Efficacy of alogliptin in preventing non-alcoholic fatty liver disease progression in patients with type 2 diabetes

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Abstract. Non-alcoholic fatty liver disease (NAFLD) represents one of the most common causes of chronic liver disease worldwide and is characterized by chronic liver inflammation and fibrosis leading to cirrhosis and increased risk of liver cancer in a proportion of patients. Effective anti-fibrotic agents have yet to be approved for the treatment of NAFLD. The present study aimed to evaluate the efficacy of dipeptidyl peptidase 4 inhibitors (DPP4-I) in the prevention of NAFLD progression in NAFLD patients with type 2 diabetes. The study was a single arm, multi-centre, non-randomised study of NAFLD patients with type 2 diabetes. NAFLD was diagnosed according to ultrasonographic findings. All the patients received 25 mg/day of alogliptin for 12 months. The efficacy of alogliptin in preventing NAFLD progression was assessed using overall NAFIC scores [non-alcoholic steato-hepatitis (NASH), ferritin, insulin and type IV collagen 7S] and individual component scores according to baseline haemoglobin A1c (HbA1c) levels. Of the 39 patients enrolled in the study, 16 patients (40.3%) had NAFIC scores >2 points, indicating the presence of NASH. NAFIC scores markedly decreased following 12 months of alogliptin administration, but remained >2 points in 10 patients, indicating that NASH may have persisted in these patients. The relative risks for persistent NASH were 4.92 (95% confidence interval, 0.61-40.0) in the highest HbA1c tertile group compared with those in the lowest group. However, no statistically significant linear trend was observed across all HbA1c categories (P=0.145). DPP4-I may have efficacy against NAFLD progression in patients with type 2 diabetes with relatively lower HbA1c levels. DPP4-I may represent a potential new therapeutic strategy for the prevention of disease progression in NAFLD patients with type 2 diabetes.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease worldwide (1). NAFLD encompasses a wide spectrum of liver diseases ranging from simple steatosis to non-alcoholic steatohepatitis, which may progress to liver cirrhosis in ≤30% patients, potentially leading to decompensated liver disease (2). As liver fibrosis progresses over a long period of time, therapies should be tolerable and safe over decades, with good targeting to the liver and few adverse effects on other organs.

However, no anti-fibrotic agents have yet been approved for clinical practice (3). Several studies have evaluated the efficacy of dipeptidyl peptidase 4 inhibitor (DPP4-I) administration in the treatment of NAFLD and non-alcoholic steatohepatitis (NASH) patients with type 2 diabetes. In NAFLD patients, DPP4-I administration has been shown to decrease serum alanine aminotransferase (ALT) levels in a positive correlation with haemoglobin A1c (HbA1c) levels (4), and ameliorate liver enzyme abnormalities and hepatocyte

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ballooning in NASH patients (5). Although our previous study reported DPP4-I as a potential new therapeutic agent against liver fibrosis in an experimental model of liver fibrosis via suppression of activated hepatic stellate cell (HSC) proliferation and collagen synthesis (6), the efficacy of DPP4-I for the prevention of NAFLD progression in clinical settings remains to be elucidated.

The aim of the present study was to evaluate the efficacy of DPP4-I in the prevention of NAFLD progression according to decreases in NAFIC scores. We further hypothesize that, in a similar manner to baseline HbA1c levels being a strong predictor of HbA1c change, the efficacy of DPP4-I in preventing NAFLD progression would be more significant in patients with higher HbA1c levels compared to those with lower levels.

Materials and methods

Study design. The study was a single arm, multi-center, non-randomized study of NAFLD patients with type 2 diabetes that were recruited from 8 centers in Japan between June 2012 and December 2013. All the patients received 25 mg/day of alogliptin (Takeda, Osaka, Japan) for 12 months.

Patients. Patients enrolled were previously diagnosed with type 2 diabetes according to the diagnostic criteria of the Japan Diabetes Society (JDS) with ALT levels >30 IU/L, which is the cut-off level used to screen for NAFLD (7,8). Patients with a current daily alcohol intake of >20 g or with known liver disease of other aetiology were excluded. Following confirmation of eligibility, receipt of informed consent and screening procedures, eligible patients were administered alogliptin and followed up over a 1-year period. NAFLD was defined according to characteristic ultrasonographic findings, such as increased hepatorenal contrast or enhanced liver brightness (9). All the patients provided written consent to participate in the study. The protocols used were approved by the Ethics Committee of Nara Medical University (Nara, Japan; UMIN000008068) and other facilities.

Data collection. On the date of alogliptin treatment initiation, patients underwent laboratory tests, routine medical history inquiry and physical examinations, including age, gender and body weight and medical history. Laboratory tests at baseline included overnight fasting measurements of aspartate aminotransferase, ALT, γ-glutamyltranspeptidase, total cholesterol, triglyceride, high-density lipoprotein cholesterol, fasting plasma glucose, HbA1c, immunoreactive insulin (IRI), ferritin, hyaluronic acid, type IV collagen 7S and type III procollagen-N-peptide. These parameters were measured by the standard techniques used in clinical chemistry laboratories (SRL, Tokyo, Japan). One year after registration, IRI, ferritin and type IV collagen 7S levels were obtained for the estimation of NAFIC scores. HbA1c levels were expressed in accordance with the National Glycohemoglobin Standardization Program, as recommended by the JDS (10).

Study outcome. One year after registration, the NAFIC (NASH, ferritin, insulin and type IV collagen 7S) score and each individual variable was used to evaluate the efficacy of alogliptin in the prevention of NAFLD progression in all the included patients.

NAFIC score. The original NAFIC score is a simple clinical scoring system allowing the differentiation of NASH from NAFLD using a cut-off score of 2. The NAFIC score is a weighted sum of three clinical variables: Serum ferritin ≥200 ng/ml (female) or ≥300 ng/ml (male), 1 point; serum fasting insulin ≥10 IU/ml, 1 point; and serum type IV collagen 7S ≥5.0 ng/ml, 2 points (11).

Statistical analyses. All the variables are expressed as medians and inter quartile ranges. Differences between the groups were evaluated using the unpaired Student's t-test for normally distributed variables and the Mann-Whitney U test for variables with skewed distributions. The trend test was used to evaluate differences between category variables. Relative risk regression analyses were performed to estimate relative risks [95% confidence interval (CI)] of persistence of NAFIC scores of >2 points at the end of the study (i.e. possible persistence of NASH) in comparison with the lowest tertile of HbA1c levels as a reference group. All the reported P-values were 2-sided and $P<0.05$ was considered to indicate a statistically significant difference. All the analyses were performed using Stata/MP version 13.0 (Stata Corporation, College Station, TX, USA).

Results

Patients. A total of 59 patients were enrolled in the study between June 2012 and December 2013. A total of 20 patients were excluded due to drop out prior to completion (5 patients) and absence of blood examination results (15 patients). The remaining 39 patients met the inclusion criteria and were included in the analysis. Table I shows the patient demographics and laboratory data according to the NAFIC scores. The median patient age, HbA1c level and body mass index were 61 years, 6.8% (53.0 mmol/mol) and 28.6 kg/m^2, respectively. In 16 patients (41.0%), the NAFIC score was >2 points, indicating the presence of NASH in 41.0% of patients with type 2 diabetes with ultrasonographic fatty liver and ALT levels of >30 U/l.

Changes in NADIC scores. Fig. 1A shows the changes in the NAFIC scores following treatment with alogliptin. NAFIC scores were significantly decreased at 52 weeks after the initiation of DPP4-I therapy. Fig. 1B shows NAFIC score changes according to baseline NAFIC scores. NAFIC scores decreased in 13 patients and remained >2 points in 10 patients, indicating NASH had possibly persisted in these patients. NAFIC scores had increased in only 1 patient at the end of the study compared to baseline scores.

Subsequently, the individual components of the NAFIC score at the baseline and the end of the study were evaluated, stratified according to the baseline HbA1c tertile (Table II). No significant changes in fasting IRI or type 4 collagen 7S levels were observed; however, ferritin levels were significantly decreased after 52 weeks administration of DPP4-I. Stratified analysis according to the HbA1c tertile demonstrated significant changes in the ferritin levels in the lowest HbA1c category only.

Table I. Baseline characteristics of the participants stratified by the NAFIC score.

Characteristics	Total (n=39)	NAFIC score 0 to 1 (n=23)	NAFIC score 2 to 4 (n=16)
Age (years)	61 (52-66)	60 (52-64)	62 (49-66)
Body mass index, kg/m^2	28.6 (26.9-31.2)	28.2 (21.9-31.2)	30.1 (27.7-33.9)
Female, %	51.3	52.2	50.0
PLT, 10^4 μl	19.5 (17.1-21.8)	19.6 (17.4-25.0)	18.7 (11.1-21.4)
AST, IU/l	40 (29-53)	31 (27-46)	46 (37-61)
ALT, IU/l	49 (38-66)	47 (34-64)	55 (42-68)
γGTP, IU/l	55 (38-86)	50 (32-77)	72 (44-106)
HbA1c, %	6.8 (6.4-7.9)	6.8 (6.4-7.5)	7.1 (6.3-8.0)
FPG, mg/dl	130 (115-159)	130 (112-154)	138 (122-172)
Fasting IRI, μU/ml	12.6 (6.5-19.3)	10.4 (5.8-16.5)	17.3 (12.0-25.9)
HOMA-IR	4.4 (2.5-7.7)	2.8 (1.9-5.2)	7.0 (4.2-9.9)
TG, mg/dl	176 (123-227)	183 (125-213)	172 (114-256)
HDL-C, mg/dl	46 (41-53)	45 (41-53)	51 (42-54)
TC, mg/dl	186 (168-207)	182 (167-202)	193.5 (170-211)
Ferritin, ng/ml	155.0 (47.0-342.0)	99.3 (43.3-179.0)	314.0 (52.1-380.0)
Hs-CRP	1,010 (568-1,680)	1,135 (471-2,110)	938 (680-1,585)
Hyaluronic acid, ng/ml	57 (24-86)	34 (24-81)	75 (28-113)
P-3-P, U/ml	0.53 (0.49-0.65)	0.50 (0.43-0.59)	0.63 (0.51-0.72)
Type 4 collagen 7S, ng/ml	3.7 (3.1-5.2)	3.3 (2.6-3.7)	5.4 (4.5-6.7)
OADs use, %			
Sulfonylurea	5.1	0.0	12.5
Thiazolidinedione	5.1	6.3	4.4
Metformin	2.6	4.4	0.0
α-glucosidase inhibitor	2.6	0.0	6.3
Hypertension, %	18.0	21.7	12.5
Dyslipidemia, %	18.0	13.0	25.0
Smoker, %	15.4	13.0	18.8

Data are median values (interquartile range) for continuous variables and % for categorical variables. ALT, alanine aminotransferase; AST, aspartate aminotransferase; FPG, fasting plasma glucose; γPG, γ-glutamyltranspeptidase; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; Hs-CRP, high-sensitivity C-reactive protein; IRI, immunoreactive insulin; P-3-P, type III procollagen-N-peptide; PLT, platelet count; TC, total cholesterol; TG, triglyceride; OADs, oral antidiabetic agents.

Table II. Changes in fasting IRI, type 4 collagen 7S and ferritin stratified according to baseline HbA1c levels.

	HbA1c range, %			
Changes	5.5-6.5 (n=14)	6.6-7.2 (n=12)	7.3-11.8 (n=13)	Total
Fasting IRI, μU/ml				
Month 0	12.7 (6.1-48.6)	11.5 (6.5-17.2)	13.6 (11.5-19.3)	12.6 (6.5-19.3)
Month 12	9.6 (8.0-17.4)	9.3 (6.2-23.1)	12.1 (9.1-16.0)	10.4 (8.0-17.7)
P-value	0.6378	0.4328	0.1330	0.5029
Type 4 collagen 7S, ng/ml				
Month 0	3.7 (2.8-4.7)	3.7 (2.6-4.8)	4.8 (3.5-6.8)	3.7 (3.1-5.2)
Month 12	3.4 (3.3-3.7)	3.6 (3.1-4.0)	3.7 (3.3-4.5)	3.7 (3.3-4.5)
P-value	0.2547	0.5828	0.8887	0.6249
Ferritin, ng/ml				
Month 0	124.7 (85.2-205.0)	211.5 (53.0-380.0)	155.0 (31.5-286.0)	155.0 (47.0-342.0)
Month 12	100.7 (54.5-137.0)	137.0 (40.3-251.5)	102.0 (19.0-251.0)	108.0 (36.0-191.0)
P-value	0.0035	0.0844	0.0869	0.0003

Data are the median values (interquartile range). HbA1c, haemoglobin A1c; IRI, immunoreactive insulin.

Table III. Association between the possibly persistent NASH at the end of the study and baseline glycemic control.

	HbA1c tertiles			
Possibly persistent NASH	First (n=201)	Second (n=168)	Third (n=151)	P-value for trend
RR for possibly persistent NASH (crude)	Reference	1.17 (0.28-4.83)	1.79 (0.52-6.15)	0.347
RR for possibly persistent NASH (model 1)	Reference	1.29 (0.14-11.62)	4.92 (0.61-40.0)	0.145

Model 1 was adjusted for age, gender and body mass index. HbA1c, haemoglobin A1c; NASH, non-alcoholic steatohepatitis; RR, relative risk.

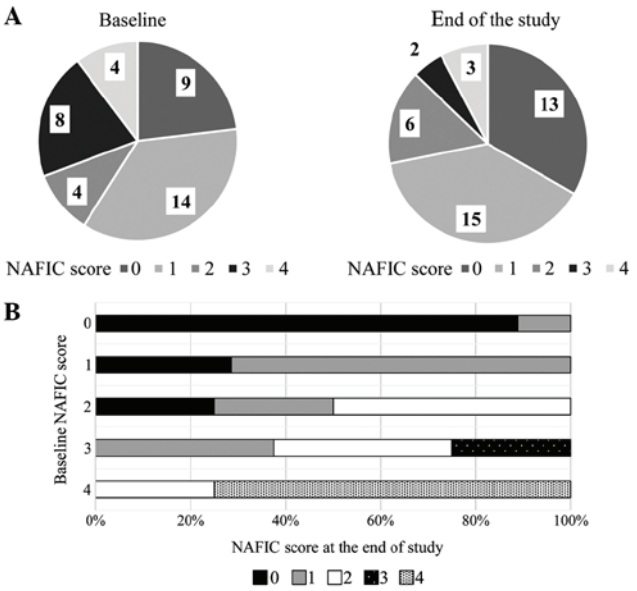

Figure 1. Changes in NAFIC scores. (A) The changes in the NAFIC scores following treatment with alogliptin. NAFIC scores were significantly decreased at 52 weeks after the initiation of dipeptidyl peptidase 4 inhibitors (DPP4-I) therapy. (B) The NAFIC score changes according to baseline NAFIC scores. NAFIC scores decreased in 13 patients and remained >2 points in 10 patients.

NASH and HbA1c tertiles. The association between possibly persistent NASH at the end of the study and HbA1c tertiles are shown in Table III. The relative risks for possibly persistent NASH was 4.92 (95% CI, 0.61-40.0) in the highest HbA1c tertile group compared to the reference category of the lowest HbA1c tertile. However, no statistically significant linear trend was observed across all HbA1c categories (P=0.145).

Discussion

To the best of our knowledge, this is the first clinical study to demonstrate the efficacy of administering DPP4-I for 12 months in order to decrease serum ferritin levels. According to the NAFIC scores, this would prevent the progression of NAFLD in patients with type 2 diabetes. The effects were observed only among patients in the lower HbA1c tertile at baseline, which was different from the efficacy in order to use for lowering HbA1c level. Therefore, we speculate that the reduction in oxidative stress, resulting from a decrease in serum ferritin levels, suppressed the extent of liver injury induced by inflammatory cytokines in NAFLD patients with early type 2 diabetes. DPP4-I may therefore be efficient in preventing the progression of NAFLD to NASH in NAFLD patients with early type 2 diabetes.

A number of previous studies have reported the effect of DPP4-I on liver dysfunction in NAFLD patients. Iwasaki *et al* (12) first reported that 4 months of sitagliptin administration resulted in improved liver enzyme abnormalities in NAFLD patients with type 2 diabetes. Yilmaz *et al* (13) reported the effect of DPP4-I in patients with biopsy-proven NASH with type 2 diabetes. This study demonstrated that administration of sitagliptin for 12 months ameliorated liver enzyme abnormalities and hepatocyte ballooning in patients whose body weight decreased during the study period. Fukuhara *et al* (4) reported that administration of sitagliptin for 12 months in patients with biopsy-proven NAFLD with type 2 diabetes improved the liver enzyme abnormalities in parallel with decreases in HbA1c levels. However, the present study did not evaluate the association between changes in body weight, HbA1c levels and NAFIC scores during the study period. This study demonstrated that administration of DPP4-I for 12 months significantly reduced NAFIC scores in NAFLD patients with type 2 diabetes. Furthermore, in patients in the highest HbA1c tertile, NAFIC scores were observed that had remained >2 points during the study period, indicating the persistence of NASH. Self-care activity in patients with lower HbA1c levels was known to be higher compared to those with higher HbA1c levels (14); therefore, patients with lower HbA1c levels may have had ideal lifestyles and body weights preventing worsening of HbA1c levels.

In the liver, DPP4 is expressed on the surface of HSCs and may contribute to activated HSC-induced ECM accumulation (15). Kaji *et al* (6) reported that DPP4-I inhibited liver fibrosis and production of hepatic transforming growth factor-β1 (TGF-β1), along with attenuation of α-smooth muscle actin-positive activated HSCs. These results indicate that the suppression of activated HSC function may underlie the anti-fibrotic effect of DPP4-I. As high glucose levels and high insulin levels stimulate the proliferation of activated HSCs in a dose-dependent manner (16), DPP4-I may be more effective against NASH progression in comparatively low glucose conditions. These experimental studies may explain the greater efficacy of DPP4-I in the prevention of NASH progression in patients in the lower HbA1c category in the present study.

Reductions in serum ferritin levels were observed only among NAFIC score components. Experimental models have demonstrated that iron increases hepatocyte apoptosis and contributes to the development of fibrosis directly and indirectly via induction of TGF-β1 production by hepatocytes

and macrophages (17). By contrast, iron depletion inhibits the pancreatic TGF signal, thus inhibiting the phosphorylation of Smad2 (18). Kajikawa *et al* (19) demonstrated that eicosapentaenoic acid reduces hepatic reactive oxygen species levels and serum ferritin in the methionine- and choline-deficient diet rat model in parallel with hepatic TGF-β1. DPP4-I reduced serum ferritin levels, which may lead to reduced levels of hepatic TGF-β1 and consequent inhibition of NASH progression.

The present study had several limitations. Firstly, NAFLD and NASH progression were evaluated using ultrasonography and a non-invasive scoring system. The NAFIC score was established to differentiate NASH from NAFL in a cross-sectional study. It is not clear whether the NAFIC score can be used to evaluate longitudinal outcome, which may have resulted in misclassification. Although liver biopsy is the gold standard for the diagnosis of NAFLD and assessment of disease progression, it is unrealistic to perform liver biopsies in all NAFLD patients with type 2 diabetes (20). Secondly, as this was a single arm study with a small number of patients and a short observation period for this type of study, to assessing the effect of significant potential confounding factors, such as calorie intake and exercise status, could not be performed.

In conclusion, DPP4-I administration resulted in decreased NAFIC scores, demonstrating the efficacy of DPP4-I against NAFLD progression. These results indicate that DPP4-I may represent a potential new therapeutic strategy for the prevention of NAFLD progression in NAFLD patients with type 2 diabetes in the future.

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