
Clinical Study Protocol

Drug Substance <<◇>>

Study Code <<◇>>

Edition Number <<◇>>

Date <<June 11th, 2018>>

<< Impact on glycaemic variability after treatment with dapagliflozin as dual therapy with metformin on Mexican type 2 diabetes patients. A randomized, open-label study.>>

1. Project Title				
Impact on glycaemic variability after treatment with dapagliflozin as dual therapy with metformin on Mexican type 2 diabetes patients. A randomized, open-label study				
2. Protocol Version				
Version 2, December 4th, 2018				
3. Type of Investigation				
Type of investigation		Select an option		
Pharmacologic		x		
Biological				
Epidemiologic				
Interchangeability				
Other				
4. Investigators				
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5. Participant Institutions

Institution	Did the institution accepted the protocol?
Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán Vasco de Quiroga 15 Belisario Domínguez, Sección XVI, 14080 Mexico City, México	Yes

6. Grant

6a. Sponsor

Astra Zeneca México will provide the resources defined in the contract to acquire investigational drug, laboratory data, medical writing, costo f publication and glucose lowering sensors.

6b. Specify if investigators will receive payment for their participation in the protocol.

No

7. Summary

Glycaemic variability is refered as swings in glucemic concentration throughout the day, including preprandial and postprandial glucose, and it has been proposed that could be determinant in the development in microvascular complications of type 2 diabetes (Brownlee and Hirsch 2006)

SGLT2 inhibitors (SGLT2i) are a novel group of medications for treating type 2 diabetes patients but their effect on glucose variability has not been determined as a primary endpoint in clinical trials of type 2 diabetes mellitus patients. The aim of this study is to compare the effect of SGLT2 inhibition on glucose variability on new onset type 2 DM patients. Methods: We will include 88 patients with type 2 diabetes diagnosis with an HbA1c $\geq 7.5\%$ and $\leq 9\%$, with BMI > 25 and $< 45 \text{ kg/m}^2$, drug-naïve subjects.

There will be a pre-randomization run-in period in which subjects will receive metformin 500mg twice daily for two weeks and then patients that tolerate this dose will be randomized 1:1 to either receive a daily dosage of dapagliflozin 10 mg and 2000 mg metformin for 12 weeks (n=40) or 2000 mg metformin (n=40). Patients who do not tolerate metformin at 2000mg dose will be downtitrated to 1500 mg daily, patients who do not tolerate metformin at 1500mg daily, will be downtitrated to 1000 daily. In case patients do not tolerate 1000 mg daily, they will be excluded.

Both groups will be monitored for 7 days using either iPro™ CGM system (Medtronic, Northridge, CA) or Dexcom G6 CGM (Dexcom Inc, San Diego, CA). Basal continuous glucose monitoring will start at week 1 (first visit), and removed at day 7 and final continuous glucose monitoring will start at week 11 and removed 7 days after (final visit).

The main variables of interest are going to be in order of importance the delta of change (before and after study entry) of: 1.- glycaemic variability, 2.- Change in insulin level concentrations 3.- change in HbA1c. 4.- change in weight, 5.- Blood pressure and 6.- waist circumference; before and after SGLT2i. The expected results are: compared to standard treatment, dapagliflozin arm will have lower glycaemic variability, higher reduction in HbA1c and lower blood pressure.

8. Background

Glycaemic variability is referred to as swings in glucose concentration throughout the day, including preprandial and postprandial glucose, and it has been proposed that it could be a determinant in the development of microvascular complications in type 2 diabetes (Brownlee and Hirsch 2006). SGLT2 inhibitors (SGLT2i) are a novel group of medications for treating type 2 diabetes patients, but their effect on glucose variability has not been determined as a primary endpoint in clinical trials of type 2 diabetes mellitus patients.

9. Problem definition

There is little scientific evidence regarding the dapagliflozin effect on glycaemic variability in patients with type 2 diabetes.

10. Justification

Patients with type 2 diabetes regularly have either overweight and obesity, and hypertension. In patients with these characteristics, dapagliflozin is often used as adjuvant treatment. This therapeutic decision is supported by international diabetes guidelines.

11. Hypothesis

Compared to standard treatment, the dapagliflozin arm will have lower glycaemic variability, higher reduction in HbA1c and lower blood pressure.

12. Objectives.

Primary objective:

To describe the effect of dapagliflozin therapy on glycaemic variability in type 2 diabetes patients.

Outcome measure:

Mean difference of glycaemic variability (MAGE) in mmol/L

Secondary objectives

1. To describe the effect of dapagliflozin on insulin serum concentration, HbA1c, on weight, blood pressure, waist circumference after 12 weeks of treatment on subjects with type 2 diabetes

Outcome measures:

- Mean difference insulin serum concentrations $\mu\text{U/mL}$
- Mean difference of %HbA1c
- Mean difference in kilograms
- Mean difference in mmHg
- Mean difference waist measured in centimeters

13. Methodology: Design.

The aim of this study is to compare the effect of SGLT2 inhibition on glucose variability in new-onset type 2 DM patients. Methods: We will include 88 patients with type 2 diabetes diagnosis with an HbA1c $\geq 7.5\%$ and $\leq 9\%$, with BMI > 25 and $< 45 \text{ kg/m}^2$, drug-naïve subjects.

There will be a pre-randomization run-in period in which subjects will receive metformin 500mg twice daily for two weeks and then patients that tolerate this dose will be randomized 1:1 to either receive a daily

dosage of dapagliflozin 10 mg and 2000 mg metformin for 12 weeks (n=44) or 2000 mg metformin (n=44). Patients who do not tolerate metformin at 2000mg dose will be downtitrated to 1500 mg daily, patients who do not tolerate metformin at 1500mg daily, will be downtitrated to 1000 daily. In case patients do not tolerate 1000 mg daily, they will be excluded. Patients who do not achieve glycaemic control, another antihyperglycaemic drug can be used (See section 20, rescue therapy).

Both groups will be monitored for 7 days using either iPro™ CGM system (Medtronic, Northridge, CA) or Dexcom G6 CGM (Dexcom Inc, San Diego, CA), or other available in Mexico. Basal continuous glucose monitoring will start at week 1 (first visit), and removed at day 7 and final continuous glucose monitoring will start at week 11 and removed 7 days after (final visit).

The main variables of interest are going to be in order of importance the delta of change (before and after study entry) of: 1.- glycaemic variability, 2.- Change in insulin level concentrations 3.- change in Hba1c. 4.- change in weight, 5.- Blood pressure and 6.- waist circumference; before and after SGLT2i. The expected results are: compared to standard treatment, dapagliflozin arm will have lower glycaemic variability, higher reduction in Hba1c and lower blood pressure.

14. Temporality.

Type of study	Select an option
Retrospective	
Prospective	X

15. Process of assignment of the groups under study

Maneuver	Yes	No	NA
Randomization	Simple randomization (using randomization.com)		
Open study	X		
Blind study			
Double blind study			
Triple blind study			

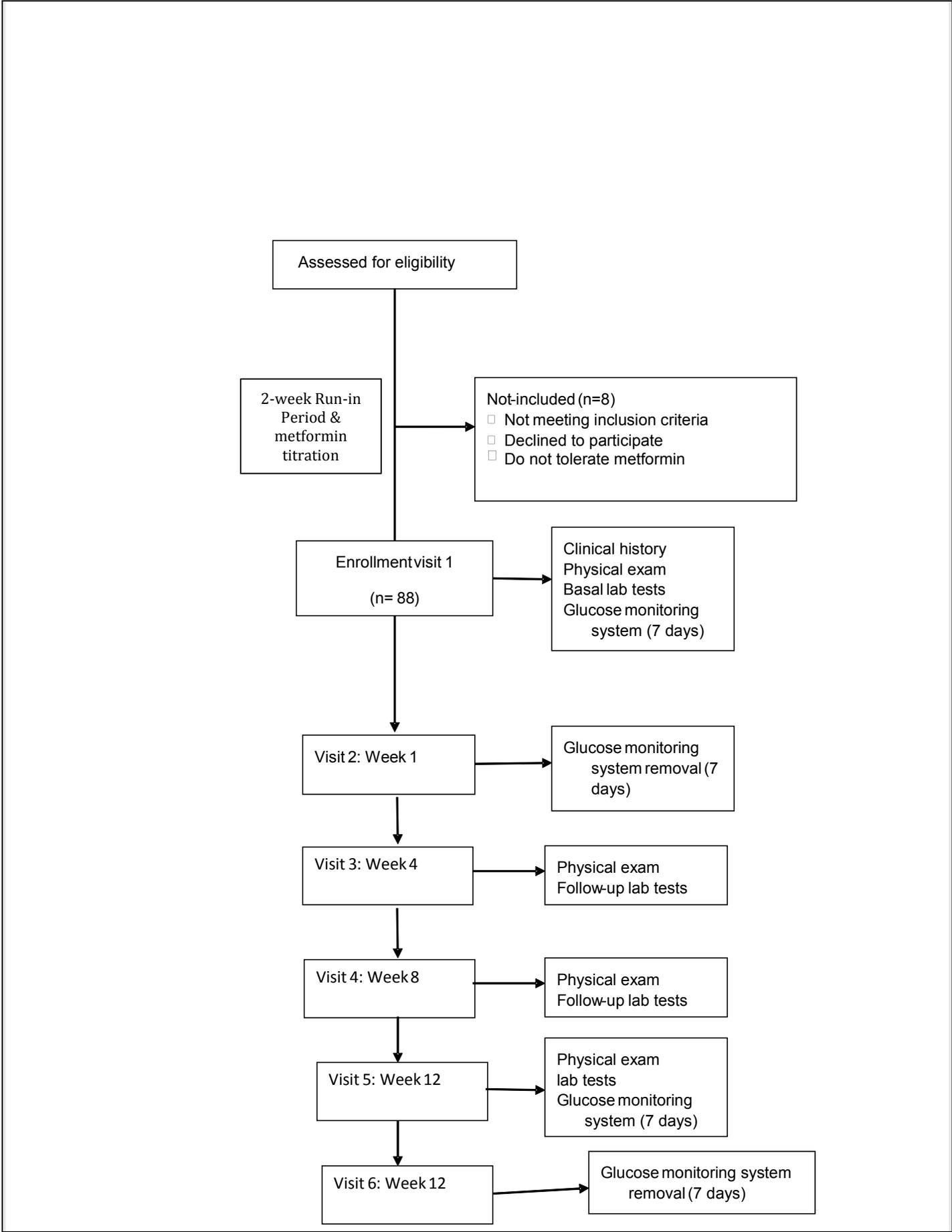
16. Description of maneuvers or interventions

After a pre-randomization run-in period in which subjects will receive metformin 500mg twice daily for two weeks, subjects enrolled will be randomized 1:1 to either receive a daily dosage of dapagliflozin 10 mg and 2000 mg metformin for 12 weeks (n=18) or 2000 mg metformin (n=18). Patients who do not tolerate metformin at 2000mg dose will be downtitrated to 1500 mg daily, patients who do not tolerate metformin at 1500mg daily, will be downtitrated to 1000 daily. In case patients do not tolerate 1000 mg daily, they will be excluded.

Both groups will be monitored for 7 days using either iPro™ CGM system (Medtronic, Northridge, CA) or Dexcom G6 CGM (Dexcom Inc, San Diego, CA). Basal continuous glucose monitoring will start at week 1 (first visit), and removed at day 7 and final continuous glucose monitoring will start at week 11 and removed 7 days after (final visit).

The main variables of interest are going to be in order of importance the delta of change (before and after study entry) of: 1.- glycaemic variability, 2.- Change in insulin level concentrations 3.- change in Hba1c. 4.- change in weight, 5.- Blood pressure and 6.- waist circumference; before and after SGLT2i. The expected results are: compared to standard treatment, dapagliflozin arm will have lower glycaemic variability, higher reduction in Hba1c and lower blood pressure.

The timemetable is shown below:



17. Treatments (if applicable)			
Drug 1	Information	No	NA
Name	Dapagliflozin		
Does it comply with "Good manufacturing practices"?	YES		
Codes, labeling, storage, retention and protection of drug samples	YES		
Pharmaceutical form	Tablets		
Dose	10 mg/day		
Interval of administration	Daily		
Route of administration	Oral		
Velocity of administration	With 1 glass of water, immediate		
Duration of treatment	12 weeks		
Drug 2	Information	No	NA
Name	Metformin		
Does it comply with "Good manufacturing practices"?	YES		
Codes, labeling, storage, retention and protection of drug samples	YES		
Pharmaceutical form	Tablets		
Dose	1000 mg/day		
Interval of administration	Twice daily		
Route of administration	Oral		
Velocity of administration	With 1 glass of water, immediate		
Duration of treatment	12 weeks		
18. Follow-up			
	Information	No	NA
Number of phases of the study	1		
Number of visits and Schedule	6		
Duration of each phase of the study	12 weeks		
Lab tests that will be used.	BH, QS, HbA1c, glycaemic variability, insulin .		
Total duration of follow-up	12 weeks		
Methods Métodos de muestreo	Randomized		
Treatment options that will be offered at the end of the study.	Metformin and standard of care		
19. Overdose management.			
If a Patient for accident ingests more tablets than recommended, blood pressure, glucose will be assessed closely every 4 hours. Dapagliflozin, due to its mechanism of action, do not cause hypoglycemia.			
20. Rescue therapy			

Group 1: Dapagliflozin 10 mg/day + metformin 2000mg/day: glimepiride, glibenclamide, DPP4i (1st line) or insulin (2nd line).

Group 2: Metformin 2000mg/day: glimepiride, glibenclamide, DPP4i (1st line) or insulin (2nd line).

21. Concomitant therapies allowed.

Group 1 (Dapagliflozin + metformin): NSAIDs, acetaminophen, antibiotics, statin, ezetimibe, ACE/ARB2 inhibitors, Serotonin recapture inhibitors, bezafibrate, amlodipine, b-blocker, and other pharmacologic groups that according to investigators does not interfere with the protocol.

Group 2 (metformin): NSAIDs, acetaminophen, antibiotics, statin, ezetimibe, ACE/ARB2 inhibitors, Serotonin recapture inhibitors, bezafibrate, amlodipine, b-blocker, and other pharmacologic groups that according to investigators does not interfere with the protocol.

22. Concomitant therapies prohibited.

Group 1 (Dapagliflozin + metformin):GLP-1 RA, corticosteroids, other SGLT2i.

Group 2 (metformin): GLP-1 RA, corticosteroids, other SGLT2i.

23. Definition of the monitoring variables

The main variables of interest are going to be in order of importance the delta of change (before and after study entry) of: 1.- glycaemic variability, 2.- Change in insulin level concentrations 3.- change in Hba1c. 4.- change in weight, 5.- Blood pressure and 6.- waist circumference; before and after SGLT2i. The expected results are: compared to standard treatment, dapagliflozin arm will have lower glycaemic variability, higher reduction in Hba1c and lower blood pressure.

Outcome measure for main variable:

Mean difference of glycaemic variability (MAGE) in mmol/L

Outcome measure for other variables:

- Mean difference insulin serum concentrations $\mu\text{U/mL}$
- Mean difference of HbA1c (in %)
- Mean difference in kilograms
- Mean difference in mmHg
- Mean difference waist measured in centimeters

24. Methods that will be used to collect information

A physical data capture sheet will be made, only the initials of patients and the internal number of the protocol will be saved.

25. Criteria of failure and success

Fail: There is no decrease in glycaemic variability with the use of dapagliflozin

Success: There exists decrease in glycaemic variability with the use of dapagliflozin

26. Sample size

Sample size was determined using t distribution:

$$n = (Z_{\alpha/2} + Z_{\beta})^2 * 2 * \sigma^2 / d^2,$$

Where $Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$ (e.g. for a confidence level of 95%, α is 0.05 and the critical value is 1.96), Z_{β} is the critical value of the Normal distribution at β (e.g. for a power of 80%, β is 0.2 and the critical value is 0.84), σ^2 is the population variance, and d is the difference you would like to detect.

We substitute the critical values of the Z distribution to the T distribution using 71 degrees of freedom

$T_{\alpha/2}$

1.99/2 critical value p 0.05 of α
Critical value 0.20 (β) = 1.29

$N = (0.995 + 1.29)^2 * 2 * 30^2 / 15.3^2$
 $N = 5.221225 * 2 * 900 / 234.09$
N= 40 per group

Total 80 subjects + considering 10% of loss = **88 subjects**

27. Description of techniques, instruments and appliances that will be used in measurements

Blood pressure will be measured considering the mean of two determinations after seating for at least 5 minutes. Mean arterial pressure (MAP) will be calculated with the formula: (systolic blood pressure * 0.33) + (diastolic blood pressure * 0.66). Anthropometric variables will be measured using standardized techniques; these included height, weight, waist and hip circumferences, and quantification of body fat using bioelectrical impedance with the Jawon scale model IOI 353 - JMW160 . BMI will be calculated using the standard equation, weight/height² (kg/m²). After at least eight-hour fasting, glucose, lipid profile (total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides), creatinine, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albuminuria in a 24 h urine collection will be measured using an automated International Journal of Nephrology 3 analyzer (Synchron CX Beckman, Fullerton CA). LDL cholesterol (LDL) will be estimated by the Friedewald equation (LDL= total cholesterol -HDL - triglycerides /5)[23]. Glycated hemoglobin (A1c) was measured using HPLC (Variant II Turbo, Biorad), certified by the National Glycohemoglobin Standardization Program (NGSP). Creatinine clearance will be calculated using the CKD-EPI equation. All samples Will be measured in the Laboratorio Central of Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran (<http://www.innsz.mx/opencms/contenido/departamentos/labcentral/>)

Insulin levels will be measured using ELISA Kits (Merck Millipore, USA)

100% of the samples are certified by the College of American Pathologists (71893-07-01)

28. Description of evaluation sheets, questionnaires, etc, that will be used.

We will use the traditional medical history datasheet. An exercise questionnaire will be used (GPAQ questionnaire, World Health Organization: www.who.int/chp/steps) and a 7-days homemade food registry.

29. Does the protocol involve the handling and labeling of biological samples? If applicable, mention the procedures that will be used.

Yes, with patients initials.

30. Corresponding information to ensure that the biological samples obtained will not be used for permanent or immortal cell lines or purposes unrelated to the study.

N/A

31. Description of treatment groups.

We will include 88 patients with type 2 diabetes diagnosis with an HbA1c $\geq 7.5\%$ and $\leq 9\%$, with BMI > 25 and < 45 kg/m², drug-naïve subjects. Subjects enrolled will be randomized 1:1 to either receive a daily dosage of dapagliflozin 10 mg and 2000 mg metformin for 12 weeks (n=18) or 2000 mg metformin (n=18). Patients who do not tolerate metformin at 2000mg dose will be downtitrated to 1500 mg daily, patients who do not tolerate metformin at 1500mg daily, will be downtitrated to 1000 daily. In case patients do not tolerate 1000 mg daily, they will be excluded.

Both groups will be monitored for 7 days using either iPro™ CGM system (Medtronic, Northridge, CA) or Dexcom G6 CGM (Dexcom Inc, San Diego, CA). Basal continuous glucose monitoring will start at week 1 (first visit), and removed at day 7 and final continuous glucose monitoring will start at week 11 and removed 7 days after (final visit).

32. Mechanisms for treatment assignment

Randomization.com tool will be used.

A Randomization Plan

from

<http://www.randomization.com>

1. metformina + tratamiento estándar _____
2. Dapagliflozina + metformina _____
3. metformina + tratamiento estándar _____
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86. metformina + tratamiento estándar _____
87. metformina + tratamiento estándar _____
88. Dapagliflozina + metformina _____

88 subjects randomized into 22 blocks
To reproduce this plan, use the seed 9476

along with the number of subjects per block/number of blocks
and (case-sensitive) treatment labels as entered originally.
Randomization plan created on 22/5/2019 13:46:39

33. If you include a placebo group, include the justification below.

NA

34. Criteria for the premature withdrawal of the study

Allergie due to Dapagliflozin, severe illness that compromise life, pregnancy during the study. Patients who present acute kidney injury (decrease in >40% of GFR) Patients that do not tolerate at least 1000 mg /day metformin.

35. Procedures for the removal of a patient from the study

The patient will be notified of the withdrawal from the study and will be given the standard treatment of any patient with type 2 diabetes mellitus.

36. Criteria for the premature (partial or complete) suspension of the study

N/A

37. Selection criteria

a) Inclusion criteria

- Subjects > 18-77 years-old
- Both Male and female
- Hba1c $\geq 7.5\%$ and $\leq 9\%$
- BMI > 25 and <45 kg/m²
- Type 2 diabetes diagnosis, drug-naïve

b) Exclusion criteria

- Hba1c > 9%
- Creatinine clearance CKD-EPI: < 60 mL/min
- LADA or Type 1 diabetes
- Gestational diabetes
- Clinically significant disease like: hepatic, hematological, oncological, psychiatric or rheumatic disease.>>
- Symptoms of marked uncontrolled diabetes: (marked poliuria or polydipsia + 10% weight loss prior the last 3 months enrollement)
- Known hypersensitivity to dapagliflozin or any of the excipients of the product
- eGFR persistently <45 mL/min/1.73 m²
- Unstable or rapidly progressing renal disease
- Patients with severe hepatic impairment (Child-Pugh class C)
- Any major CV event/Vascular Disease within 3 months prior to signing the consent at enrolment, as assessed by the investigator
- For women only - currently pregnant (confirmed with positive pregnancy test) or breast-feeding.

c) Elimination criteria

- Allergie due to Dapagliflozin, severe illness that compromise life, pregnancy during the study. Patients who present acute kidney injury (decrease in >40% of GFR). Patients that do not tolerate at least 1000 mg /day metformin.
- For women only – pregnancy confirmed with serum chorionic gonadotropin. In case of pregnancy during the study, the subject has to be eliminated from the trial, will be offered standard treatment and Pregnancy Outcome Report (Annex) has to be filled to record the mother's essential details. Part I should be filled in after the pregnancy has been identified and Part II is to record the outcome of the pregnancy.

38. Outcome and variables

The main variables of interest are going to be in order of importance the delta of change (before and after study entry) of: 1.- glycaemic variability, 2.- Change in insulin level concentrations 3.- change in Hba1c. 4.- change in weight, 5.- Blood pressure and 6.- waist circumference; before and after SGLT2i. The expected results are: compared to standard treatment, dapagliflozin arm will have lower glycaemic variability, higher reduction in Hba1c and lower blood pressure.

Outcome measure for main variable:

Mean difference of glycaemic variability (MAGE) in mmol/L

Outcome measure for other variables:

- Mean difference insulin serum concentrations $\mu\text{U/mL}$
- Mean difference of HbA1c in %
- Mean difference in kilograms (weight)
- Mean difference in mmHg (Blood pressure)
- Mean difference waist measured in centimeters

39. Methods that will be used to contact patients

Personal data will be obtained for contact as phone number, mobile phone number, email through the data collection form.

40. Statistical analysis plan.

Normality of variables are going to be analyzed using Kolmogorov-Smirnov test. According to the distribution of variables, they are going to be expressed as mean and standard deviation (DE) or median and interquartile range (IQR) for variables with normal or biased distribution, respectively.

Glucose variability is going to be determined using the MAGE and CONGA indexes. Comparison between the two groups is going to be evaluated using ANCOVA.

A p value based on two-sided tests ≤ 0.05 is going to be considered significant. All analyses are going to be performed with SPSS 20.0 (Chicago, IL).

41. Sample size justification

Expecting a mean difference of 20 mg/dL (1.1 mmol/L) in the MAGE index with and standard deviation of 30 mg/dL (1.7 mmol/L), with an alpha value of 0.05 and a power of 0.80, 40 subjects in each group will be needed (+10% for losses) $\rightarrow 88$

Sample size was determined using t distribution:

$$n = (Z_{\alpha/2} + Z_{\beta})^2 * 2 * \sigma^2 / d^2,$$

Where $Z_{\alpha/2}$ is the critical value of the Normal distribution at $\alpha/2$ (e.g. for a confidence level of 95%, α is 0.05 and the critical value is 1.96), Z_{β} is the critical value of the Normal distribution at β (e.g. for a power of 80%, β is 0.2 and the critical value is 0.84), σ^2 is the population variance, and d is the difference you would like to detect.

We substitute the critical values of the Z distribution to the T distribution using 71 degrees of freedom

$T_{\alpha/2}$

1.99/2 critical value p 0.05 of α

Critical value 0.20 (β) = 1.29

$N = (0.995 + 1.29)^2 * 2 * 302 / 15.32$

$N = 5.221225 * 2 * 900 / 234.09$

N= 40 per group

Total 80 subjects + considering 10% of loss = 88 subjects

Diabetes Technol Ther. 2018 Nov;20(11):715-724. doi: 10.1089/dia.2018.0052. Epub 2018 Sep 14. Effects of Dapagliflozin on 24-Hour Glycemic Control in Patients with Type 2 Diabetes: A Randomized Controlled Trial. Henry RR1,2, Strange P3, Zhou R4, Pettus J1,2, Shi L3, Zhuplatov SB5, Mansfield T5, Klein D4, Katz A5.

SUPPLEMENTARY TABLE S3. KEY SECONDARY END POINTS

Secondary end points	Overall population		Metformin stratum		Insulin stratum	
	Dapagliflozin (n = 50)	Placebo (n = 50)	Dapagliflozin (n = 23)	Placebo (n = 25)	Dapagliflozin (n = 27)	Placebo (n = 25)
Fructosamine, mg/dL						
Baseline mean (SD)	293.5 (6.7)	307.9 (7.3)	279.3 (8.9)	310.0 (11.5)	305.5 (9.4)	305.8 (9.3)
Adjusted mean (SE) change from baseline to week 4	-20.4 (3.2)	-9.6 (3.2)	-20.2 (4.9)	-2.2 (4.6)	-21.8 (4.3)	-16.3 (4.4)
Adjusted mean (SE) difference vs. placebo		-10.8 (4.6)		-18.1 (6.9)		-5.5 (6.1)
P value for treatment difference		0.019		0.012		0.373
24-h MAGE, mg/dL						
Baseline mean (SD)	102.7 (31.0)	108.6 (29.9)	89.3 (24.8)	98.9 (22.6)	114.0 (31.5)	118.2 (33.4)
Adjusted mean (SE) change from baseline to week 4	-10.0 (4.1)	+5.3 (4.1)	-7.3 (6.2)	+10.4 (5.8)	-12.7 (5.5)	+0.2 (5.7)
Adjusted mean (SE) difference vs. placebo		-15.3 (5.8)		-17.7 (8.5)		-12.9 (7.9)
P value for treatment difference		0.010		0.040		0.105
“Distance traveled,” mg/dL						
Baseline mean (SD)	793.9 (267.5)	779.7 (167.0)	781.9 (338.9)	749.7 (184.8)	804.2 (193.6)	809.7 (144.6)
Adjusted mean (SE) change from baseline to week 4	-28.0 (26.3)	+9.5 (25.9)	-50.0 (39.5)	+54.1 (36.9)	-5.9 (34.8)	-35.0 (36.2)
Adjusted mean (SE) difference vs. placebo		-37.5 (36.9)		-104.1 (54.1)		+29.1 (50.2)
P value for treatment difference		0.312		0.057		0.564
SD of 24-h glucose, mg/dL						
Baseline mean (SD)	42.9 (13.1)	43.8 (11.9)	36.7 (10.7)	39.0 (8.9)	48.1 (12.9)	48.5 (12.7)
Adjusted mean (SE) change from baseline to week 4	-3.4 (1.6)	+1.3 (1.6)	-1.6 (2.4)	+5.1 (2.2)	-5.1 (2.1)	-2.5 (2.2)
Adjusted mean (SE) difference vs. placebo		-4.7 (2.2)		-6.8 (3.3)		-2.7 (3.0)
P value for treatment difference		0.037		0.041		0.382

Study was powered only for the overall population; although P values are supplied for the individual strata, inferences for treatment differences should not be made. MAGE, mean amplitude of glucose excursion; SE, standard error.

Result

$\mu_1 - \mu_2 = (M_1 - M_2) = 20.3$, 95% CI [8.393, 32.207].

You can be 95% confident that the difference between your two population means ($\mu_1 - \mu_2$) lies between 8.393 and 32.207.

42. Subject expected to be enrolled

88 subjects

43. In case of multicenter study, include global number and local number of sample

N/A

44. Procedures for reporting a deviation from original statistical plan

A letter from the research committee will be sent for approval

45. Possible discomforts resulting from the study

Attending follow-up visits, blood samples.

46. Potential risks

Inherent in blood sample

47. Anticipated risk detection methods

Patients will be asked to report, 24 hours a day, the appearance of an adverse effect such as nausea, vomiting, diarrhea, fever or dysuria, or any other.

Adverse events will be recorded and managed prospectively according local legislation (NOM 220 SSA1 2016) and standardized adverse reaction forms, in case of a Serious Adverse Event, the subject will be referred to the emergency department of the Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran and treated accordingly local procedures and PV Legislation in addition, all SAEs should be submitted to the AstraZeneca Product Safety mailbox, AEMailboxClinicalTrialTCS@astrazeneca.com, and patientSafety.mexico@astrazeneca.com via "T02 format " (See annex 1) in no more than 24 hours, this submission should include the evidence of submission to the Ministry of Health (MoH).

In case of receiving any pregnancy report during the study, the pregnancy format (Pregnancy outcome report) should be completed for the purpose of documenting the follow-up appropriately during pregnancy and once it has been resolved.

In case to receive any emerging safety issues or unanticipated problems, this should be communicated in no more than 24 hours and at least in parallel with correspondence to regulators, IRB /IEC and Investigators.

The investigator must communicate the initiation of the clinical stage in Mexico (data collection/first visit of the first patient) of the study and a notice of Completion of the clinical stage in Mexico of the study (end of data collection/last visit of the last patient), by means of a submission in a maximum of 15 working days from the beginning or end of the clinical stage in Mexico.

Notice shall be given to the PV National center of cancellation, suspension, discontinuation and/or resumption (including the reasons for this), the document should be performed according the local pharmacovigilance guidelines. The notice shall be within a maximum of 15 working days after cancellation, suspension and/or discontinuation.

The evidence of submission of the communications must be sent to PatientSafety.Mexico@astrazeneca.com in a period of 48 hours after the submission. If the investigator receives a response from MoH this response should be sent to PatientSafety.Mexico@astrazeneca.com in a period of 48 hours after the document acknowledgment.

Adverse Event: An AE is any untoward medical occurrence in a patient administered a study drug which may or may not have a causal relationship with the study drug. Therefore, an AE is any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease, which is temporally associated with the use of a study drug, whether or not considered related to the study drug. An AE also includes any worsening (ie, any clinically significant change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the study drug.

Serious Adverse Event: A SAE is any untoward medical occurrence that at any dose:

Results in death – includes all deaths, even those that appear to be completely unrelated to study drug (eg, a car accident in which a patient is a passenger).

Is life-threatening – in the view of the investigator, the patient is at immediate risk of death at the time of the event. This does not include an AE that had it occurred in a more severe form, might have caused death.

Requires in-patient hospitalization or prolongation of existing hospitalization. In-patient hospitalization is defined as admission to a hospital or an emergency room for longer than 24 hours. Prolongation of existing hospitalization is

defined as a hospital stay that is longer than was originally anticipated for the event, or is prolonged due to the development of a new AE as determined by the investigator or treating physician.
 Results in persistent or significant disability/incapacity (substantial disruption of one's ability to conduct normal life functions).
 Is a congenital anomaly/birth defect.
 Is an important medical event - Important medical events may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the patient or may require intervention to prevent 1 of the other serious outcomes listed above (eg, intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse).

48. Security measures for timely diagnosis and prevention of risks

Patients who present a side effect to revision will be cited for assessment.

49. Procedures to be followed to resolve the risks in case they arise

Treatment of the complication or side effect will be given, either by prescription on an outpatient basis or it will be referred to the service of urgencies to the patient for its assessment and treatment. The emergency expenses will be borne by the patient since there is no budget to insure the subjects of study. Dapagliflozin is on the market and approved by FDA, EMA and COFEPRIS.

50. Expected direct benefits

Improvement in glycemic variability

51. Indirect benefits expected

Improvement in weight, pressure, uric acid

52. Overall weighting of risks against benefits of the proposed study

Greater benefits with respect to risks. SGLT2i are recommended as a second line after metformin for its additional benefits in weight loss, blood pressure and cardiovascular safety, with an acceptable safety profile.

53. Specify costs (direct/indirect, monetary, in time of participation, visits) that the investigation will require from study subjects

Patients will be required to attend 1 enrollment visit and 6 follow up visits in the next 12 weeks (transportation costs)

54. Specify if the consultations, laboratory / cabinet examinations and medical / surgical treatments generated during the study will be covered by the patient / research subject

Because there is no budget to insure the patient, the expenses for emergencies or associated complications will be covered by the subject of the study.

55. Who will cover investigation associate costs.

Astra Zeneca México will provide the resources defined in the contract to acquire investigational drug, laboratory data, medical writing, cost of publication and glucose lowering sensors.

TOOL	Trademark	Presentation	Unit Price	Quantity	Total
			USD		USD
iPro™2 Continuous glucose monitor.	Medtronic®	1	\$ 1,622.00	4	\$6,488.00
Sensors	Medtronic®	10	\$ 378.50	14	\$5,299.00
glucose	immunometric	88 samples	\$ 1,000.00	2	\$2,000.00
insulin	Merck Millipore®	Kit/88 samples ELISA	\$ 1,892.00	2	\$3,784.00
Medical writing			\$ 2,600.00	1	\$2,600.00

Cost of publication			\$ 4,000.00	1	\$4,000.00
FORXIGA 10mg	AstraZeneca	28 tablets	0 (will be covered by local market)	264	\$0.00
TOTAL					\$24,171.00

56. If applicable, specify the incentives that will be offered (incentive is understood as an offer or influence that compels to perform an action without implicitly a significant deviation with our general plan of life; Gr.: Give a book for having participated)

Note: A compensation / incentive outside the proportion is considered a coercive attitude

N/A

57. References.

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Li FF1, Gao G1, Li Q1, Zhu HH1, Su XF1, Wu JD1, Ye L2, Ma JH1.

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Annex

Annex 1. T02 Format



Formato_T02.-
Reporte de Reaccion /

Annex 2. T02 Filling instructions.



Instructivo de
Llenado T02.docx

Annex 3. Pregnancy Outcome Report format.



AZ pregnancy
outcome report.pdf