Effect of low dose combination of linagliptin + metformin on the function of pancreatic beta cells, resistance to insulin and cardiovascular function on patients with prediabetes and overweight/obesity. Randomized Clinical trial

Research Protocol

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BACKGROUND

Epidemiological impact of type 2 diabetes

Diabetes mellitus type 2 (DM2) one of the most frequent endocrine diseases in the general population, being in Mexico the main cause of death. The DM2 is considered a syndrome with an altered intermediate metabolism, caused by inadequate insulin secretion, resistance to the effect of the same or the combination of both, which as a consequence cause hyperglycemia in both fasting and postprandial .(1)

The risk factors for DM2 are: overweight and obesity, first-degree relatives with DM2, ≥ 45 years of age, women with a history of macrosomic products (>4kg) and/or with a history of gestational diabetes, women with a history of polycystic ovaries, arterial hypertension ≥140/90mmhg, dyslipidemia (cholesterol HDL ≤40mg/dl, triglycerides ≥250mg/dl, and cardiovascular disease.

It is estimated that around 11.7 million Mexicans will have DM2 for the year 2025, which means an increase of 207.8% occupying 7th place with more places worldwide.(2) According to the national health survey conducted in the year 2000, found a prevalence of DM2 of 8.2%, reporting that this increase is partly due to the greater number of patients with DM2 under 40 years, and the high prevalence of risk factors that contribute to the development of chronic complications is what explains why it is the main cause of death.(3) However, a global prevalence of DM2 was reported in the 2006 National Health and Nutrition Survey (NHNUTS) with greater prevalence in the center and center-west of the country.(4); Likewise, this survey found a significant increase in the number of people <40 years of age with a diagnosis of DM2.(5)

In countries like Mexico, DM2 is a pathology that has marked an epidemiological transition. The impact of this pathology is very important since more than 180,000 new cases are registered annually and cause around 36,000 deaths. It is estimated that the annual cost of diabetes for Mexicans is 430 million dollars per year, figures that represent an approximate cost of 45 dollars per person per year and this corresponds to three quarters of the entire budget granted for Health.(6)

The complications of DM2 can be microvascular: retinopathy, neuropathy and nephropathy, or macrovascular: heart disease, cerebral vascular disease and peripheral vascular disease, with cardiovascular disease being the main cause of death in more than 75% of patients with diabetes, since they have a high risk of coronary artery disease, with a higher incidence of ischemic events and death after an acute myocardial infarction; In the absence of ischemic heart disease, the risk of presenting it is the same as those without diabetes but with a history of ischemic heart disease.

This implies that prevention measures for asymptomatic diabetic patients should be similar to secondary prevention measures for non-diabetic patients with ischemic heart disease.(7) Prediabetes has also been associated with increased cardiovascular morbidity and mortality, so it is suggested that endotheliopathy also begins in the early stages of the disease, also affecting renal function(8–10).

PHYSIOPATHOLOGY OF TYPE 2 DIABETES

The pathogenesis of DM2 is multifactorial. The knowledge established so far indicates a strong environmental influence, with an important contribution of the genetic factor(11). The two most important factors that contribute to the pathophysiology of DM2 are insulin resistance and beta cell dysfunction. There is a complex interrelation between the different components involved in the process of production, consumption and storage of energy. The sequence of pathophysiological events seems to indicate that the first thing to appear is insulin resistance, conditioned by several factors such as lack of physical activity, the westernized diet high in simple carbohydrates and saturated fatty acids, the ethnic group and perhaps a genetic influence so far not well determined(12).

Several pathophysiological mechanisms have been described in insulin resistance. At the liver level it has been observed that there is a decrease in the number and activity of insulin receptors, a decrease in glucose uptake due to a decrease also in the number and activity of the glucotransporters and due to the effect of the glycolytic pathway that secondarily

prevent the entry of glucose, as well as an effect of free fatty acids at this level interfering with insulin signaling. On the other hand, at the muscular level this insulin resistance is also very important, because as previously mentioned this is the place where the highest insulin-dependent glucose uptake is carried out (80%). Here there has also been a decrease in glucotransporters (in this case GLUT4), accumulation of triglycerides and excess free fatty acids that compete with glucose as an energy substrate and increase levels of Malonyl-CoA it has the ability to inhibit carnitine palmitoyltransferase type 1, an important enzyme for beta oxidation of fatty acids. In addition, both at the liver, muscle level and mainly in adipose tissue, an interrelation between different cytokines has been found, such as tumor necrosis factor alpha (TNFA) which determines insulin resistance by causing phosphorylation of the intracellular part of the insulin receptor substrate 1 (IRS-1), and its counterpart, adiponectin which is the only protective cytokine against insulin resistance.

Due to this insulin resistance, glucose (muscle) uptake decreases at the peripheral level and at the liver level it is not able to suppress glucose production, therefore, as a compensatory mechanism the beta cell increases insulin secretion and thanks to this hyperinsulinemia, equilibrium is achieved and euglycemia is maintained, so hyperinsulinemia is considered as an indirect marker of insulin resistance.

With the passage of time and the persistence of insulin resistance and the increase of some toxic factors for the beta cell (glucose and lipids), it begins to deplete and decrease insulin secretion, so that ability to maintain suppressed the hepatic glucose production is lost and fasting hyperglycemia appears, until reaching diagnostic levels of DM2 where we have a 50-80% reduction in functional capacity and in the volume of beta cells(11–14)

DYSFUNCTION OF PANCREATIC ISLOTS

When insulin resistance appears, there are many factors that determine whether or not the dysfunction of the beta cell and pancreatic islets appear as a functional unit that lead the beta cell to that inability to maintain normoglycemia and normotolerance to glucose(15). The factors are many, genetic factors not well established, alteration in the cell cycle

regeneration-apoptosis with decrease in the first and increase in the last, a toxic effect of the same glucose (glucotoxicity), prolonged exposure to free fatty acids(16), deficiency or resistance to the effect of incretins(17), oxidative stress and increased production of superoxide at the beta cell level(18,19), and a factor identified for many decades but perhaps undervalued, abnormalities in the secretion of amylin or IAPP (islet amyloid plypeptide), conditioning amyloid formation and deposition at the level of pancreatic islets as well as dysfunctional remodeling thereof(20–23).

All pathophysiological alterations in the natural history of diabetes mellitus, occur progressively during the course of the disease, its identification and characterization being vital, as well as knowing the risk factors associated and involved with each of them that we allowed to focus prevention strategies and timely diagnosis towards stages prior to the onset of DM2 and thus be able to impact on the occurrence of chronic complications and morbidity and mortality in these.

PREDIABETES

Since the natural history of the disease is a continuous spectrum, and as such, DM2 is only a limited part of that spectrum, something known as prediabetes was created, which is identified with fasting glucose levels between 100 and 125mg/dl, and is known as impaired fasting glucose, or with glucose levels between 140_199mg/dl at 2 hours after an oral load of 75g of glucose, known as glucose intolerance; In both entities the presence of complications, both macro and microvascular, have been described.

In patients with prediabetes, the presence of pathophysiological phenomena similar to those occurring in DM2 but in less severity have been described(24); Patients with prediabetes have insulin and muscular resistance to insulin(23,25–27), pancreatic beta cell dysfunction(23,26,28–30), pancreatic alpha cell dysfunction with the consequent absolute or relative hyperglycagonemia(30–32), reduction of the incretin effect(33,34); Adipose tissue is an active endocrine organ that secretes several adipocytokines (leptin, adiponectin, resistin, visfatin) and cytokines (TNF Alfa, IL-1, IL-6) which are also involved in insulin resistance and inflammation present in subjects with obesity and prediabetes(35,36). We

could, then, summarize the pathophysiology of prediabetes as follows: subjects with impaired fasting glucose predominantly have insulin resistance at the liver level and decrease in the first phase of insulin resistance at the peripheral level, specifically at the muscle level, and deterioration in the first and second phase of insulin secretion(37,38).

Both entities, impaired fasting glucose and glucose intolerance, identify different populations, since fasting glucose reflects the rate of hepatic glucose secretion and in varying degrees the secretion of insulin by the pancreas, since one of its main effects is to inhibit hepatic glucose production; On the other hand, glucose intolerance evaluates the rate of glucose uptake (in muscle) and the ability of the pancreas to compensate for this defect.

The prevalence of prediabtes in Mexico, according to ENSANUT 2006, refers to a prevalence of impaired fasting glucose greater than 30%(39).

The transition from prediabetes (GAA and ITG) to DM2 can take a long time, however, the existing information indicates that the majority of these patients (70-80%) will develop at some point in their DM2 life. People with GAA + ITG and who are also overweight and other risk factors for DM2 are the ones most likely to have this disease progression, and factors such as reduced insulin secretion and severe insulin resistance, serve as markers to identify these types of patients. These patients have a slightly elevated cardiovascular risk (relative risk 1.1-1.4), which rises up to 2 to 4 times more with progression to DM2(40), in the same way, in patients with prediabtes a prevalence of retinopathy of 8%has been observed, which rises to 13% when progressing to DM2(41).

With the above, and considering again the continuous spectrum of the disease, we can realize that there is another stage that also belongs to a prediabetic state, this is when there is only insulin resistance without fasting or postprandial hyperglycemia, which in fact is the initial stage of the disease, and that commonly occurs in patients with obesity(42).

A chronological sequence has been proposed in the appearance of the different types of prediabetes(27,42), Which would be: I) insulin resistance isolated without fasting

hyperglycemia or postprandial hyperglycemia, II) isolated GAA, III) isolated ITG, and IV) GAA + ITG, for the appearance of these 4 states the presence of resistance to the insulin (in liver, muscle and adipose tissue) and pancreatic beta cell dysfunction; It is clear, by definition, that the diagnosis o the last three is made with the fasting glucose measurement and the performance of the CTOG with an oral load of 75g of glucose, on the other hand, for the diagnosis of the first (resistance to isolated insulin), there are several methods. The hyperinsulinemic-euglycenic clamp is the most accurate method to measure insulin resistance, while the hyperglycemic clamp is mainly used to evaluate the function of the pancreatic beta cell, but it has the advantage that it can also be used to evaluate the function of the pancreatic islet, when glucagon levels are also measured, and also allows a reliable assessment of insulin resistance at the same time(43).

TREATMENT OF PREDIABETES

Different pharmacological and non-pharmacological strategies have been used to try to prevent the progression of prediabetes to DM2. In general, there are two types of preventive strategies, those that are carried out at the population level and whose objective is to avoid the presence of modifiable risk factors and that which is carried out in high-risk population, such as patients with GAA, ITG and gestational diabetes, this strategy aims to improve insulin resistance and pancreatic beta cell dysfunction.

One of the investigations that best show the impact of lifestyle modifications and the use of metformin on the prevention of DM2, is the Diabetes Prevention Program (DPP); This study was a randomized clinical trial with an average follow-up of 2.8 years, which included 3,234 patients with BMI> 24 and GAA and/or ITG that were randomized in 3 study groups: 1) Metformin 850mg daily treated during the first month and then 850mg twice a daily plus routine recommendations (orally and written) annually on lifestyle, 2) placebo treated group plus routine lifestyle recommendations, and 3) group treated with an intensive lifestyle modification program, which consisted of performing 150min of physical activity per week and reducing fat intake and calories in the diet, in order to lose 7% of

body weight. This group had 16 personalized sessions during the first 24 weeks, and then every month. Is worth mentioning that in this study, only 50% of the participants in the intervention group to modify the lifestyle managed to lose 7% of body weight within 24 weeks of initiation, and only 38% maintained it until the end of the study; 74% maintained the required physical activity 24 weeks after the study and only 58% until the end of the study; at 24 weeks of the study, the intervention group achieved a reduction in energy intake of 450 kcal/day compared to approximately 250 kcal/ day in the other two groups The results showed that with intensive changes in lifestyle, the risk of DM2 was reduced by 58%, and with metformin by 31%; even so, the cumulative incidence of DM2 in 3 years was 14.4% in the intervention group, 21.7% in the metformin group, and 28.9% in the placebo group(44). However, the long-term results (15 years) of this type of interventions have shown that the effect is lost over time and that eventually most patients will develop DM2.

Other antidiabetic medications that have been used are: acarbose, which in a cohort of 1429 patients with ITG showed a reduction in the risk of DM2 of 25% with a necessary number to treat of 11 to avoid a case of DM2, however, more than a quarter of patients did not complete the follow-up due to side effects of the medication(45); rosiglitazone in the DREAM study that included 5269 patients with GAA and/or ITG randomized 4-8mg of rosiglitazone or placebo, at an average follow-up of 3 years, the rosiglitazone-treated group showed a DM2 risk reduction of 62%(46); The ACT NOW study that used pioglitazone 45mg a day in subjects with GAA and/or ITG shows a risk reduction of DM2 of about 80% compared to placebo(47).

Currently, the pharmacological interventions used in patients with DM2 , are directed towards the pathophysiological alterations, while in prediabetes, if we try to avoid the progression of the disease, it is clear that the options that can greatly impact each of the pathophysiological alterations are those that could have greater effect to prevent disease progression(28,29); within these pharmacological interventions, those that could be more effective, due to the pharmacological profile, are:

1) METFORMIN: Metformin is the most widely used antidiabetic drug in the world. Although its main effect is exerted at the liver level, it also has an effect on the tissue such as muscle, adipose and endothelial tissue; most of its effects are related to the control of insulin resistance and/or hyperinsulinemia. At the liver level, metformin activates an enzyme known as AMP kinase (AMPK), which causes an inhibition of lipogenic and gluconeogenesis enzymes, decreasing lipolysis, increasing fatty acid oxidation, reducing hepatic glucose production (gluconeogenesis), increasing hepatic glucose uptake and glycolysis, all this is because increases the sensitivity to the effect of insulin. At the muscle level, metformin also works by improving muscle glucose uptake, among other effects. It is the only medication approved for use in people with prediabetes; it is associated with weight reduction in a variable percentage, as monotherapy is very unlikely to cause hypoglycemia. The disadvantage of metformin is that it has been observed in long-term studies in prediabtes (15 years of follow-up) that the effect is almost equated to a placebo and that eventually the majority of treated patients will develop DM2.

2) PIOGLITAZONE: Pioglitazone belongs to a group of medicines known as thiazolidinediones; this type of drug activates the peroxisome proliferator receptor (PPAR), by binding to this receptor they form a heterodimer together with the receptor of retinoic acid and other molecules, through this mechanism regulate the expression of several genes that affect the oxidation of fatty acids, increases the transcription of the gene encoding for glucotransporter 4 (Glut4), affects the production of cytokines (increasing the secretion of adiponectin and reducing that of alpha TNF), and improves mitochondrial function; it is through these mechanisms that pioglitazone increases insulin sensitivity in muscle, adipose tissue and liver. (48) Pioglitazone is the only medicine in this family that persists on the market as an antidiabetic. Pioglitazone manages to reduce glycosylated Hb in patients with DM2 by 0.5 – 1.4% and has shown great utility in delaying (47)
3) INCRETINES: As already mentioned, patients with DM2 and prediabetes have dysfunction of the pancreatic islets (beta cell dysfunction with consequent reduction

in insulin secretion, alpha cell dysfunction with increased glucagon production, and amyloid deposits) and this is a crucial event for the progression of prediabetes disease to DM2, so medications that can improve the functionality of this islet, increasing insulin secretion and reducing glucagon, could have a major impact on the disease. There are two incretins, GLP-1 and GIP, of these two, the first being found reduced in patients with DM2 and with prediabetes(33,34,49,50) and both are produced and released by intestinal cells. The pharmacological effects of incretins are: increased insulin secretion, decreased glucagon secretion, delay gastric emptying, and reduced appetite leading to weight reduction. In general there are two ways to increase the incretin effect, by applying incretin analogues, which are injectable, and by inhibiting the enzyme that degrades to endogenous GLP-1, which is dipeptidil-peptidase 4. As the incretin effect is reduced in patients with DM2 and with prediabetes, and knowing that the effect of incretinomimetic medicinal products improves pancreatic islet function and could have some effect on the peripheral action of insulin(51–54), it is interesting to know the impact they could have on DM2 prevention, especially when combined with insulin sensitizers. There are few studies that evaluate the effect of incretins on people with prediabetes; with GLP-1 analogues, one study randomized 38 patients with GAA or ITG to receive exenatid 10 g every 12 hrs subcutaneously for 24 weeks, finding that in addition to the weight-reducing effect, a large percentage of patients with prediabetes treated with exenatide reverted to normal (77% vs. 56% for the placebo group)(55). Another randomized clinical trial compared the effect of liraglutide, another LPG-1 analogue, on glucose metabolism, insulin resistance and insulin secretion in 24 patients treated with liraglutide and 27 with placebo, of which 10 and 11, respectively, they had altered fasting glucose + glucose intolerance; achieved a 29% reduction in peripheral insulin resistance and a 21% increase in insulin secretion(56). In another study, 22 patients with GAA were randomized to receive 100 mg of sitagliptin, DPP-IV inhibitor, for 8 weeks reporting no effect on glucose metabolism, although reports of some clinical cases mention important effect glucose in patients with prediabetes.

Other non-diabetic drugs have also been used and shown variable results on the prevention of DM2 (valsartan, statins, orlistat, fibrates, estrogens, etc.)(57).

DIFFERENCES BETWEEN DPP-IV INHIBITORS

Power and Efficacy to Inhibit the Enzyme DPP-IV

It is important to mention that all DPP-IV inhibitors are competitive reversible inhibitors and it is difficult to compare their effects when analyzing studies, experimental conditions may be different; however, there is a study in which the inhibitors were compared under identical conditions showing similar efficacy (maximum effect) to inhibit DPP-IV in-vitro, although there were differences in potency, the most potent being linagliptin(58).

Half Life

As for the half-life, vildagliptin and saxagliptin (59) have shorter half-lives, on the other hand, linagliptin (60) and sitagliptin(61) have longer duration of effect, which gives the latter two the ability to inhibit the DPP-IV enzyme for 24h at 80%; although it should be noted that in the original study that evaluated the half-life and potency of sitagliptin to inhibit DPP-IV was performed on healthy volunteers(62), unlike the original study evaluating the half-life and potency of linagliptin to inhibit DPP-IV that was performed in patients with type diabetes mellitus 2 (DM2)(60), scenario in which medicines are usually used. However, it is important to mention that the activity in these trials is evaluated ex vivo and is generally not corrected by the dilution of the sample, so the actual inhibition of DPP-IV is expected to be even greater than the measure.

Selectivity and disposal pathway

The selectivity of the different DPP-IV inhibitors by this enzyme has been evaluated in in-vitro studies and has been reported to have both linagliptin (58) like sitagliptin(62) are the ones who have the most selectivity by the enzyme DPP-IV; linagliptine has a selectivity of 10,000 per DPP-IV than for the DPP-8/9 compared to sitagliptin which is >5550; this is important because inhibition of these two DPP-8/9 enzymes is the one that has theoretically been thought to be associated with side effects of inhibition of lymphocyte activity, although this effect has not been observed in the clinic, as these 2 enzymes are at the

intracellular level (63–65). On the other hand, linagliptin only has less selectivity over fibroblast activation protein α (FAPα) which is a protein not found in adult tissue so the implications of this data are even smaller, as DPP-IV inhibitors are only indicated in adults. Sitagliptin, vildagliptin and saxagliptin are eliminated by more than 80% renally, instead, linagliptin is eliminated by more than 80% by biliary route, so it can be used in patients with any degree of renal impairment without the need to adjust the dose and without loss of the pharmacological effect.(66).

CLINICAL ASPECTS OF DPP-IV INHIBITORS

Clinical efficiency

In terms of the clinical efficacy and ability of the different DPP-IV inhibitors to reduce HbA1c, fasting glucose and postprandial glucose, various meta-analyses and clinical studies have shown similar efficacy, achieving a reduction in HbA1c of 0.5 – 1.0 % (-0.8%), with larger reductions when baseline values are higher(63–65,67,68).

Side effects and safety

So far no higher rate of side effects have been found with DPP-IV inhibitors compared to control groups, and likewise, no differences in side effects have been reported between the various DPP-IV inhibitors(63,64,67,68); In addition, it´s safe in terms of pancreatitis and pancreatic cancer risk has been validated by the FDA and the European Medicines Agency recently (69).

TABLE 1. Differential characteristics between DPP-IV inhibitors

NAME	POTENCY (IC50)	DOSIS	EFICIENCY TO INHIBIT DPP-IV x 24h	HALF LIFE (hrs)	ELIMINATION	SELECTIVITY	USE IN RENAL INEFFICIENCY	CLINIAL EFFICIENCY ON HBA1C
Linagliptin(60)	1Nm	5mg/24h	> 80 %	10 – 40	Bile > 80 %	High	Recommended	0.5 – 1.0 %
Sitagliptin(61)	19nM	100mg/24h	> 80 %	8 – 24	Renal > 80 %	High	Not recommended or adjust doses	0.5 – 1.0 %
Vildagliptin(59)	62nM	50mg/12h	> 80 %	1.5 – 4.5	Renal > 80 %	Half	Not recommended	0.5 – 1.0 %
Sitagliptin	50nM	5mg/24h	> 70 %	2 – 4	Renal > 80 %	Half	Not recommended or adjust doses	0.5 – 1.0 %

LINAGLIPTIN

From the above we can summarize that the mechanism of action of linagliptin is to inhibit the enzyme DPP-IV (dipeptidil peptidase type IV); this enzyme (DPP-IV) has the biological effect of inactivating the peptide similar to glucagon type 1 (GLP-1), so by being inhibited by linagliptin this favors endogenous GLP-1 levels to rise up to 3.2 times above the previous values (same as being reduced in patients with type 2 diabetes mellitus, which partly explains that these patients have a reduction in the incretin effect), which conditions the biological effects of GLP-1 as stimulation of insulin secretion by beta cells pancreatic and inhibition of glucagon secretion by pancreatic alpha cells. This effect on stimulation in insulin secretion is totally dependent on glucose levels, so it does not cause hypoglycemia. Linagliptin has a half-life of 12 hrs so it can be used every 12 or 24 hrs, it is eliminated by biliary by more than 70% so it can be used in patients with nephropathy without the need to adjust the dose, offers a power to achieve a sustained inhibition 90% in DPP-IV for 24 hrs and is highly selective for inhibiting DPP-IV compared to other enzymes such as DPP-8 and DPP-9. Linagliptin is indicated in the treatment of patients with uncontrolled type 2 diabetes mellitus, which does not cover control targets such as: B glucosyl less than 7%, fasting glucose less than 110mg/dl and postprandial glucose less than 140mg/dl. It can be used as therapy in combination with metformin, sulfonylureas, thiazolidinedionas and insulins, either as double or triple therapies.

It is clear that the use of a placebo to compare any drug is no longer justifiable, in all studies the comparison has been made based on a control group with or without placebo, and moreover, some studies have only used as a point of comparison to the placebo group, which obviously increases the chances that the drug in comparison may show some beneficial effect; on the other hand in Mexico, until today, there are no studies on primary prevention of DM2, and if pathophysiological alterations occur from prediabetes stages, it is important to evaluate the effect of drugs that combined may have the greatest impact on alterations and therefore on the progression of the disease; It would also be useful to assess the impact that the combination of medications, with different mechanisms of action and at

low doses, can have a greater impact on the progression of the disease and reduce the cardiovascular risk that occurs since prediabetes.

JUSTIFICATION

DM2 is a chronic disease that has reached the proportions of a global epidemic from the increasing number of patients in all countries; has become the disease that causes the most chronic and acute complications to patients, consuming a large percentage of health spending in countries such as Mexico, it generates a large number of disabilities in productive ages and is one of the main causes of cardiovascular morbidity. The pathophysiology of the disease is complex and multifactorial, with great influence of the environment that the appearance of pathophysiological phenomena that as manifestation intermediation have hyperglycemia, and as a final manifestation, complications; and it is precisely the sequence of pathophysiological events that gives the disease a continuous spectrum in the natural history of it, unfortunately, when the diagnosis of DM2 is made we are identifying patients in very advanced stages of the disease, at which point up to 50% of them may have any of the known complications, and this together with the fact that we do not have a good diagnostic tool and that most patients with DM2 are outside the goals of cardiometabolic control, explains the great significance, exponential growth and difficulty that has conferred on health systems to control it. For all of the above, it seems clear that the best strategies will be those that are aimed at the early stages of the disease, because it will involve more awareness in the population and the impact on the stability and/or reversibility of the pathophysiological alterations, which will eventually translate into a lower incidence DM2. The studies on prevention of DM2 that have been conducted have not compared the effect of some of the drugs that have so far been more effective for this purpose and in some of these studies the doses used are frequently associated with the appearance of side effects; of these studies, none have been conducted in Mexico, and we are convinced that if we use the combination of drugs with additive pathophysiological impact plus cardiovascular protection in early stages, we can obtain better, longer-lasting and more impactful results on the natural history of the disease with a view to long-term follow-up that shows measures that may have an applicability in clinical practice, in order

to contribute to the control of this pathology. **.** Therefore, combining medications with different mechanisms of action, in low doses, could be a useful strategy not only to prevent DM2, but also to prevent macro and microvascular complications early.

PROBLEM STATEMENT

¿What is the effect of low-dose combination of linagliptin + metformin compared to metformin alone on pancreatic beta cell function, insulin resistance and cardiovascular function in patients with prediabetes and overweight/obesity?

HYPOTHESIS

Of Work

The low-dose combination of linagliptin + metformin has a greater effect on pancreatic beta cell function, insulin resistance and cardiovascular function compared to metformin alone in patients with prediabetes and overweight/ Obesity.

Null

The low-dose combination of linagliptin + metformin has no greater effect on pancreatic beta cell function, insulin resistance and cardiovascular function compared to metformin alone in patients with prediabetes and overweight/ Obesity.

OBJETIVES

General: Evaluate the effect of low-dose combination of linagliptin + metformin against metformin alone on pancreatic beta cell as function, insulin resistance and cardiovascular function in patients with prediabetes and overweight or overweight or Obesity.

Specific

- Evaluate insulin resistance, pancreatic beta cell function and cardiovascular function in a group of patients with prediabetes, treated with Metformin 1700 mg/day for 6 months, at the start of surgery and at 6 months.
- Evaluate insulin resistance, pancreatic beta cell function, and cardiovascular function in a group of patients with prediabetes treated with the combination of linagliptin 2.5mg every 24 h + metformin 1700 mg every 24 h for 6 months, at the start of intervention and at 6 months.
- Compare markers of cardiovascular risk before and after treatments in the 2 study groups.
- Compare insulin resistance and pancreatic beta cell function between study groups at the end of the intervention.
- Know glucose metabolism by oral glucose tolerance curve at baseline and end of treatment in the study groups.
- Determine changes in body composition.

Secondary objectives

- Evaluate therapeutic adherence in study groups.
- Know the incidence of type 2 diabetes mellitus in each study group during follow-up to this project.
- Know renal function by depuration of creatiline and albuminuria at the beginning and end of the study in the study groups.

STUDY DESIGN.

Type of Study: Clinical trial (randomized, controlled and triple-blind).

MATERIAL AND METHODS.

Study universe: Mexican patients with prediabetes and overweight/obesity.

Study population: Mexican patients with prediabetes and overweight/obesity, from the State of Guanajuato.

Place of realization: Hospital Metabolism Laboratory of Division of Health Sciences of the University of Guanajuato, León, Gto.

Type of sampling: Non-probabilistic, of consecutive cases.

Sample size: The calculation of the sample size was based on the formula for comparing proportions. We hope that the low-dose combination of linagliptin + metformin will improve the outcome variables (insulin resistance, pancreatic beta cell function and cardiovascular function) by at least 75%, metformin use alone achieves this only by 30%(44.46), so the study is designed to detect a minimum difference of 45% between study groups with a type I (alpha) error of 0.05 and a sample power of 80% , and according to the following formula(70):

$$n = \frac{(Z\alpha/2+Z\beta)^2\, p^{-}(1-p^{-})(r+1)}{(d)^2 r}$$

Where,

$(Z\alpha/2+Z\beta)^2 = 7.9$

p^1 = proportion of individuals in the best treatment who failed to achieve improvement = 0.25

p^2 = proportion of individuals in the worst treatment who failed to achieve improvement = 0.70

r = reason for individuals in treatment groups = 1

d = difference you're expected to find = 0.45

n = 14 + 20% of losses = 17 patients per group

SELECTION CRITERIA TO PARTICIPATE

- Women and men aged 18 to 65 overweight or obese (BMI 25 kg/m2)
- Patients with prediabetes, defined by:
    1) Glucose intolerance (ITG = glucose at 2h en la CTOG between 140-199mg/dL).

2) The coexistence of fasting impaired glucose (GAA = fasting glucose between 100 and 125mg/dl) and glucose intolerance (ITG = glucose at 2h in CTOG between 140-199mg/dL).

- Those who agree to participate in the study sign an informed consent letter.

NOT SELECTED TO PARTICIPATE

- Patients with DM2 previously diagnosed or detected during sampling and CTOG.
- People currently being treated or for the past 3 months with metformin, pioglitazone, or any other antidiabetic drug, including insulin.
- Serum creatinine >1.6mg/dl.
- Very high hipertriglyceridemia (>500mg/dl).
- Pregnant women.
- Uncontrolled arterial hypertension (systólic >180mmhg o diastolic >105mmHg).
- Excessive alcohol intake, acute or chronic.
- Medications or medical conditions affecting glucose homeostasis (thiazides, beta blockers, systemic glucocorticoids, weight-reducing or anorexigenic drugs, Cushing syndrome, thyrotoxicosis)

ELIMINATION

- Intolerance to the medicine used.
- Absence to more than 20 % of follow-up appointments.
- That they do not finalize the study and monitoring protocol.

VARIABLES

INDEPENDENT VARIABLES

1) Intervention

DEPENDENT VARIABLES

1) *Glucose homeostasis.*
2) *Sensitivity to insulin.*
3) *Pancreatic beta cell function.*
4) *Cardiovascular function.*

OPERATION OF VARIABLES, MEASUREMENT SCALES AND MEASUREMENT UNITS.

DEPENDENT VARIABLE	CONCEPTUAL DEFINITION	OPERATIONAL DEFINITION	CLASIFICATION	MEASURE-MENT LEVEL	INDICATOR
INTERVENTION	It is the established therapeutic strategy to prevent the progression of Prediabetes to type 2 diabetes mellitus and its complications.	A. Administration of 1 tablet of linagliptin 2.5 mg /metformin 850 mg every 24 hours + 1 tablet of metformin 850 mg every 24 hours. B. Administration of 1 tablet of metformin 850 mg every 12 hours.	Qualitative	Nominal	A. Group with low dose treatment of Linagliptin/ Metformin. B. Metformin treatment group.

DEPENDENT VARIABLE	CONCEPTUAL DEFINITION	OPERATIONAL DEFINITION	CLASIFICATION	MEASUREMENT LEVEL	INDICATOR
FUNCTION OF THE PANCRETIC BETA CELL	Pancreatic beta cell's ability to secrete enough Insulin to keep blood glucose levels at normal levels.	Value obtained by the CTOG, when applying the formula $(\Delta I_{0-30})/(\Delta G_{0-30})$, (ΔI_{0-30}) = difference in insulin value measured at 0 and 30 minutes. (ΔG_{0-30}) = difference in glucose value measured at 0 and 30 minutes.	Quantitative	Reason	Pmol/mmol,
HOMEOSTASIS OF GLUCOSE	Ability of therapeutic interventions to reverse glucose intolerance.	It shall be considered as **YES** when the fasting glucose level at 6 months after the intervention begins, either at 2 h during CTOG is <140mg/dL, and **NO** when glucose levels at 2h during CTOG are 140mg/dL.	Qualitative	Dichotomous Nominal	Yes No
SENSITIVITY TO INSULIN	Insulin's ability to promote glucose uptake by muscle tissue, and suppress glucose production by the liver and avoid the lipolysis in adipose tissue.	It will be evaluated using Matsuda's index with glucose and insulin values during CTOG using the following formula: Insulin sensitivity = $10000 / \sqrt{(G_0 \times I_0)} \times (\widetilde{G}_{0-120} \times \widetilde{I}_{0-120})$	Quantitative	Reason	Natural numbers

CARDIO-VASCULAR FUNCTION	Function of the system made up of heart and blood vessels for transport activities.	Measurement of ejection fraction, diastolic and systolic preloads and ejection fraction.	Quantitative	Continues	Percentage %

METHODS.

Identification of patients at risk

During this initial phase, overweight or obese patients will be identified with and at least 2 risk factors for DM 2, who will be invited to the screening of the study and as a first step will answer a survey of nutrition and physical activity.

Recruitment of patients

Metabolic evaluation and classification of patients: Once obtaining weight and size, we identify overweight or obese patients and risk factors for DM2, they will be invited to continue the counting phase; The next step will be to cite patients for fast glucose measurement, and only patients who have a glucose level of 100mg/dL will have CTOG performed to determine the presence of prediabetes.

Glucose tolerance curve: The tolerance curve consists of citing the patient with an 8-12 hrs fast, the vein is channeled to the ulnar with 0.9% 250cc saline solution, basal blood samples are taken and 75g of glucose is given to drink and subsequently take blood samples every 30 minutes for the next two hours (0, 30, 60, 90 and 120min), for glucose determination at that time and for subsequent measurement of insulin, glucagon and GLP-1. As mentioned in the selection criteria, patients who are diagnosed with DM2 during screening (CTOG) will not be included in the study.

According to glucose values within 2 hours of oral loading, patients will be classified as:

1) Glucose-tolerant norms when glucose at 2hrs during CTOG is <140mg/dl,
2) Patients with glucose intolerance when glucose at 2hrs of CTOG is between 140 and 199mg/dL,

3) Recently diagnosed DM2 patients when glucose is 2 hrs. CTOG is 200mg/dL.

Patients with CTOG glucose intolerance are those who will be invited to participate in the trial and those who accept will continue with a more thorough metabolic evaluation. For this purpose, patients will have completed a medical history and the following studies:

1) Fasting basal blood samples for measurement of:
   - Blood count
   - Lipid profile
   - Insulin for homa-IR determination, HOMA-B, insulin sensitivity, insulin secretion and pancreatic beta cell function.

2) Determination of body composition (% visceral fat, % total body water, bone mineral density, etc.) using DEXA analysis. Here the patient scrutiny ends and if at this time the patient agrees to participate in the phase of intervention, the following will be carried out, after explanation and signature of informed consent letter.

1) Cardiovascular function assessment: This will be done through standard echocardiography area of the High Specialty Regional Hospital.
2) Evaluation of renal function: Through 24-hour urine collection, which will measure creatinine clearance and 24-hour albuminuria.
3) Randomization: It will be done by using a table of random numbers generated by a computer program. Patients will be randomized to receive, for 6 months, one of the following 2 interventions: 1) Dosis of *linagliptin 2.5 mg* every 24 h + *metformin 1700 mg* every 24 h y 2) 1 tablet every 12 h of Metformin 850mg.

THIRD PHASE: *Intervention and follow-up*

All patients will receive nutritional guidance and physical activation during the study. Once patients have all baseline measurements and have met the selection criteria, they will be randomized to one of the two interventions. All patients will have monthly follow-up and global clinical evaluation and treatment attachment, and anthropometry.

FOURTH PHASE: *Ending of the study (final measurements).*

At 6 months of treatment, patients will be quoted again in fasting, on two different occasions,

1) FIRST APPOINTMENT: For measurement of glucose and fasting insulin, determination of body composition by imdoanciometry and performance of CTOG.
2) SECOND APPOINTMENT: Evaluation of cardiovascular function by echocardiography, and renal function by depurating creatiline and albumin in urine of 24h.

STATISTIC ANALYSIS

The data will be presented with descriptive statistics, mean and standard or median deviation and interquartile travel, as well as percentages and incidence. For comparisons of numeric variables between study groups, we will use Student t if the distribution of the data follows a normality pattern, otherwise the Mann-whitney U test will be used; to compare qualitative variables we'll use square Chi. Analysis by intent to be treated and analyzed by protocol shall be performed and considered as significant when the p-value is <0.05. For the analysis and presentation of the data we will use the sPSS Version 15 and GraphPad Prism program 5.

ETHICAL ASPECTS

Ethical standards, Regulations of the General Health Law on Health Research and the Helsinki Declaration of the World Medical Association of the 64th General Assembly, StrongHold, Brazil in 2013 and current international codes and standards of good clinical research practices. Special attention will be given to taking care of the privacy and

autonomy of the patient. Once the patient has been informed about the study and agrees to participate in it, and since it involves a greater risk to a minimum, the signature of informed consent letter will be requested.

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