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Original

Ipragliflozin, a Sodium-Glucose Cotransporter 2 Inhibitor, Ameliorates Nonalcoholic Fatty Liver Disease in Japanese Patients with Type 2 Diabetes Mellitus

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Aim: Sodium-glucose cotransporter 2 inhibitors are novel antidiabetic agents that inhibit glucose reabsorption in the renal proximal tubules. We assessed the potential effects of sodium-glucose cotransporter 2 inhibitors on body weight, hepatic fat accumulation, and liver stiffness by using transient elastography (TE) and measuring biochemical markers associated with nonalcoholic fatty liver disease (NAFLD) with type 2 diabetes mellitus (T2DM).

Methods: For 12 weeks, patients with T2DM and NAFLD were treated by ipragliflozin as an add-on medication. Physical findings, biochemical blood and urinary analyses, meal tolerance test, and TE were assessed before and 12 weeks after the administration of ipragliflozin.

Results: Fifteen patients were enrolled in this study. From baseline to 12 weeks, body mass index (BMI; $p < 0.0001$), hemoglobin A1c ($p < 0.01$), and fasting and postprandial plasma glucose ($p < 0.05$ and $p < 0.01$, respectively) were significantly reduced. Fasting C-peptide immunoreactivity index ($p < 0.05$), urinary glucose ($p < 0.001$), and hematocrit ($p < 0.01$) were significantly increased. Uric acid ($p < 0.01$), γ-glutamyl transpeptidase ($p < 0.05$), ferritin ($p < 0.001$), hepatic fat accumulation (i.e., the controlled attenuation parameter [CAP]; $p < 0.05$), and liver stiffness (E; $p < 0.05$) were significantly decreased, as measured by TE. The percent change in BMI and the change in the aspartate aminotransferase, alanine aminotransferase (ΔALT), and type IV collagen 7s levels, and in the aspartate aminotransferase-to-platelet ratio index and fibrosis-4 index values were correlated with the change in the CAP (ΔCAP). The ΔALT was the only independent predictor of ΔCAP, based on multivariate analysis ($p < 0.01$).

Conclusions: The administration of the sodium-glucose cotransporter 2 inhibitor ipragliflozin may be associated with the amelioration of hepatic steatosis and elasticity in patients with T2DM and NAFLD.

Key Words: liver fibrosis, liver steatosis, nonalcoholic fatty liver disease, sodium-glucose cotransporter 2 inhibitor, type 2 diabetes mellitus

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a significant health problem, and 20% of affected individuals have nonalcoholic steatohepatitis (NASH), which is the most severe form of NAFLD.[1] NASH predicts incidental cardiovascular disease—independent of metabolic syndrome or insulin resistance—and should be aggressively treated.[2,3] Lifestyle interventions that result in weight loss decrease the onset of type 2 diabetes mellitus (T2DM) and cardiovascular events.[4,5] Hepatic fat reduction by lifestyle intervention using exercise and restricted diet usually requires at least a 5% loss in total body weight. A decrease in body weight of approximately 3% may be associated with a significant improvement in hepatic steatosis.[6,7] Simple weight loss by lifestyle intervention is difficult to achieve; therefore, bariatric surgery is an alternative treatment for body weight reduction.[8,9] Other pharmacotherapeutic treatments are useful for some people with fatty liver or NASH/NAFLD, although the effectiveness of this approach is relatively limited or controversial.

Sodium-glucose cotransporter 2 (SGLT2) inhibitors are novel antihyperglycemic agents. Through an insulin-independent mechanism, these agents reduce the plasma glucose concentration with a low risk of hypoglycemia, and are associated with the loss of body weight.[10,11] We focused on the potential effect of SGLT2 inhibitors on reducing body weight and aimed to assess their effects on hepatic fat accumulation and liver stiffness by using transient elastography (TE) and by measuring biochemical markers associated with NAFLD in patients with T2DM.

Materials and Methods

Fifteen patients with T2DM and NAFLD who attended Yachiyo Medical Center at Tokyo Women's Medical University (Chiba, Japan) were recruited. All patients were diagnosed as having NAFLD, based on the guidelines of the American Association for the Study of Liver Diseases (Alexandria, VA), American College of Gastroenterology (Bethesda, MD), and American Gastroenterological Association (Bethesda, MD).[7] Patients were excluded if they had liver cirrhosis, viral hepatitis, autoimmune hepatitis, α-1 antitrypsin deficiency, primary biliary cirrhosis, hemochromatosis, Wilson disease, or other secondary causes such as alcohol ingestion of more than 20 g/day. Patients were also excluded if they had chronic kidney disease (i.e., serum creatinine concentration >1.5 mg/dL) or had been treated with SGLT2 inhibitors. Abdominal ultrasonography was used to confirm whether the echogenicity of the liver exceeded that of the renal cortex and spleen. In addition, there was an attenuation of the ultrasound wave, loss in the definition of the diaphragm, and poor delineation of the intrahepatic architecture, based on the standard criteria.[12] The SGLT2 inhibitor ipragliflozin (50 mg/day) was added to the patient's current treatment. The patient's diet and exercise therapy were not changed during this study. Laboratory data, which included biochemical markers for NAFLD and TE to measure the controlled attenuation parameter (CAP) and liver stiffness (E), were obtained before and 12 weeks after the administration of ipragliflozin. The C-peptide immunoreactivity index (CPI) was calculated as 100 times the C-peptide level (ng/mL) divided by the plasma glucose level (mg/dL). The aspartate aminotransferase-to-platelet ratio index (APRI) and the fibrosis-4 (FIB-4) index were calculated, as described in previous reports.[13,14]

The CAP and E were measured with the FibroScan 502 Touch system (Echosens, Paris, France) by using an M-probe (3.5 MHz), as described previously.[15,16] The CAP and E are expressed in decibels/meter (dB/m) and kilopascals (kPa), respectively. The mean values of the CAP and E measurements were reliable when 10 valid measurements were obtained and the interquartile range/median ratio was <30%.[17] Transient elastography is a noninvasive method used to detect liver steatosis and fibrosis; its findings are correlated with the staging of liver steatosis and fibrosis detected by using liver biopsy.[15,18]

A test meal (JANEF E460F18; Kewpie Corp., Tokyo, Japan) consisted of chicken in white sauce, crackers, and soft pudding, and contained 450 kcal of total energy (51.4% carbohydrates, 33.3% fat, and 15.3% protein). It was developed by the Japanese Diabetes Society and Kewpie Cooperation (Tokyo, Japan).[19] Meal tolerance tests were administered, as previously described.[20,21] In brief, fasted patients ingested the test meal for 20 minutes at breakfast. Blood samples were then collected at 0 minutes (i.e., before) and 60 minutes after the test meal load-

Table 1 Clinical parameters at baseline.

Parameter	Number of patients (n = 15)
Sex, male/female [no (%)]	11 (73%) /4 (27%)
Age (y)	47.4±8.0 (36-64)
Body weight (kg)	98.7±21.8 (70-147)
BMI (kg/m^2)	35.2±6.1 (25.4-49.1)
Duration (y)	4.3±2.7 (1-10)
HbA1c (NGSP) (%)	7.5±1.3 (6.1-11.1)
Fasting plasma glucose (mg/dL)	145±40 (96-229)
Hypertension	13 (86%)
Dyslipidemia	10 (66%)
Diabetic neuropathy	4 (27%)
Diabetic retinopathy	2 (13%)
Diabetic nephropathy	4 (27%)
Smoking history	10 (67%)
Concomitant medications	
Sulfonylurea	1 (7%)
Glinide	1 (7%)
Dipeptidyl peptidase-4 inhibitor	5 (33%)
α-Glucosidase inhibitor	3 (20%)
Biguanide	12 (80%)
Thiazolidinedione	1 (7%)
GLP-1 receptor agonist	2 (13%)
Insulin	0 (0%)

BMI, body mass index; GLP-1, glucagon-like peptide-1; HbA1c, hemoglobin A1C.
All data are presented as the mean ± the standard deviation (range) or as the number (percentage).

ing. These steps were repeated before and at 12 weeks after the administration of ipragliflozin.

The protocol of this study conformed to the ethical guidelines of the 2008 Declaration of Helsinki and was approved by the ethics committee of Tokyo Women's Medical University (Tokyo, Japan; approval #3300). Each patient provided written informed consent.

Statistical analysis

The data are expressed as the mean ± the standard deviation. The paired t-test was used to analyze significant differences between the clinical data at baseline and at 12 weeks after ipragliflozin treatment. Correlation coefficients were calculated by using Pearson's analysis for a normal distribution and by using Spearman analysis for a skewed distribution. Multivariate stepwise regression analysis was used to identify factors affecting the decrease in hepatic fat accumulation by using changes (Δ) in the CAP as the dependent variable and other parameters that were detected with univariate analysis. Statistical analysis was conducted using JMP 12 (SAS Institute Inc., Cary, NC, USA). For each parameter, the delta symbol (Δ) represents the difference in values before and after initiating ipragliflozin. The percent change (%Δ) represents the ratio of the change in each parameter to its basal value before the administration of ipragliflozin.

Results

The characteristics of the studied patients are shown in **Table 1**. The mean age was 47.4 years. The mean body mass index (BMI) was 35.2 kg/m^2 (range, 25.4-49.1 kg/m^2).

The BMI and hemoglobin A1c (HbA1c) levels decreased by 0.6 kg/m^2 ($p < 0.01$) and 0.6% ($p < 0.001$), respectively (**Table 2**). The urinary glucose level had increased by 3,064 mg/dL ($p < 0.001$) at 12 weeks after the administration of ipragliflozin. After the administration of ipragliflozin, fasting plasma glucose concentrations significantly decreased by 16 mg/dL ($p < 0.05$) and the fasting CPI significantly increased by 0.25 ($p < 0.05$). Postprandial plasma glucose levels and the postprandial CPI during the meal tolerance tests were significantly decreased by 26 mg/dL ($p < 0.01$) and by 0.05 ($p < 0.05$),

Table 2 The changes in metabolic parameters at baseline and 12 weeks after the administration of ipragliflozin.

	Baseline	12 weeks
BMI (kg/m^2)	35.2±6.1	34.6±5.9**
Systolic blood pressure (mmHg)	135.0±16.0	130.0±16.0
Diastolic blood pressure (mmHg)	88.0±10.0	86.0±12.0
HbA1c (NGSP) (%)	7.5±1.3	6.9±0.9***
Fasting plasma glucose (mg/dL)	145±40	129±24*
Postprandial plasma glucose (mg/dL)	219±41	193±35**
Fasting CPI	2.32±0.87	2.57±1.22*
Postprandial CPI	3.00±1.57	2.95±1.01*
Urinary glucose/Cre (mg/gCre)	62±138	3,126±2,081***
HDL cholesterol (mg/dL)	47±10	47±8
LDL cholesterol (mg/dL)	111±25	109±29
Triglycerides (mg/dL)	188±142	182±100
Uric acid (mg/dL)	6.4±1.3	5.8±1.2**
Hematocrit (%)	43.8±3.1	47.1±3.2***
AST (IU/L)	43±21	32±11
ALT (IU/L)	59±28	49±28
γGTP (IU/L)	75±72	55±34*
eGFR (mL/min)	87.2±21.4	82.2±19.0
NAG (IU/L)	21±25	12±8
Serum β2MG (mg/L)	1.7±0.2	1.8±0.2
Urinary β2MG (μg/L)	253±311	182±150
Ferritin (ng/mL)	128±58	77±39***
Hyaluronic acid (ng/mL)	27±17	30±15
Type IV collagen 7s (ng/mL)	4.2±1.0	3.9±0.7
APRI	0.49±0.26	0.40±0.11
FIB-4 index	1.03±0.43	0.95±0.29

β2MG, β2 microglobulin; γGTP, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CPI, C-peptide immunoreactivity index; CPR, C-peptide immunoreactivity; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAG, N-acetylglucosamine; APRI, AST to platelet ratio index.

CPI (CPR index) =100×CPR/plasma glucose.

*$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

All data are presented as the mean ± the standard deviation.

respectively, at 12 weeks after the administration of ipragliflozin, compared with the baseline values. Blood pressure, high-density lipoprotein and low-density lipoprotein cholesterol concentrations, and the triglyceride concentration were not significantly different. However, the uric acid level was significantly decreased by 0.6 mg/dL ($p < 0.01$) and the hematocrit was significantly increased by 3.3% ($p < 0.001$).

The estimated glomerular filtration rate and levels of serum β2 microglobulin, urinary N-acetylglucosamine, and urinary β2 microglobulin were not significantly different. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) tended to decrease, although there was no statistical difference. The serum γ-glutamyl transpeptidase (γ-GTP) and ferritin levels were significantly decreased by 20 IU/L ($p < 0.05$) and 51 ng/mL ($p < 0.001$), respectively, whereas the concentrations of serum hyaluronic acid and type IV collagen 7s, and the APRI and FIB-4 index were not significantly different.

The CAP and E were measured by TE. These values were significantly decreased by 14 dB/m and 1.35 kPa, respectively, at 12 weeks after ipragliflozin treatment ($p < 0.05$), compared with the baseline values (**Figure 1**). There were no serious adverse events attributable to ipragliflozin during this study.

The change in CAP (ΔCAP) was correlated with the percent change in BMI (%ΔBMI; $p < 0.05$); the change in the serum levels of aspartate aminotransferase (ΔAST; $p < 0.05$), alanine aminotransferase (ΔALT; $p < 0.01$), and type IV collagen 7s ($p < 0.01$); and the change in the values of the aminotransferase-to-platelet ratio index (ΔAPRI; $p < 0.05$) and fibrosis-4 (ΔFIB-4) index ($p < 0.05$) (**Table 3**). However, it was not correlated with parameters associated with HbA1c, glucose levels, and fer-

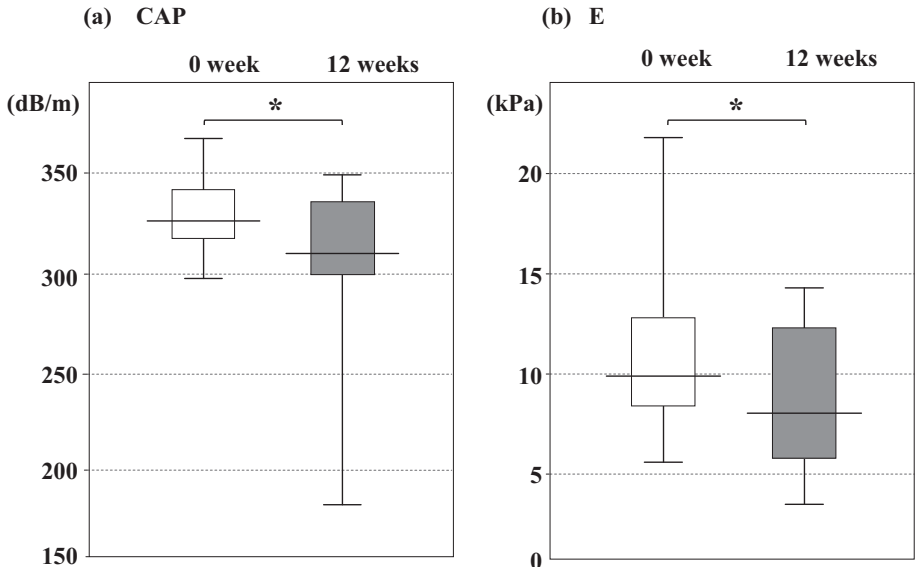

Figure 1 The controlled attenuation parameter (CAP) and stiffness (E) values, as measured by transient elastography at baseline and 12 weeks after the administration of ipragliflozin (the mean ± the standard deviation).
*p < 0.05.

Table 3 Relationships between Δ CAP and changes in metabolic parameters.

Variables	Correlation coefficient	p
%ΔBMI	0.598	0.040*
ΔHbA1c	0.206	0.520
ΔFasting plasma glucose	0.119	0.712
ΔFasting CPI	−0.423	0.188
ΔHematocrit	−0.005	0.987
ΔUric acid	0.043	0.895
ΔAST	0.674	0.016*
ΔALT	0.716	0.009**
ΔγGTP	0.001	0.997
Δferritin	0.170	0.598
ΔType IV collagen 7s	0.752	0.008**
ΔAPRI	0.691	0.013*
ΔFIB-4 index	0.585	0.046*

*p < 0.05, ** p < 0.01.

ritin.

Multivariate regression analyses revealed that ΔALT was the only independent predictor of ΔCAP (β = 0.716; t = 3.24; 95% CI, 0.447-2.416; p = 0.009). However, no parameters were identified as an independent determinate of the change in liver stiffness (ΔE).

Discussion

In our study, at 12 weeks after treatment with the SGLT2 inhibitor ipragliflozin, we used TE and observed an improvement in hepatic fat accumulation and elasticity in patients with T2DM and NAFLD. The BMI was associated with the CAP. A predictor for improving CAP could be ALT and type IV collagen 7s.

A reduction in nutritional intake and in body weight effectively treats NAFLD.[22,23] However, hepatic fat reduction only by lifestyle interventions such as maintaining a reduced body weight is difficult to achieve and is sometimes unsustained.[22,23] The effects of some pharmacotherapeutic drugs such as vitamin E,[24] metformin,[25,26] thiazolidinedione derivatives,[27] and glucagon-like peptide-1

analogs[28,29] are relatively limited or controversial. In a previous report[27] of patients with T2DM, histological findings, which were obtained after 6 months of treatment with a hypocaloric diet (a reduction of 500 kcal/day in relation to the calculated daily intake required to maintain body weight) plus pioglitazone (45 mg/day) or a placebo, revealed that liver steatosis, ballooning necrosis, and intrahepatic inflammation improved with pioglitazone, compared with a placebo. However, pioglitazone induced an increase in the body weight from the baseline (i.e., pretreatment) weight, whereas the placebo group had no significant change in the body weight.

Vitamin E (800 IU/day) is also efficient in treating NASH, compared with a placebo and pioglitazone.[24] The glucagon-like peptide-1 analog liraglutide has been studied for T2DM treatment.[30] In addition, liraglutide monotherapy (1.2 mg/day or 1.8 mg/day) reduced body weight by approximately 2.0-3.2 kg.[30] In one study,[30] the liver-to-spleen attenuation ratio increased after treatment with liraglutide (1.8 mg) and metformin. This increase indicated reduced hepatic steatosis. These medical treatments for fatty liver also need to accompany diet therapy and exercise.

In recent years in Japan, obesity has been rapidly increasing in younger patients with T2DM.[31] Therefore, early intervention for obesity with T2DM and fatty liver may be necessary for preventing cardiovascular disease and cancer, which are expected to increase in the future. Based on this background, we studied the effect of ipragliflozin on hepatic fat content and liver stiffness measurements of patients who did not change their diet and exercise therapy.

The SGLT2 inhibitors are extensively used worldwide to treat T2DM. They effectively reduce the blood glucose level and body weight owing to the excretion of approximately 50-60 g/day urinary glucose, which is nearly equivalent to a daily caloric loss of 200-240 kcal/day. In our study, the BMI was reduced by 0.6 kg/m^2 after 12 weeks of treatment, and the serum AST and ALT levels tended to decrease. Additional improvement in body weight and aminotransferase levels may be expected at more than 12 weeks after the administration of ipragliflozin, followed by an improvement in the HbA1c level.

Changes in the ALT levels were strongly correlated with the liver fat content but not with inflammation, hepatocyte ballooning, or fibrosis.[32] Therefore, it has been proposed that serum ALT may enable the detection of NAFLD.[33] These reports support our findings that decreasing the CAP was associated with decreasing the ALT level, after the potential amelioration of NAFLD.

After administering ipragliflozin, the intrahepatic fat content and liver elasticity improved, based on the TE results. The expression of SGLT2 has been identified to a much lesser extent in the liver than in the kidney,[34] which suggests that decreased body weight is partly associated with a decline in the hepatic fat content that is part of ectopic fat accumulation.

Liver biopsy remains the gold standard for diagnosing NASH and staging fibrosis in patients with NAFLD; however, repeated liver biopsy is difficult to perform because it is an invasive method. Measuring liver stiffness and elasticity with TE is a noninvasive and effective method for screening patients with NAFLD. However, the main limitation of TE is its failure to obtain reliable liver stiffness measurements.[35] In this study, to avoid this factor as much as possible, at least 10 valid measurements were obtained to determine reliable mean values for CAP and E.

A substantial amount of previous data indicates that liver fibrosis is potentially reversible in chronic liver diseases.[36] Adipokines, chemokine, and/or insulin resistance contribute to and are associated with liver fibrosis in NAFLD.[37] Leptin signaling promotes hepatic stellate cell activity and amplifies fibrogenic activity through suppressing peroxisome proliferator-activated receptor gamma or downregulating adiponectin.[38] Serum leptin levels are much higher in patients with NASH or NAFLD than in control patients, which suggests leptin resistance.[38] A mouse model of NAFLD exhibited insulin resistance and increased free fatty acid (FFA) levels, which have an important role in liver steatosis; the administration of SGLT2 inhibitors ameliorated these levels.[39]

Some previous research indicates that SGLT2 inhibitors may be beneficial for the prevention and progression of liver fibrosis in vivo;[39,40] however, no large clinical trials have concluded that SGLT2 inhibitors have an antihepatic fibrosis effect in humans. Leptin and FFA levels improve after the administration of SGLT2 inhibitors in humans.[41] Therefore, SGLT2 inhibitors may ameliorate

liver steatosis. In our study, leptin and FFA were not measured. In addition, the amelioration of liver elasticity, as measured by TE, was incongruous with the lack of change in the APRI and FIB-4 index values. This incongruous finding may have been caused by the fact that liver fibrosis in our patients was mild at the beginning of the study and the study period was short. The details of the mechanisms for the improvement in liver elasticity in humans remain unclear. Therefore, further investigations on this issue are needed.

The serum ferritin concentration decreased significantly. In women, the serum ferritin concentration varies because of menstruation. Therefore, we analyzed ferritin data only in men, and found a significant difference (133 ± 57 versus 70 ± 43 ng/mL, $p < 0.001$). Serum ferritin concentration is not only a clinical marker of iron storage, but inflammation in several tissues, and is especially increased in NASH.[42]

Renal tubular function, as indicated by β2 microglobulin and N-acetylglucosamine levels, did not change in our study. In an in vitro study,[43] the inhibition of SGLT2 was protective against oxidative stress induced by high glucose concentrations in incubated cultured tubular cells. The average baseline renal tubular functions were near normal in our study. The protective effects of ipragliflozin on renal tubular function may be more prominent in people with injured tubular function and during a long period of treatment with SGLT2 inhibitors.

There were several limitations in our study. First, this single-arm clinical study involved no placebo and control groups. Second, only a small number of patients was studied. Third, histological examinations did not include liver biopsies, which may have directly revealed the amelioration of hepatic fat accumulation and elasticity. We recognize that a multi-arm clinical trial with a larger number of patients and longer study periods are needed to clarify the potential effect of an SGLT2 inhibitor on ameliorating hepatic steatosis and elasticity.

In Japan, SGLT2 inhibitors can be used for treating T2 DM. We previously reported that people with type 1 diabetes and a fatty liver have more cardiovascular risk factors.[44] In the future, this drug may be indicated for people with fatty liver or NASH/NAFLD to prevent cardiovascular diseases.

Conclusion

The treatment for NAFLD, especially diet therapy, is clinically difficult for people with T2DM. Through their effects on reducing body weight and ameliorating hepatic steatosis and fibrosis, SGLT2 inhibitors may be a medical intervention that can be added to ongoing treatment with medicine, diet, and exercise therapy.

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Conflicts of Interest: No author has any conflicts of interest to declare.

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