in addition to their continued metformin regimen. A baseline visit will be performed during each of the two treatment periods, at which the patient will be assessed and the study medication will be dispensed for two weeks of outpatient treatment. After two weeks of treatment, a mixed meal will be served. The standardized mixed meal must, which will be consumed in 15 minutes, consists 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 decaffeinated coffee/tea. The meal consists of 524 kcal with 60% from carbohydrate, 20% from fat and 20% from protein.

The study medication will then be discontinued and a four-week washout period will occur before the next two week treatment period is started. This two-week treatment period will end with ingestion of a mixed meal, as after the first treatment period.

Figure of Study Design



Each patient will therefore receive two treatments in a randomized design:

Vildagliptin 50 mg twice daily (BID) during weeks 0-2 and Dapagliflozin 10 mg once daily (QD) during weeks 6-8 or

Dapagliflozin 10 mg QD during weeks 0-2 and Vildagliptin 50 mg BID during weeks 6-8

The study medication will not be blinded.

5. Study population

The study population will consist of male and female patients with metformin-treated type 2 diabetes and HbA1c 6.0-9.0% (inclusive) (43-72 mmol/mol; inclusive), aged 20-75 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 type 2 diabetes treated with a stable dose of metformin during the last three months
3. Age 20-75 years.
4. HbA1c 6.0-9.0% (43-72 mmol/mol) 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 within preceeding 6 months 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 vildagliptin or dapagliflozin or any compound in the tablet core

6. Study medication

6a. Study drug

The study drug vildagliptin 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 type 2 diabetes. Vildagliptin is a small molecule which is taken orally 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 2008 and is a well-established glucose-lowering therapy in type 2 diabetes world-wide, including in Sweden.

The study drug dapagliflozin is a SGLT-2 inhibitor meaning that it inhibits the enzyme SGLT-2. This enzyme is responsible for reabsorbing glucose in the renal tubule and therefore to prevent glucosuria. Inhibition of this enzyme, therefore, inhibits glucose reabsorption which increases glucose excretion in the urine which in turn reduces circulating glucose in subjects with type 2 diabetes which results in lowering of HbA1c. Dapagliflozin is a small molecule which is taken orally once daily. In terms of adverse events, it has been shown to increase the risk of genital infections but apart from that to be safe in studies undertaken so far. The study drug was approved by EMA in 2012 and is now a well-established glucose-lowering therapy in type 2 diabetes world-wide, including in Sweden.

6b. Handling of study medication.

Vildagliptin and dapagliflozin are distributed to the Clinical Research Department from the Pharmacy at the University Hospital. Patients will be given a box with for vildagliptin 28 tablets à 50 mg which covers the 2 week treatment period at the dosage of BID. For dapagliflozin, patients will be given a box with 14 tablets à 10 mg which covers the 2 week treatment period at the dosage of QD. The boxes will be given to the patients at the Clinical Research Department by the Study Nurse. At randomization, subject will receive a written dosage card explaining the dosage of the study medications. 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 2, 6 and 8. 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 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	2	6	8
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				
Urine pregnancy test (for premenopausal women)	X	X	X	X	X
Mixed meal test			X		X
Physical examination	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

A mixed meal will be ingested at Week 2 and Week 8 following. 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. Study medication (vildagliptin or dapagliflozin) 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.

Sampling of blood for determination of plasma levels of glucose, glucagon, insulin, C-peptide, intact and total GLP-1, intact and total GIP will be performed at fixed time points as described in Table 2.

Table 2. Sampling in the mixed meal test

Time (min)	Procedures	Blood sample for analysis of hormones
-30		
-25		
-20		X
-15	Drug intake	
-10		
-5		X
0	Meal served	
5		X
10		X
15		X
20		
30		X

45		X
60		X
75		X
90		X
120		X
150		X
180		X
240		X

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 study medication will not be blinded and all patients will receive both vildagliptin and dapagliflozin in a cross-over design. The randomization, therefore, is in which order the study medication will be taken.

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 mixed meal test. 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 within 24 hours.

DSUR (Development Safety Update Report) and Serious Unexpected Suspected Adverse Reaction (SUSAR) will be reported to the Medical Products Agency (through the EudraVigilance database) by the sponsor.

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 on the area under the curve (AUC) of the 180 min glucagon levels after mixed meal ingestion

Secondary end-points are the effect on AUC_{180} of GLP-1, GIP, insulin, C-peptide and estimated ISR (insulin secretory rate).

12.Sample size calculation

The sample size calculation was based on detecting a difference between vildagliptin and dapagliflozin in the AUC of glucagon level during the 300 min after mixed meal ingestion after 2 weeks of treatment. Previous studies on the effect of vildagliptin (3) and dapagliflozin (4) showed a reduction of glucagon by vildagliptin and an increase of glucagon by dapagliflozin. Based on these data and expecting similar variation in the data in the population studied here, a power analysis aiming at showing a 30% difference in glucagon levels by the two drugs 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 will be analyzed using a 2-sided paired test. 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 response to a mixed meal ingestion after two weeks treatment with the DPP-4 inhibitor vildagliptin and the SGLT2-inhibitor dapagliflozin in patients with metformin-treated type 2 diabetes. Since the study is a mechanistic study, it 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 pathophysiological treatment to be prescribed to subjects with type 2 diabetes. The risk for the patients participating in the study is minimal since both drugs have been shown to be safe with low risk of adverse events, even in long-term studies, with the exception of genital infections with dapagliflozin. Furthermore, the risk with the mixed meal test 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:

- Q2/2015: Obtaining all permissions and planning of the logistics
- Q3/2015: Screening of patients
- Q4/2015-Q2 2016: Performing the study with FPFV planned for October 2015 and LPFV planned for April 2016
- Q2/2016: Analyses

18. Publication

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

19. References

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Lund, March 28, 2016

Bo Ahrén, sponsor