Effect of Linagliptin *versus* Metformin on Insulin Secretion, Insulin Sensitivity and Glucose Control in Subjects with Impaired Glucose Tolerance

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ABSTRACT

Aim: The aim of the study is to evaluate the effect of linagliptin versus *metformin on insulin secretion, insulin sensitivity and glucose control in patients with impaired glucose tolerance (IGT).*

Patients and methods: A randomized, double-blind, clinical trial with parallel groups was per-formed on 16 adults with IGT. Lipid profile and haemoglobin (HbA_{1c}) were evaluated prior to and after the intervention. Glucose and insulin were measured at 0, 30, 60, 90 and 120 minutes after a 75–g oral dextrose load. Eight patients received metformin (500 mg) twice a day before meals for three months. The remaining eight patients received placebo *(500 mg) in the morning and linagliptin (5 mg) in the evening before meals. The area under the curve (AUC) of glucose and insulin, total insulin secretion, first-phase of insulin secretion, and insulin sensitivity were assessed.*

Results: After linagliptin administration, a significant decrease in glucose at 90 minutes (10.8 ± 2.6 vs *7.9 ± 2.2 mmol/L,* p < 0.05*), 120 minutes (8.8 ± 0.9* vs *6.5 ± 2.1 mmol/L,* p < 0.05*) and AUC of glucose (1168 ± 210* vs *953 ± 207 mmol/L,* p < 0.05*) were observed. Metformin administration decreased insulin significantly at 0 minutes (94.8 ± 25.8* vs *73.8 ± 24.6 pmol/L,* p < 0.05*).*

Conclusion: Three-month administration of linagliptin in patients with IGT decreased glucose at 90 and 120 minutes after a 75–g oral dextrose load and AUC of glucose. Metformin decreased insulin at 0 minutes.

Keywords: Glucose intolerance, insulin, linagliptin, metformin

Efecto de la Linagliptina Frente a la Metformina en la Secreción de Insulina, Sensibilidad a la Insulina y Control de la Glucosa en Sujetos con Intolerancia a la Glucosa

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RESUMEN

Objetivo: El objetivo del estudio es evaluar el efecto de la linagliptina frente a la metformina en la secreción de insulina, la sensibilidad a la insulina, y el control de la glucosa en pacientes con intolerancia a la glucosa (IG).

Pacientes y métodos: Se realizó un ensayo clínico aleatorio de doble ciego con grupos parale-los a 16 adultos con IG. El perfil lipídico y la hemoglobina (Hba_{1c}) se evaluaron antes y después de la intervención. La glucosa y la insulina se midieron a los 0, 30, 60, 90 y 120

minutos después de un carga oral de 75–g dextrosa. Ocho pacientes recibieron metformina (500 mg) dos veces al día antes de las comidas por tres meses. Los ocho pacientes restantes recibieron placebo *(500 mg) por la mañana y linagliptina (5 mg) por la noche antes de las comidas. El área bajo la curva (ABC) de la glucosa y la insulina, la secreción total de insulina, la primera fase de la secreción de insulina, y la sensibilidad a la insulina, fueron evaluadas.*

Resultados: *Luego de la administración de la linagliptina, se observó una disminución significativa de la glucosa a los 90 minutos (10.8 ± 2.6 vs 7.9 ± 2.2 mmol/L, p < 0.05), 120 minutos (8.8 ± 0.9 mmol/L p < 0.05) y el ABC de la glucosa (1168 ± 210 vs 953 ± 207 mmol/L, p < 0.05). La administración de metformina redujo significativamente la insulina a los 0 minutos (94.8 ± 25.8 vs 73.8 ± 24.6 pmol/L, p < 0.05).*

Conclusión: *Tres meses de administración de linagliptina en pacientes con IG disminuyó la glucosa a los 90 y 120 minutos después de una carga oral de dextrosa de 75–g y el ABC de la glucosa. La metformina disminuyó la insulina en 0 minutos.*

Palabras clave: Intolerancia a la glucosa, insulina, linagliptina, metformina

INTRODUCTION

Impaired glucose tolerance (IGT) represents a state that not only increases the risk for Type 2 diabetes but also for cardiovascular disease (1). Early physiological studies have shown that IGT is an insulin-resistant state where there is an impaired insulin response to glucose (2). The concept of IGT is a combination of insulin resistance and insulin deficiency, characterized by the abnormal rise in postprandial glucose plasma concentrations (3, 4). Multiple defects in insulin action and beta-cell function characterize the IGT state, but the dominant role in its pathogenesis is ascribed to the impaired ability of pancreatic islets to sense glucose as a stimulus for appropriate insulin release (5).

Metformin therapy for prevention of Type 2 diabetes may be considered in those patients with IGT, impaired fasting glucose (IFG), or HbA_{1c} 5.7–6.4% (6). Dipeptidyl peptidase-4 (DPP-4) inhibitors are a relatively new class of oral antidiabetic agents that enhance insulin secretion by reducing the degradation of endogenous glucagon-like peptide-1 (GLP-1). Controversy exists about the findings observed with several DPP-4 inhibitors such as vildagliptin and sitagliptin in patients with pre-diabetes. Vildagliptin markedly increases post-meal levels of active GLP-1, improving cell function and decreasing postprandial hyperglycaemia and HbA_{1c} levels in patients with IGT (7). Furthermore, in another study, vildagliptin improved insulin sensitivity and cell function, leading to a decrease in postprandial glycaemia in patients with IFG. Thus, vildagliptin may prevent progression to diabetes in high-risk subjects (8). In contrast, sitagliptin did not show improvements in fasting or postprandial glucose turnover in subjects with IFG (9).

To the best of our knowledge, there is no current information about the use of linagliptin, another DPP-4 inhibitor, in patients with pre-diabetes. Therefore, the aim of this study was to evaluate the effect of linagliptin *versus* metformin on insulin secretion, insulin sensitivity and glucose control in patients with IGT.

SUBJECTS AND METHODS

A randomized, double-blind, clinical trial in parallel groups was performed in 16 adults (30–60 years of age) with IGT and overweight or obesity (body mass index (BMI): 25–34.9 kg/m²). Weight of the participants was stable for at least three months. Exclusion criteria were pregnant patients; those who were breastfeeding; patients with diabetes mellitus, hypertension, thyroid or liver disease, chronic renal disease, dyslipidaemia (triglycerides ≥ 5.6 mmol/L, low-density lipoprotein cholesterol (LDL-C) ≥ 4.5 mmol/L), or any other chronic disease. In addition, patients were excluded from the study if they exhibited any contraindication to the use of metformin or linagliptin or if they were on any medications with known effects on glucose or lipoprotein metabolism. All individuals were non-smokers and denied having a history of diabetes mellitus.

After random allocation of the intervention, eight patients received an oral dose of metformin (500 mg twice/day) before breakfast and dinner. The other group

with eight patients received 5 mg of linagliptin (Trajenta, Boehringer Ingelheim–Lilly, Mexico) before breakfast and 500 mg of *placebo* before dinner. Both groups followed the treatments for three months. All patients received general nutritional recommendations and were instructed to not modify their usual exercise habits.

Before testing, an isocaloric diet of at least 250 g of carbohydrates per day was given for three days. Women were in the first phase of their menstrual cycle (3–8 days). Testing was initiated at 8:00 am after a 12-hour fast. Height and weight were recorded with the individuals wearing shoes. Values were used to calculate BMI according to the following formula: weight (kg)/height (m²). Waist circumference was taken at the middle between the highest point of the iliac crest and the lowest rib in the mid-axillary line. Adiposity (% of fat mass) was assessed by bioelectrical impedance analysis, using a contact electrode foot-to-foot body fat analyser system (TBF-300 A, Tanita Corporation of America, Arlington Heights, IL, USA). The investigator evaluated blood pressure after a five-minute resting period with the individual sitting in a chair, and determined using a digital sphygmomanometer.

A venous blood sample was obtained with the subject in a supine position in a quiet room. A catheter was placed in order to accomplish sampling at 0, 30, 60, 90 and 120 minutes after a 75–g oral dextrose load. After that, samples were centrifuged.

The resulting serum was placed into two aliquots: one of the aliquots was immediately used for glucose determination; the second was frozen at -20 ºC for insulin measurement within the following 30 days. At time 0 minutes, an extra blood tube was taken to measure HbA_{1c} and high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and triglyceride (TG) concentrations.

Glucose concentration was determined by the glucose oxidase method; TC, TGC and HDL-C were measured enzymatically. In particular, HDL-C was assessed after selective precipitation of non-HDL-C fractions. Determinations were performed with commercially available equipment (Vitro Ortho-Clinical Diagnostics, Johnson and Johnson, Rochester, NY) with an intra- and inter-assay coefficient of variation of < 2%. Insulin concentrations were measured by a chemiluminescent immunoassay technique (Beckman Coulter, Fullerton, CA) with an intra- and inter-assay coefficient of variation of 3.8 and 4.2, respectively. Haemoglobin was determined in whole blood using ion exchange HPLC (normal range: 4–6%). The area under the curve, 0–120

minutes of glucose and insulin, was calculated with the polygonal formula. Total insulin secretion was evaluated with the insulinogenic index (ΔAUC insulin/ΔAUC glucose). The first phase of insulin secretion was estimated using the Stumvoll index (1283 + 1.829 x insulin 30' - 138.7 x glucose 30' + 3.772 x insulin 0') and insulin sensitivity with Matsuda index [10 000/ √ (glucose 0' x insulin 0'), (mean glucose taken from the oral glucose tolerance test [OGTT] x mean insulin OGTT)] (10, 11). Low-density lipoprotein cholesterol (LDL-C) concentration was estimated using the Friedewald formula (12). Sample size was calculated using a formula for clinical trials (13), with a statistical confidence of 95%, statistical power of 80%, standard deviation (SD) for the two-hour plasma glucose of 0.89 mmol/L and an expected difference of at least 1.27, obtaining a total of eight patients for each group. Calculation for insulin secretion and insulin sensitivity yielded a smaller sample size. Values were converted to the International System of Units and are presented as mean ± SD. Shapiro-Wilk test was used to evaluate normal and intra- and inter-group distribution. Differences were tested using the Wilcoxon signed-rank and Mann-Whitney U-test with SPSS v.20; $p < 0.05$ was considered significant.

The study was reviewed and approved by the local Institutional Ethics Committee and all participants gave written, informed consent prior to any procedures.

RESULTS

The 16 eligible subjects with IGT identified during the screening process completed the three-month period of pharmacological intervention with an adherence > 80%. Three males were included in each group. There was no significant difference in age between groups (53.9 ± 5.4 *vs* 48.9 ± 5.1 years old; $p = 0.101$). No significant differences were shown at baseline in clinical and laboratory characteristics between groups.

Metformin significantly decreased insulin at 0 minutes. In the linagliptin group, there were significant reductions of glucose at 90 and 120 minutes after a 75–g oral dextrose load and glucose AUC (Table).

There were no significant differences in adverse events observed between groups; abdominal pain was reported in one patient and diarrhoea in another patient of the metformin group. In the linagliptin group, no adverse events were observed.

DISCUSSION

Metformin is the main drug used to treat IGT (6), because its administration has prevented or delayed

Table: Clinical and laboratory characteristics in both groups.

	Metformin		Linagliptin	
	Baseline	Final	Baseline	Final
Weight (kg)	81.9 ± 12.2	81.0 ± 12.8	84.2 ± 18.2	86.4 ± 17.7
Body mass index (kg/m^2)	31.0 ± 2.4	30.8 ± 2.6	31.3 ± 3.7	32.3 ± 3.8
Waist circumference (cm)	100 ± 9	97 ± 7	100 ± 13	102 ± 10
Systolic blood pressure (mmHg)	120 ± 12	121 ± 6	123 ± 19	121 ± 23
Diastolic blood pressure (mmHg)	80 ± 8	78 ± 4	80 ± 14	78 ± 17
Glucose 0-min (mmol/L)	5.7 ± 0.7	5.3 ± 0.5	5.3 ± 0.6	5.5 ± 2.5*
Glucose 120-min (mmol/L)	9.3 ± 0.9	8.6 ± 2.3	8.8 ± 0.9	6.5 ± 2.1*
Glycosylated haemoglobin HbA$_{1c}$ (%)	6.2 ± 0.4	6.1 ± 0.4	6.2 ± 0.6	5.9 ± 0.5
Insulin 0 min (pmol/L)	94.8 ± 25.8	73.8 ± 24.6*	87.6 ± 36.0	91.2 ± 43.2
Total cholesterol (pmo/L)	6.3 ± 1.1	6.1 ± 1.4	5.4 ± 1.4	5.0 ± 0.6
LDL-C (pmol/L)	3.9 ± 1.0	3.8 ± 1.0	3.1 ± 1.6	2.6 ± 1.0
HDL-C (pmol/L) (female)	1.0 ± 0.2	1.1 ± 0.2	1.1 ± 0.1	1.1 ± 0.2
HDL-C (pmol/L) (male)	1.1 ± 0.2	1.0 ± 0.3	1.2 ± 0.2	1.0 ± 0.3
Triglycerides (pmoL/L)	3.1 ± 1.6	2.8 ± 1.6	2.5 ± 1.6	2.9 ± 2.2
AUC glucose (mmoL/L)	21843 ± 3993	20943 ± 2571	21039 ± 3782	17169 ± 3724*
AUC insulin (mmoL/L)	8562 ± 5783	7915 ± 3961	7697 ± 3832	8701 ± 4852
Insulinogenic index	0.40 ± 0.26	0.40 ± 0.25	0.39 ± 0.20	0.49 ± 0.24
Stumvoll index	1002 ± 418	898 ± 549	958 ± 693	1073 ± 463
Matsuda index	2.0 ± 0.7	2.4 ± 0.7	2.3 ± 1.1	3.1 ± 2.4

HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; AUC: area under the curve; mmol/L: millimoles per litre; pmol/L: picomoles per litre.
*$p < 0.05$.

the incidence of diabetes in high-risk subjects in 31% (14–16). It has been shown to have effects on insulin secretion and sensitivity (17), which is the reason why it was chosen as the drug to compare with linagliptin in this study. However, the dose of metformin that has been used in patients with IGT has been in the range from 1 to 3 g (15–17), higher than the dose we administered. We therefore, consider that the minimum dose of metformin selected for our study may be considered as a study limitation. We did not find a decrease in insulin secretion and insulin sensitivity or glycaemic control, only a decrease of insulin at 0 minutes. This would be consistent with some beneficial effect on insulin metabolism that may be related to the activation of AMPK-dependent protein kinase, an important enzyme involved in the insulin-signaling pathway (18).

Moreover, the use of linagliptin has not yet been approved in pre-diabetes. It is only indicated for glycaemic control in patients with diabetes (19); however, it has been shown that the use of DPP-4, including vildagliptin, had a beneficial effect in patients with pre-diabetes through improvements in insulin secretion and sensitivity, among other pharmacological effects (7, 8). Therefore, linagliptin may be useful in the management of patients with impaired glucose.

In this study, improvement in insulin secretion and sensitivity using linagliptin was expected. It has been shown that GLP-1 increases pancreatic insulin secretion (20), but that was not the case because no significant changes were observed. This can be explained because the equations for the Stumvoll and Matsuda indices require insulin and glucose in the first minutes and, at this stage, no changes were observed in addition to a possible metabolic compensation in this group of patients (5). Although both formulae have good correlation with the gold standard, they are generally considered as estimations of insulin metabolism.

No decrease in fasting glucose was observed. This was perhaps because linagliptin acts after food intake and its effect is primarily on postprandial glucose (21). A decrease in HbA$_{1c}$ was expected, which was only 0.3%, with no statistical significance. This may be due to the fact that patients entered the study with HbA$_{1c}$ values < 6.5%.

Linagliptin showed significant glucose reductions at 90 and 120 minutes after a 75–g load of oral dextrose and diminished glucose AUC. This may be explained by the incretin effect mechanism of DPP-4. Linagliptin provides a longer shelf life than the insulinotropic hormones such as glucose-dependent insulinotropic peptide

and GLP-1, which act following the ingestion of food and increased pancreatic insulin secretion following the ingestion of food (20, 21). These data are comparable with those reported in patients with pre-diabetes who used vildagliptin and demonstrated improvements in postprandial glucose (7, 8).

The decrease in glucose AUC in this study is similar to that reported in diabetic patients given sitagliptin-pioglitazone. The area under the curve glucose reduction was observed together with a postload glucose reduction (22). Despite our results, further long-term studies with a larger sample size are necessary in order to recommend the use of linagliptin in patients with glucose intolerance.

In conclusion, three-month administration of linagliptin in patients with IGT decreased glucose at 90 and 120 minutes after an oral 75–g dextrose load and AUC of glucose. Metformin administration only decreased insulin at 0 minutes.

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AUTHORS' NOTE

The authors state that there is no conflict of interest with regard to this manuscript. The authors declare no competing interests with the mentioned pharmaceutical company.

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