Effects of sitagliptin in diabetic patients with nonalcoholic steatohepatitis

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Abstract

Background & Aims : **Preliminary evidence suggests that inhibition of dipeptidyl peptidase (DPP)-IV preserves pancreatic beta cell function in patients with type 2 diabetes (T2D). However, its effects on liver histology in nonalcoholic steatohepatitis (NASH), hepatic complication of diabetes, have not yet been adequately explored. The present open-label, single-arm observational pilot study investigated the effects of one year of treatment with a dipeptidyl peptidase-IV inhibitor, sitagliptin, on liver histology, body mass index (BMI), and laboratory parameters in NASH patients with T2D.**

Patients and Methods : **Paired liver biopsies from 15 diabetic patients with NASH (7 males, 8 females ; mean age : 49.7 ± 8.1 years (range : 36-62)) before and after one year of therapy with sitagliptin 100 mg once daily were studied. Clinical and laboratory parameters were recorded.**

Results : **Treatment with sitagliptin resulted in a significant decrease in ballooning (*P* = 0.014) and NASH scores (*P* = 0.04), while the reduction in the steatosis score was of borderline statistical significance (*P* = 0.054). These effects were accompanied by a significant reduction in body mass index, AST, and ALT levels.**

Conclusion : **Our study suggests that sitagliptin ameliorates liver enzymes and hepatocyte ballooning in NASH patients with T2D and may have therapeutic implications.** (Acta gastroenterol. belg., **2012**, 75, **240-244**).

Key words : sitagliptin, diabetes, nonalcoholic steatohepatitis, dipeptidyl peptidase-IV inhibitor, DPPIV.

Introduction

A novel approach in the treatment of type 2 diabetes (T2D) is to harvest the actions of incretin hormones such as glucagon like peptide (GLP)-1 and glucose-dependent insulinotropic peptide (GIP). GLP-1 and GIP are gut peptides secreted in a nutrient-dependent manner, stimulating glucose-dependent insulin secretion. In rodents, they promote beta cell proliferation and inhibit apoptosis, leading to expansion of beta cell mass (1,2). Furthermore, GLP-1 improves glucose homeostasis via the inhibition of gastric emptying, food intake, and glucagon secretion (3). In this regard, GLP-1 administration potently stimulates insulin secretion and reduces blood glucose in human subjects with T2D (3). Dipeptidyl peptidase IV (DPP-IV) is the enzyme responsible for the degradation of endogenous GLP-1 and GIP by rapidly cleaving and inactivating both peptides into inactive metabolites (4). Hence, a recent glucose-lowering approach is to enhance incretin hormone levels and their activities through the development of selective DPP-IV inhibitors (5,6). This approach in both preclinical and clinical studies of T2D has been shown to increase the levels of intact GLP-1 and GIP, ultimately leading to significant improvements in overall glucose homeostasis (7-9). Nonalcoholic steatohepatitis (NASH) is a subset of nonalcoholic fatty liver disease that may progress to cirrhosis in up to 30% of patients and may lead to decompensated liver disease (10). T2D and NASH are highly correlated disorders, as insulin resistance is a common pathophysiological hallmark in both conditions (11). Nevertheless, recent data indicate that the insulin resistance is likely to be the consequence rather than the cause of NASH (12). Therefore, targeting the insulin resistance for NASH management is not feasible (13). Since the DPP-IV inhibitors are shown to be safe in patients with impaired liver function, their usefulness in NASH should be elucidated (14,15). In a recent animal study, sitagliptin has decreased the liver steatosis, β-cell apoptosis and insulin resistance in fructose-fed rats that developed metabolic syndrome (16). To our knowledge, there is no clinical study conducted in humans examining the effects of sitagliptin on NASH patients with T2D, despite the promising evidence in animal studies.

The present open-label, single-arm, single-centre pilot study investigated the effects of one year of treatment with sitagliptin on liver histology in NASH patients with T2D.

Methods

This was an open-label, single-arm, single-centre pilot study that evaluated the effects of one-year treatment with sitagliptin on liver histology in NASH patients with T2D.

The primary endpoints included the changes in steatosis, lobular inflammation, ballooning, fibrosis, and histological NASH score in paired liver biopsy. The secondary endpoints were the changes in body mass index (BMI), liver enzymes, and glycemic indexes. Safety was assessed via regular monitoring of treatment-emergent adverse events and laboratory tests.

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Patients were deemed eligible for the study if they had a diagnosis of T2D and histological proof of NASH and they were scheduled for sitagliptin 100 mg once daily for the treatment of T2D. The diagnosis of T2D was made according to the ADA criteria (17). Suitable patients, identified from the review of case notes and/or computerized clinic registries, were contacted by the investigators in person, were followed-up, and those who gave consent were scheduled for a second liver biopsy after one year of treatment with sitagliptin. Twenty T2D patients aged ≥ 18 years of either sex who had biopsy-proven NASH within the previous six months were recruited into the study. Of them, two denied consent for the second biopsy, two required insulin treatment during follow-up, and in one case the biopsy material was not sufficient for the second histological assessment. Consequently, the final data set included a total of 15 patients.

Patients with viral hepatitis, hemochromatosis, Wilson's disease, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, biliary obstruction, alpha-1 antitrypsin deficiency, ischemic cardiac or cerebrovascular disease, impaired renal function or malignancies were excluded. Subjects using angiotensin II receptor blockers, estrogens, amiodarone, steroids, tamoxifen, lipid lowering agents, and other antidiabetic medications (insulin, thiazolidinediones, metformin, sulfonylureas, alpha-glucosidase inhibitors) were not eligible for this study. Patients with a Child-Pugh score > 5, history of pancreatitis, pregnancy, alcohol intake exceeding 20 g/day, or previous abdominal surgery were also excluded. Standard diet advice for T2D was given by a dietician. Physical activity was left at the patient's discretion but none of the study participants joined any special fitness program aiming to lose weight throughout the entire study period.

All subjects underwent physical examination, anthropometric measurements and biochemical screening before and after one year of sitagliptin therapy. Body mass index (BMI) and blood pressure were recorded. The estimate of insulin resistance was calculated using the HOMA-IR index, with the following formula : insulin resistance = fasting plasma insulin × fasting plasma glucose/22.5.

Ultrasonography-guided liver biopsies were obtained using a 16-gauge Hepafix needle (Braun Malsungen, Germany). All biopsy specimens were placed in formalin solution for fixation and embedded in paraffin blocks. Serial sections (sectioned at 4 mm intervals) were stained with haematoxylin-eosin and Masson's trichrome. An experienced pathologist blinded to the clinical data scored the liver biopsies according to the NIDDK NASH Clinical Research Network scoring system (18). We preferred using this scoring system because it has the advantage of encompassing the entire histological spectrum of NAFLD, not only NASH, thus enabling the comparative analysis of pre-treatment and post-treatment biopsies. Additionally, in comparison with the previous scoring systems, stage 1 is examined in a more detailed manner as it is further subdivided into three substages (19). Steatosis was scored from 0 to 3 with a four grades scoring system from S0 to S3 : S0 : no steatosis or steatosis in less than 5%, S1 : 5-33%, S2 : 33-66%, S3 : > 66% of hepatocytes. Lobular inflammation was graded as follows : stage 0, no foci ; stage 1 : < 2 foci ; stage 2 : 2-4 foci ; stage 3 : > 4 foci per 200 × field. Ballooning degeneration of liver cells was evaluated as : grade 0, absent ; grade 1, few cells ; grade 2, many cells. The histological NASH score was defined as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and ballooning (0-2) ; thus ranging from 0 to 8. Cases with scores of 0-2 were considered as having simple steatosis ; whereas, cases with scores of 5 or greater were diagnosed as definite NASH. Cases with activity scores of 3 and 4 were considered as borderline NASH (18). Only subjects with a baseline NASH score ≥ 3 were eligible for the study. Fibrosis was staged as follows : stage 0 : no fibrosis ; stage 1 : perisinusoidal or periportal fibrosis with 3 different patterns : 1A : mild, zone 3, perisinusoidal ; 1B : moderate, zone 3, perisinusoidal fibrosis, and 1C portal/periportal fibrosis ; stage 2 : perisinusoidal and portal/periportal fibrosis ; stage 3 : bridging fibrosis ; stage 4 : cirrhosis.

The study protocol was approved by the local ethics committee (approved on February 27, 2009 ; MAR-YÇ-2009-0010), and conducted in compliance with good clinical practice and the principles of the Declaration of Helsinki. All patients gave written informed consent to participate in the study.

Statistical analysis

The sample size was not based on any statistical calculations for power, as this was an exploratory pilot study. Similarly, no power calculation was performed for safety measures, because there was no statistical analysis to be performed without a comparison group. No patient was lost to follow-up. Data were tested for normality using a Shapiro-Wilk calculation. Variables were expressed as means ± standard deviations or medians and interquartile ranges, as appropriate. Changes in quantitative measures from baseline to one year of treatment were tested for significance using paired t-tests (for Gaussian data) or Wilcoxon signed-rank test (for skewed variables). In the case of missing data points, we utilized last observation carried forward. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 14.0 (SPSS Inc., Chicago, IL, USA). A P value of < 0.05 (two-tailed) was considered statistically significant.

Results

A total of 15 T2D patients with NASH (7 males, 8 females ; mean age : 49.7 ± 8.1 years (range : 36-62)) participated. At baseline, there were 5 patients with

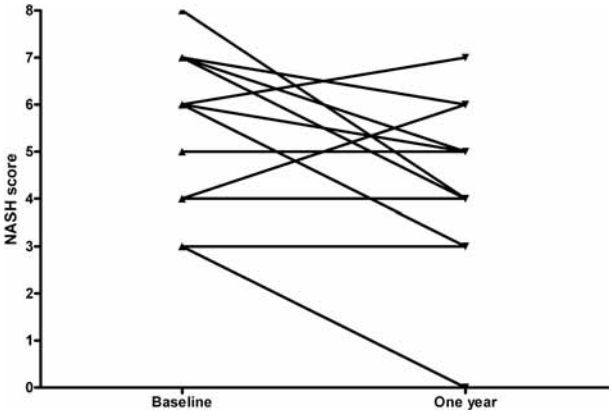

Fig. 1. — Changes in NASH scores in paired liver biopsies (n = 15) before and after one year of treatment with sitagliptin.

borderline NASH and 10 with definite NASH. The mean waist and hip circumferences were 100.2 ± 10.2 and 106.1 ± 10.2 cm, respectively. The mean systolic and diastolic blood pressure values were 130.7 ± 18.7 and 83.1 ± 11.3 mmHg, respectively. All patients were evaluated for the one-year endpoints.

After one year of treatment, sitagliptin treatment caused a significant reduction in ballooning ($P = 0.014$, Fig. 1) and NASH score ($P = 0.04$), while the reduction in steatosis score was of borderline statistical significance ($P = 0.054$; Table 1). By contrast, fibrosis and lobular inflammation did not improve. These effects on liver histology were accompanied by a significant reduction in body mass index ($P = 0.044$,) serum AST ($P = 0.003$), and serum ALT ($P = 0.001$). No significant changes in the other variables were observed (Table 2).

Aiming to test whether the difference in NASH score is parallel to the decreased BMI, we examined the correlation between the change in NASH score and change in BMI pre-treatment and after treatment with sitagliptin. We did not find any correlation between the two variables (change in NASH score ; -16.49 ± 36.5% vs. change in the BMI ; -3.06 ± 5.23% ; $P = 0.28$).

Treatment was generally well tolerated ; only one patient noted appreciable diarrhea, which was not sufficient to necessitate dose reduction or cessation of the drug.

Discussion

The current pilot study has demonstrated that one-year treatment with sitagliptin, a DPP-IV inhibitor, ameliorates hepatocyte ballooning and a trend toward improved liver steatosis was observed in NASH patients with T2D. These two changes lead to a significant reduction in the NASH score in paired biopsy samples. Treatment with sitagliptin also associated with a significant reduction in BMI and serum liver enzymes. Taken together, these data suggest that sitagliptin decreases serum AST and ALT levels together with a significant

Table 1. — **Histological scores at baseline and after one year of treatment with sitagliptin in 15 patients with type 2 diabetes and NASH**

	Baseline	One year	P
Steatosis	2.33 ± 0.72	1.93 ± 0.88	0.054
Lobular inflammation	1.73 ± 0.70	1.47 ± 0.83	0.33
Ballooning	1.53 ± 0.64	1.07 ± 0.70	0.014
Fibrosis	1.67 ± 1.54	1.60 ± 1.59	0.77
NASH score	5.64 ± 1.60	4.50 ± 1.83	0.04

beneficial effect on the extent of hepatocyte swelling as reflected by the ballooning score. If independently validated by randomized, placebo-controlled trials, these findings raise the possibility that sitagliptin could offer a novel agent for the treatment of NASH in patients with T2D.

Several lines of evidence support the use of DPP-IV inhibitors in NASH. Serum DPP-IV activity was found to be significantly increased in a study involving 31 NASH patients compared to controls (20). Notably, both serum activity and the intensity of DPP-IV immunostaining in the liver were found to be significantly associated with the intensity of fatty infiltration and the histologic grading (20). These results suggest a direct participation of DPP-IV in the pathophysiology of NASH, thus providing an evidence for the use of DPP-IV inhibitors to prevent or slow down the progression of hepatosteatosis and inflammation (14). It is also worth noting that evidence from animal studies suggests an increased serum DPP IV activity in experimental liver cirrhosis (21). In line with these results, an increased expression of DPP-IV in liver has been reported in rats with experimental diabetes and fed a high-fat diet (22). In the light of these findings, the use of DPP-IV inhibitors in the setting of NASH is promising and may have several inherent advantages in terms of efficacy and tolerability compared with thiazolidinediones, which have been shown to induce weight gain (23).

The most significant changes in hepatic histology induced by sitagliptin in our study are reflected by the decrease in the grade of hepatocyte ballooning and, to a lesser extent, steatosis. There was no change in fibrosis for which treatment periods of longer than one year would probably be needed. Ballooning degeneration which has been considered a hallmark of steatohepatitis, associates with cell swelling (24,25) and has been linked to cytoskeletal injury in NASH (24). Therefore, it is tempting to speculate that DPP-IV inhibitors may exert a protective effect on the cytoskeletal hepatocyte proteins.

Elevated ALT is a biochemical surrogate marker of NASH. In a study conducted in NASH patients who underwent bariatric surgery changes in aminotransferases were positively correlated with the changes in histological grades (26). The amelioration of ALT observed

Table 2. — **Clinical and biochemical characteristics at baseline and after one year of treatment with sitagliptin in 15 patients with type 2 diabetes and NASH**

	Baseline	One year	P
BMI, kg/m²	30.7 ± 5.6	29.7 ± 5.2	0.044
AST, U/L	46 ± 19	30 ± 10	0.003
ALT, U/L	65 ± 22	40 ± 21	0.001
Serum glucose, mg/dL	126 ± 37	125 ± 32	0.97
Serum insulin, μU/mL	17.2 (7.84-33.20)	17.32 (11.04-23.24)	0.91*
HOMA-IR	5.66 (2.18-8.52)	5.09 (4.16-7.23)	0.73*
Triglycerides, mg/dL	158 (126-190)	153 (121-247)	0.27*
Total cholesterol, mg/dL	223 ± 58	221 ± 54	0.76
HDL cholesterol, mg/dL	45 ± 14	43 ± 15	0.46
LDL cholesterol, mg/dL	137 ± 52	142 ± 41	0.31
HbA1c, %	6.7 ± 1.3	6.5 ± 1.7	0.61

Data are given as means ± standard deviations or medians (interquartile ranges). P values were calculated using paired Student's t test unless otherwise indicated. * Wilcoxon paired signed-rank test. BMI : body mass index ; AST : aspartate aminotransferase ; ALT : alanine aminotransferase ; HOMA-IR : homeostasis model of assessment-insulin resistance ; HDL : high-density lipoprotein ; LDL : low-density lipoprotein ; HbA1c : glycated hemoglobin.

with sitagliptin treatment in our patients may be partially related to the decreased BMI. However it is not plausible to exclude the beneficial effects of sitagliptin although in previous studies treatment with sitagliptin had neutral effect on body weight (27). Future studies in larger samples are needed to confirm and expand these preliminary data.

Interestingly, the observed trend toward a reduction of steatosis in our study – one of the earliest events in the progression of nonalcoholic fatty liver disease – is consistent with our understanding of the efficacy of sitagliptin in animal models who develop the metabolic syndrome (16). It is noteworthy that sitagliptin did not improve the HOMA-IR in our diabetic patients with NASH. This fact is not unexpected since sitagliptin did not affect the indexes of insulin resistance and sensitivity despite the significant improvements in indexes of insulin release and β-cell function in patients with T2D (27).

Several important caveats are inherent in our study. First, we did not evaluate if the beneficial effects on liver enzymes were sustained after the cessation of therapy. Secondly, we evaluated only a limited number of insulin resistance biomarkers ; therefore, more parameters should be evaluated for assessing whether an effective improvement of insulin resistance may occur in T2D patients with NASH following sitagliptin treatment. Third, our sample included subjects of Turkish nationality, so that results might not be applicable to populations with different ethnic background. This shortcoming is common to several single-center studies where the population is genetically and ethnically homogeneous. Fourth, the intensity of DPP-IV immunostaining was not checked in paired liver biopsies. Fifth, there was no placebo arm to compare whether the end of treatment results is purely attributable to the sitagliptin or they are the results of the variations observed throughout the natural course of the disease. Finally, because this was a small trial gathering the results of only fifteen patients our findings should be translated carefully. An important strength of our study is that all participants underwent paired liver biopsy, the best standard to monitor the progression of NASH (28). Liver biopsy has the advantage of providing important information relating to the degree of liver damage, as well as of comparing histological changes before and after therapy (19).

In summary, this pilot clinical study is the first to demonstrate that sitagliptin may improve hepatocyte ballooning and liver enzymes in diabetic patients with NASH. Future large, placebo-controlled randomized clinical trials are needed to confirm and expand these findings.

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