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NOTE

Amelioration of fatty liver index in patients with type 2 diabetes on ipragliflozin: an association with glucose-lowering effects

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Abstract. In this study, we investigated the ameliorating effects of ipragliflozin on fatty liver in patients with type 2 diabetes. The factors that influenced the amelioration of fatty liver were also examined. Analysis included data of 21 Japanese patients with type 2 diabetes obtained from our prospective observational study. After obtaining patients' informed consent, once-daily ipragliflozin (50 mg/day) was given for 16 weeks. In addition to several clinical parameters, body composition was also compared before and after 16 weeks of treatment. The extent of fatty liver was estimated using a fatty liver index (FLI). After 16 weeks, FLI significantly decreased, from 70.1 ± 19.4 to 60.3 ± 25.5 ($p = 0.0009$) as well as levels of fasting plasma glucose (FPG), HbA1c, body weight, visceral adipose tissue (VAT), subcutaneous adipose tissue (SAT) and fat mass. To reveal the factors influencing the FLI changes observed on ipragliflozin treatment, correlations between changes in FLI and several other measured parameters were examined. Changes in FPG (correlation coefficient = 0.4683, $p = 0.0323$) and HbA1c (correlation coefficient = 0.4383, $p = 0.0469$) showed significant positive correlations with changes in FLI. On the other hand, no correlations of changes in FLI were observed with body weight, VAT, SAT nor fat mass. In conclusion, ipragliflozin ameliorated FLI in Japanese patients with type 2 diabetes. Improvement in FLI was associated with that of glucose intolerance.

Key words: Fatty liver, Ipragliflozin

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) is one of the most common causes of chronic liver disease in the both Western and oriental countries [1, 2]. NAFLD and type 2 diabetes frequently coexist, sharing pathogenetic requisites such as obesity, insulin resistance. Indeed, NAFLD is highly prevalent (49.6–74%) among patients with type 2 diabetes [3]. Moreover, the presence of type 2 diabetes is a risk factor not only for the development of nonalcoholic steatohepatitis (NASH), but also for the development of cirrhosis and hepatocellular carcinoma in patients with NAFLD [4, 5].

Ipragliflozin is an oral anti-diabetic agent that selectively inhibits sodium-glucose co-transporter 2 (SGLT2), which improves glucose intolerance and reduces body weight in patients with type 2 diabetes by enhancing urinary glucose excretion. Several clinical trials have shown the efficacy of ipragliflozin in patients with type 2 diabetes, being administered either as monotherapy or in combination with other drugs, without significant safety concerns and with body weight reduction [6, 7]. These findings prompted us to investigate the effect of ipragliflozin on fatty liver in patients with type 2 diabetes. Some reports have attested to the efficacy of ipragliflozin for improving hepatic steatosis in rodent models [8-10], but not in humans, although it has been reported that ipragliflozin improves the serum alanine aminotransferase levels in patients with type 2 diabetes [10-12]. The current study investigated the ameliorating effects on fatty liver in Japanese patients with type 2 diabetes before and after 16 weeks of treatment with ipragliflozin, and examined factors that influenced the amelioration of fatty liver.

Materials and Methods

This subanalyzed data were obtained from our prospective observational study [13]. Summary of this study protocol was described as below; Japanese patients with type 2 diabetes (between 20 and 75 years

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old, HbA1c level above 7.0%, body mass index (BMI) above 22 kg/m^2 and estimated glomerular filtration rate (eGFR) above 45 mL/min), who were undergoing outpatient treatment at Hokkaido University Hospital from June 2014 to February 2015, were recruited into this study. After obtaining patients' informed consent, once-daily ipragliflozin (50 mg/day) was given for 16 weeks. Several clinical parameters, including fasting plasma glucose (FPG), HbA1c, lipid profiles and liver function, were compared before and after 8 and 16 weeks of treatment. Body composition represented by fat mass, visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) volumes, were also measured using a Dual Scan® (HDS 2000®, employing dual bioelectrical impedance analysis; Omron Healthcare Company, Limited, Kyoto, Japan) and an InBody 720® (InBody Company, Limited, Tokyo, Japan, employing the Direct Segmental Multi-frequency Bioelectrical Impedance Analysis Method) before and after 8 and 16 weeks of treatment. The study was conducted with the approval of the institutional review board (013-0380) and written informed consent was obtained from all patients. This trial was registered with the University Hospital Medical Information Network (UMIN), number UMIN000014058.

The extent of fatty liver was estimated using a fatty liver index (FLI), comprising BMI, waist circumference (WC, in cm), gamma-glutamyl transferase (GGT, in IU/L) and triglycerides (TG, in mg/dL) [14]. This index was calculated using the following equation:

$$FLI = \{(\exp(0.953 * \log(TG) + 0.139 * BMI + 0.718 * \log(GGT) + 0.053 * WC - 15.745)/1 + \exp(0.953 * \log(TG) + 0.139 * BMI + 0.718 * \log(GGT) + 0.053 * WC - 15.745)\} * 100$$

Because FLI <30 can be used to rule out hepatic steatosis [15], patients with FLI <30 were excluded in the analysis. The study outcome was the change in FLI before and after 16 weeks of treatment. A set of factors affecting the change in FLI were examined.

Results are expressed as means ± SD. Differences between before and after 16 weeks of treatment were analyzed for statistical significance using the paired *t*-test. Calculations for correlation coefficients and simple linear regression analyses were performed to test for associations between variables. Multivariate analyses were performed using multiple linear regression analyses to identify factors independently associated with the outcomes. All *p* values were two-sided and values <0.05 were considered statistically significant.

Results

A total of 24 patients completed the protocol in the original prospective observational study [13]. Of these, three patients with FLI <30 were excluded and the remaining 21 (six men and 15 women) were included in the analysis. The mean age was 51.2 ± 12.0 years and the average duration of diabetes was 9.0 ± 4.4 years. Medication for type 2 diabetes included biguanide (n = 18), dipeptidyl peptidase-4 inhibitor (n = 15), sulfonylurea (n = 11), thiazolidinedione (n = 5), alpha-glucosidase inhibitor (n = 2), glucagon-like peptide-1 receptor agonists (n = 2) and insulin (n = 2).

After 16 weeks, body weight, BMI, WC, VAT, SAT and fat mass significantly decreased (Table 1). Levels of FPG, HbA1c, insulin, alanine aminotransferase and leptin also decreased significantly, but the lipid profiles did not change (Table 1). Total ketone body, acetoacetic acid and beta-hydroxybutyric acid levels tended to increase, but not significantly (Table 1). These results indicated that treatment with ipragliflozin for 16 weeks improved both obesity and glucose intolerance, although there were no correlations between the changes in body weight, VAT and fat mass, and also in FPG and HbA1c (Supplementary Table 1). As expected, FLI significantly decreased from 70.1 ± 19.4 to 60.3 ± 25.5 after 16 weeks of treatment (p = 0.0009) (Table 1).

To reveal whether the improvement of obesity or glucose intolerance could influence the FLI changes observed with ipragliflozin treatment, we examined the correlations between the changes in FLI and the changes in the parameters reflecting glucose tolerance and obesity. As shown in Table 2, the changes in FPG and HbA1c showed significant positive correlations with the changes in FLI. No significant correlations of the changes in FLI were observed with the changes in body weight, BMI, WC, VAT, SAT or fat mass. Furthermore, multiple linear regression analysis showed that the changes in FPG and HbA1c had a significant positive correlation with the changes in FLI after adjustment for the changes in fat mass (FPG: standardized partial regression coefficient = 0.4501, p = 0.0340; HbA1c: standardized partial regression coefficient = 0.4403, p = 0.0383), and the changes in HbA1c had a significant positive correlation with the changes in FLI after adjustment for the changes in VAT (standardized partial regression coefficient = 0.4134, p = 0.0474).

Table 1 Changes in the parameters examined in 21 participants after the administration of ipragliflozin

	0 week	16 weeks	*p* value
Body weight (kg)	77.3 ± 17.6	74.5 ± 18.0	<0.0001
BMI (kg/m^2)	29.7 ± 5.4	28.6 ± 5.4	<0.0001
WC (cm)	101.0 ± 10.2	97.3 ± 9.7	<0.0001
VAT (cm^2)	113.9 ± 32.7	104.8 ± 35.4	0.0049
SAT (cm^2)	275.8 ± 91.4	257.1 ± 96.2	0.0435
Fat mass (kg)	29.6 ± 11.7	27.8 ± 11.4	0.0002
FPG (mg/dL)	163.0 ± 34.7	141.2 ± 35.3	0.0025
HbA1c (%)	7.7 ± 0.7	7.3 ± 0.4	0.0052
Insulin (μU/mL) [#]	10.1 ± 4.0	6.9 ± 2.6	<0.0001
AST (U/L)	35.5 ± 17.4	32.6 ± 16.6	0.2952
ALT (U/L)	45.3 ± 28.4	37.4 ± 24.8	0.0063
GGT (U/L)	63.1 ± 101.1	49.4 ± 69.8	0.0537
TG (mg/dL)	175.3 ± 86.1	172.6 ± 117.1	0.8932
HDL cholesterol (mg/dL)	51.1 ± 11.3	53.0 ± 10.5	0.3092
LDL cholesterol (mg/dL)	100.2 ± 30.7	90.2 ± 40.2	0.3285
FFA (μEq/L)	552.0 ± 194.0	578.6 ± 207.0	0.6385
Leptin (ng/mL)	20.0 ± 16.6	16.4 ± 11.4	0.0134
Adiponectin (μg/mL)	3.3 ± 2.3	3.3 ± 2.3	0.5334
Total ketone body (μmol/L)	103.0 ± 73.9	249.0 ± 345.8	0.0602
Acetoacetic acid (μmol/L)	24.8 ± 13.4	45.8 ± 56.5	0.0871
Beta-hydroxybutynic acid (μmol/L)	78.2 ± 61.6	201.9 ± 291.1	0.0600
Fatty liver index	70.1 ± 19.4	60.3 ± 25.5	0.0009

Data are means ± SD. BMI, body mass index; WC, waist circumference; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c; AST, aspartate amino transferase; ALT, alanine amino transferase; GGT, gamma-glutamyl transferase; TG, triglycerides; HDL cholesterol, high density lipoprotein cholesterol; LDL cholesterol, low density lipoprotein cholesterol; FFA, free fatty acid. *p* values: paired *t*-test. [#] Except for two cases of insulin treatment.

Discussion

This is a preliminary but first study to show the ameliorating effects of ipragliflozin on fatty liver in human patients with type 2 diabetes. The results of our study showed that FLI, which is used as a surrogate measure for fatty liver, was significantly decreased by ipragliflozin treatment for 16 weeks in Japanese patients with type 2 diabetes. The following indicates the strengths of this study. First, we showed that ipragliflozin ameliorated the index of hepatic steatosis in humans, although it has been reported that ipragliflozin improves the serum alanine aminotransferase levels in patients with type 2 diabetes [10-12]. Second, we investigated whether improvement of obesity or glucose intolerance could influence the FLI changes observed with ipragliflozin treatment. The important point of the study is that obesity was estimated more precisely by using fat mass, VAT and SAT volumes, and not by body weight, BMI or WC. Interestingly, changes in FPG and HbA1c, but not in VAT, SAT or fat mass, showed significant correlations with changes in FLI.

Table 2 Relationships between changes in fatty liver index and the various parameters examined

	Correlation coefficient	*p* value
Δ Body weight	0.3978	0.0741
Δ BMI	0.4223	0.0565
Δ WC	0.1894	0.4110
Δ VAT	0.3918	0.0790
Δ SAT	0.3097	0.1718
Δ Fat mass	0.3285	0.1460
Δ FPG	0.4683	0.0323
Δ HbA1c	0.4383	0.0469

BMI, body mass index; WC, waist circumference; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c.

Studies in humans and rodents have demonstrated that the mechanisms leading to the excessive accumulation of hepatic triglycerides are mainly linked to increased delivery of free fatty acid from peripheral expanded adipose tissue to the liver, enhanced de novo lipid synthesis *via* the lipogenic pathway in the liver, and impaired beta-oxidation in the liver [16]. In our results, levels of FPG, HbA1c and insulin decreased significantly in the absence of changes in free fatty

acid levels (Table 1). Considering that the elevated plasma glucose and insulin levels promotes de novo fatty acid synthesis, thereby contributing to the development of hepatic steatosis [16], treatment with ipragliflozin could ameliorate hepatic steatosis *via* reduction of hepatic lipogenesis caused by decreasing plasma glucose and insulin levels. Indeed, it has been reported that ipragliflozin decreased the hepatic triglyceride content and the expression levels of lipogenic genes such as *Srebp1c*, *Fasn* and *Scd1* irrespective of body weight reduction in obese mice [10], which is consistent with our clinical results. In addition, it has been reported that decreased insulin levels *via* improving hyperglycemia by the SGLT2 inhibitor, induce the upregulation of hepatic gluconeogenesis [17]. Because gluconeogenesis requires beta-oxidation [18], treatment with ipragliflozin could enhance beta-oxidation in hepatic cells, resulting in amelioration of hepatic steatosis. This concept is corroborated by our finding that levels of total ketone bodies, acetoacetic acid and beta-hydroxybutyric acid, tended to increase with ipragliflozin treatment. To prove these hypotheses, liver biopsy samples in which patients with type 2 diabetes are treated with SGLT2 inhibitors will be necessary.

The limitations of our study were the relatively small number of participants and the lack of a placebo group. There was also the limitation of the absence of an FLI assessment. Although non-invasive techniques like magnetic resonance spectroscopy, computed tomography and ultrasound are often used for diagnosing fatty liver, these methods require radiological equipment and specialist operators. FLI is a simpler and less expensive method compared with magnetic resonance spectroscopy, and a strong correlation has been reported between FLI and hepatocellular lipid content [19, 20].

In conclusion, ipragliflozin ameliorated FLI in this population of Japanese patients with type 2 diabetes. Improvement in FLI was associated with that of glucose intolerance. Large scale prospective studies will promise to elucidate the mechanisms behind the ameliorating effects on fatty liver caused by the glucose-lowering effect following treatment with ipragliflozin.

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The contributions of the authors were as follows. T.T. contributed to the data analysis and wrote the manuscript. A.N. and H.M. designed and performed the research, and wrote the manuscript. A.N., H.M., C.Y., and T.A. contributed to discussion, reviewed and edited the manuscript. Furthermore, H.M. thanks Dr. James W. Perfield II (University of Missouri, MO, USA) for his continued support and mentorship.

Disclosure

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T.T. and C.Y. declare no conflicts of interest.

Supplementary Table 1 Relationships between the changes in body weight, visceral adipose tissue and fat mass, and those in fasting plasma glucose and HbA1c

	Δ FPG		Δ HbA1c	
	Correlation coefficient	*p* value	Correlation coefficient	*p* value
Δ Body weight	0.0253	0.9134	0.1530	0.5080
Δ VAT	0.3128	0.1674	0.0684	0.7682
Δ Fat mass	0.0603	0.7951	-0.0061	0.9789

VAT, visceral adipose tissue; FPG, fasting plasma glucose; HbA1c, hemoglobin A1c.

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