Ipragliflozin, a sodium–glucose cotransporter-2 inhibitor, ameliorates the development of liver fibrosis in diabetic Otsuka Long-Evans Tokushima Fatty rats

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Abbreviations: NASH, non-alcoholic steatohepatitis; IR, insulin resistance; SGLT2, sodium–glucose cotransporter-2; OLETF, Otsuka Long-Evans Tokushima Fatty; LETO, Long-Evans Tokushima Otsuka; HSC, hepatic stellate cell; NAFLD, non-alcoholic fatty liver disease; DM, diabetic mellitus; PPARγ, peroxisome proliferator-activated receptor gamma; DPPIV, dipeptidyl peptidase-IV; DPPIV-I, dipeptidyl peptidase-IV inhibitor; CDAA, choline-deficient L-amino acid-defined; QUICKI; quantitative insulin sensitivity check index; SR, Sirius Red; TGF-β, transforming growth factor beta; αSMA, alpha-smooth muscle actin; RT-PCR, real-time polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Alb, albumin; T-Bil, total bilirubin; ALT, alanine aminotransferase; IRS, insulin receptor substrate; PI3K, phosphatidyl inositol 3-kinase; TZD, thiazolidinediones

Key Words: SGLT2 inhibitor, liver fibrosis, insulin resistance

Abstract

It is widely understood that insulin resistance (IR) critically correlates with the development of liver fibrosis in several types of chronic liver injuries. Several experiments have proved that anti-IR treatment can alleviate liver fibrosis. A sodium–glucose cotransporter-2 (SGLT2) inhibitor is a new class of anti-diabetic agent that inhibits glucose reabsorption in the renal proximal tubules to improve IR.

The aim of this study was to elucidate the effect of an SGLT2 inhibitor on the development of liver fibrosis using obese, diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats and their littermate non-diabetic Long-Evans Tokushima Otsuka (LETO) rats. Male OLETF and LETO rats were intraperitoneally injected with porcine serum twice a week for 12 weeks to augment liver fibrogenesis. Different concentrations of ipragliflozin (3 mg/kg and 6 mg/kg) were orally administered during the experimental period. Serological and histological data were examined at the end of experimental period.

The direct effect of ipragliflozin on the proliferation of a human hepatic stellate cell (HSC) line, LX-2, was also evaluated *in vitro*. OLETF rats, but not LETO rats, received 12 weeks of porcine serum injection to induce severe fibrosis. Treatment with ipragliflozin markedly attenuated the development of liver fibrosis and expression of hepatic fibrosis markers, such as alpha smooth muscle actin, collagen1A1, and transforming growth factor-beta (TGF-β), along with improvement of IR in a dose-dependent manner in OLETF rats. In contrast, proliferation of LX-2 *in vitro* was not affected, suggesting that ipragliflozin had no remarkable direct effect on the proliferation of HSCs.

In conclusion, our dataset suggest that an SGLT2 inhibitor could alleviate the development of liver fibrosis by improving IR in naturally diabetic rats. This may be a basis to create new therapeutic strategies for chronic liver injuries with IR.

Introduction

Liver cirrhosis arises from various etiological factors, such as hepatitis virus B or C, autoimmune hepatitis, alcohol consumption, and metabolic disorders (1, 2, 3). Hepatocellular carcinoma commonly develops from patients with cirrhotic liver; therefore, prevention of liver cirrhosis is a critical issue in improving the prognosis of chronic liver injuries. Recently, with the influence of metabolic syndrome, the prevalence of non-alcoholic fatty liver disease (NAFLD) has increased rapidly (4). Among the NAFLDs, non-alcoholic steatohepatitis (NASH) is widely known as a progressive pathological phenotype, and increase of NASH-related liver cirrhosis has emerged as one of the critical issues to be solved (5). NASH is widely known as a hepatic phenotype of metabolic syndrome, particularly if it is deeply associated with type 2 diabetes mellitus (DM) (6).

In patients with type 2 DM, insulin resistance (IR) is central in the disease pathogenesis (7). Recent studies suggested a strong relationship between type 2 DM and development of chronic hepatitis C and NASH (8). For example, IR is one of the major risk factors for the progression of liver fibrosis in patients with chronic hepatitis C and NASH (9-12). In addition, rodent experiments revealed that an IR-mimic, high glucose, and high-insulin condition increased collagen and extracellular matrix production from activated hepatic stellate cells (HSCs), which played a central role in liver fibrogenesis (13,14). Therefore, particularly in NASH, several therapeutic strategies using hypoglycemic medicines have been attempted to reverse the

development of liver fibrosis. Among them, peroxisome proliferator-activated receptor γ (PPARγ) agonist and dipeptidyl peptidase 4 (DPPIV) inhibitor have been reported as promising hypoglycemic agents that can also reverse the progression of liver disease. Both PPARγ agonist and DPPIV inhibitor exerted anti-fibrogenic activity in rodent NASH-mimic models (15). Furthermore, clinical studies elucidated that PPARγ agonist alleviated the pathological progression of patients with NASH (16). Thus, IR-targeted therapy would be one of the promising choices for IR-associated chronic liver diseases.

In recent years, a sodium–glucose cotransporter-2 (SGLT2) inhibitor, which inhibits glucose reabsorption by blockade of SGLT2, has been proposed as a new promising agent for type 2 DM treatment (17). SGLT2 is expressed on the apical side of proximal tubular cells (18), where it reabsorbs approximately 90% of the urine glucose in the proximal tubules (19). This agent can lower blood glucose level by promoting urinary glucose excretion (20). Ipragliflozin is the first selective SGLT2 inhibitor that was approved in Japan (21). Several clinical trials reported the benefit of this agent in blood glucose control as both monotherapy and in combination therapy with other hypoglycemic medicines (22-24). Another rodent experiment suggested that ipragliflozin can also improve IR through pancreatic β-cell protection (25). Recently, ipragliflozin has been reported to prevent the development of liver fibrosis in choline-deficient, amino acid-defined (CDAA) diet-treated rats, which express NASH-mimic hepatic phenotypes (26). According to this report, an SGLT2 inhibitor could also be a promising hypoglycemic agent, like a PPARγ agonist and DPPIV inhibitor. However, there is a critical issue based on a previous report that treatment with CDAA diet did not exert severe IR (27, 28).

Therefore, in this study, we evaluated whether ipragliflozin can inhibit the progression of liver fibrosis using obese diabetic rats as model of liver fibrosis with severe IR both *in vivo* and *in vitro*.

Materials and methods

Animal treatment

Male Otsuka Long-Evans Tokushima Fatty (OLETF) rats and Long-Evans Tokushima Otsuka (LETO) rats were supplied by Otsuka Pharmaceutical Co. (Tokushima, Japan) (29). Ipragliflozin was supplied by Astellas Pharma Co. (Japan). At the age of 8 weeks, OLETF and LETO rats were each divided into four groups: G1–G4 for OLETF and G5–G8 for LETO rats. Each group consisted of six rats. Porcine serum (1 ml/kg) was intraperitoneally injected in all rats, except in G1 and G5 rats that served as negative controls, twice a week for 12 weeks; the same amount of saline was injected in negative control rats. Through drinking water, G3 and G7 rats were treated with low-dose ipragliflozin (3 mg/kg/day), while G4 and G8 rats were treated with high-dose ipragliflozin (6 mg/kg/day). G1, G2, G5, and G6 rats were given normal drinking water as control. At the end of this experiment, rats were anesthetized, their abdominal cavities were opened, blood samples were drawn via aortic puncture, and livers were harvested for histological evaluation. Serum biological markers were measured by routine laboratory methods. IR was evaluated with the homeostasis model assessment-insulin resistance (HOMA-R) and the quantitative insulin sensitivity check index (QUICKI), as described previously (30). All animal procedures were performed according to a standard protocol and in accordance with the standard recommendations for the proper care and use of laboratory animals.

Immunohistochemical staining and semi-quantification

The liver sections were routinely stained with Sirius Red (SR) for the detection of the development of liver fibrosis. Immunohistochemical staining of α-smooth muscle actin (α-SMA) (Dako, Kyoto, Japan) was performed, as described previously (31-33).

Semi-quantitative analyses of liver fibrosis were conducted using Adobe Photoshop software.

Real-time polymerase chain reaction (RT-PCR)

Hepatic mRNA expression levels of transforming growth factor-β (TGF-β), alpha-1 type 1 collagen (collagen 1A1), and alpha smooth muscle actin (αSMA) were evaluated by RT-PCR, as described previously (33). In brief, liver tissues were immediately flash-frozen for RNA extraction. *In vitro*, human LX-2 HSCs were plated at a density of 5 × 10^5 cells/ml on uncoated plastic dishes under three different conditions for 24 h. The first group was treated with low-glucose DMEM (100 mg/dl). The second group was treated under IR-mimicking conditions with high-glucose DMEM (280 mg/dl) plus insulin (200 nM). The last group was incubated with the IR-mimicking condition plus ipragliflozin (10 μM). After incubation, cells were harvested and RNA was extracted for RT-PCR, as described previously (33). RT-PCR was performed with the ABI Prism7700 Sequence Detection System (PE Applied Biosystems, FosterCity, CA, USA) according to the manufacturer's manual. Relative quantification of gene expression was performed using glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control. Threshold cycles and the standard curve method were used for calculating the relative amount of target RNA. Each rat primer construction is listed as follows: TGF-β, forward ATACGCCTGAGTGGCTGTCTT, reverse ATACGCTGAGTGGCTGTCTT; collagen1A1, forward TGCTGCCTTTTCTGTTCCTT, reverse AAGGTGCTGGGTAGGGAAGT; αSMA, forward ACTGGGACGACATGGAAAAG, reverse CATCTCCAGAGTCCAGCACA; and GAPDH, forward AGACAGCCGCATCTTCTTGT, reverse CTTGCCGTGGGTAGAGTCAT, and primer for human as follows: TGF-β, forward GGGACTATCCACCTGCAAGA, reverse

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CCTCCTTGGCGTAGTAGTCG; and collagen 1A1, forward CCAAATCTGTCTCCCCAGAA, reverse TCAAAAACGAAGGGGAGATG.

Water soluble tetrazolium salt (WST)-1 assay and cell counting

To evaluate the direct effect of ipragliflozin on human HSC lines, we compared cell proliferation, with or without treatment of ipragliflozin. The human HSC line LX-2 was seeded on uncoated plastic dishes at a density of 5×10^4 cells/ml. After overnight culture, the cells were treated with different concentrations of ipragliflozin (0, 0.1, 1, 5, 10, 20, and 50 µM, respectively) for 24 h. As a positive control, cells were treated with Platelet-derived growth factor-BB (PDGF-BB; 10 ng/ml, R&D system, Minneapolis, MN). Proliferation of each cell was measured by WST-1 assay according to the manufacturer's manual.

Next, LX-2 HSCs were seeded at a density of 5×10^5 cells/ml on uncoated plastic dishes in five different conditions: normal glucose (100 mg/dl), high glucose (280 mg/dl), normal glucose plus insulin (200 nM), high glucose plus insulin, and high glucose plus insulin plus ipragliflozin (10 µM). These glucose concentrations were compared with the serological data of OLETF and LETO rats, as previously reported (34). After incubation for 48 h, cells were harvested and the cell number per group was counted with a cell counter (Waken B tech Co. Ltd, Kyoto, Japan).

Western blotting for IRS1-PI3K-Akt signaling in vitro

To examine whether SGLT2-I had a direct inhibitory effect on insulin signaling in activated HSCs, Western blotting was performed using a standard protocol. LX-2 cells were seeded on uncoated plastic dishes under the following three conditions for 24 h: normal glucose (100 mg/dl), high glucose (280 mg/dl) plus insulin (200 nM), and high glucose plus insulin plus SGLT2-I (10 µM). After incubation, cells were harvested and

whole cell lysate was prepared before being subjected to SDS-PAGE (ATTO Supporting Life Research, Tokyo, Japan). Western blot analysis was performed with rabbit monoclonal anti-IRS1, anti-Akt, anti-phospho Akt, anti-β-actin antibody (Cell Signaling Technology, Danvers, MA, USA), and mouse monoclonal anti-phospho IRS1 antibody (Abcam, Cambridge, UK).

Statistical analysis

Results were expressed as mean ± SD and analyzed using analysis of variance test. A p-value of less than 0.05 was considered statistically significant.

Results

Ipragliflozin ameliorated IR in diabetic OLETF rats but not in non-diabetic LETO rats

Physical, serological, and urinary data of all experimental groups of diabetic OLETF rats (G1–4) and non-diabetic LETO rats (G5–8) are shown in Figure 1 and Table 1. The final body weight of OLETF rats was significantly higher than that of LETO rats, similar to previous reports (34). Rats receiving porcine serum injection exhibited loss of body weight. Treatment with ipragliflozin lowered the body weight of OLETF rats that received with porcine serum injection in a dose-dependent manner, but not that of the LETO rats (Figure 1A). Liver weight in the OLETF groups was altered by neither porcine serum injection nor ipragliflozin. In the LETO groups, liver weight was lowered by porcine serum injection, but not by treatment with ipragliflozin (Figure 1B).

In serological data (Table 1), the serum albumin (Alb) level of each group was similar in both OLETF and LETO rats. Both serum total bilirubin (T-Bil) and alanine aminotransferase (ALT) levels were increased by porcine serum injection in OLETF rats but not in LETO rats. Treatment with ipragliflozin did not alter these serum markers.

In diabetic OLETF rats (G1, G2), as expected, a significantly higher plasma glucose and plasma insulin level than those in non-diabetic LETO rats (G5, G6) were observed (Table 1). Porcine serum injection increased the plasma glucose and insulin levels in LETO rats, but not in diabetic OLETF rats. Treatment with ipragliflozin significantly increased excretion of urinary glucose in a dose-dependent manner in both rats. Urinary glucose level in OLETF rats was higher in diabetic OLETF rats than in LETO rats in the indicated groups. Ipragliflozin significantly lowered plasma glucose and insulin levels in a dose-dependent manner (G2 vs. G3 and G2 vs. G4). However, Ipragliflozin only lowered these in LETO rats in the high-dose group (G6 vs. G7 and G6 vs. G8). Similarly, treatment with ipragliflozin significantly improved the HOMA-R and QUICKI score in a dose-dependent manner, indicating that ipragliflozin alleviated IR in diabetic OLETF rats. In LETO rats, this finding was observed only in the high-dose ipragliflozin group (G6 vs. G7, G6 vs G8), indicating that IR states were much less affected in non-diabetic LETO rats compared with OLETF rats in this experimental model.

Ipragliflozin ameliorated porcine serum-induced liver fibrosis in diabetic OLETF rats

Histological findings were evaluated after a 12-week treatment with porcine serum and ipragliflozin. Liver fibrosis significantly developed in diabetic OLETF rats treated with porcine serum (G2) but not in non-diabetic LETO rats in each treatment group (G5–G8). Porcine serum-induced hepatic fibrosis was ameliorated by ipragliflozin treatment in a dose-dependent manner (Figure 2). Activated HSCs indicated by immunohistochemistry of αSMA were significantly increased in diabetic OLETF rats treated with porcine serum (G2), and treatment with ipragliflozin decreased αSMA-positive activated HSCs in parallel with liver fibrosis level (Figure 3A-D, and E).

Expression levels of representative liver fibrogenic genes (i.e., αSMA, collagen1A1, and TGF-β) were examined in each rat group by RT-PCR (Figure 3F-H). A significant

elevation in each gene expression level that indicates the development of liver fibrosis was observed in diabetic OLETF rats after porcine serum injection (G2) compared with that in control rats (G1). Reflecting on the microscopic findings, treatment with ipragliflozin alleviated porcine serum-induced elevation of these markers of fibrosis in a dose-dependent manner. Alteration of hepatic fibrogenesis markers was not observed in LETO rats regardless of treatment (data not shown). This experimental model indicated that liver fibrosis, which was observed exclusively in diabetic OLETF rats, may be augmented by the presence of IR, under which ipragliflozin successfully ameliorated liver fibrosis.

Ipragliflozin did not alter proliferation of HSCs in vitro

As shown in Figure 4A, ipragliflozin did not inhibit the proliferation of LX-2 in clinically compatible doses. Neither SGLT1 nor SGLT2 RNA was expressed in LX-2 cells (data not shown). High glucose or insulin levels increased the proliferation of HSCs, and the combination of both further increased proliferation. In contrast, treatment with ipragliflozin did not inhibit the proliferation of LX-2 under IR-mimicking conditions (Figure 4B, C). Also, the expressions of collagen 1A1 and TGF-β did not change in LX-2 cells under each condition, indicating that ipragliflozin does not possess direct effects on fibrosis production in HSCs (Figure 4D, E). Moreover, the IR-mimicking condition promoted phosphorylation of both IRS1 and Akt, indicating that this upregulated the activation of IRS–PI3K–Akt signaling. However, treatment with ipragliflozin did not alter this signaling pathway (Figure 4 F). These results suggested that the anti-fibrotic effect of ipragliflozin was based on the amelioration of IR but not on direct effect toward HSCs.

Discussion

In the current study, we first reported that the anti-diabetic SGLT2 inhibitor ipragliflozin successfully ameliorated liver fibrosis in a diabetic rat model. It is widely recognized that IR is a common pathological condition that underlies several chronic liver diseases like chronic hepatitis C or NASH (11, 12). Several reports indicate that IR was associated with variable events involved in the development of chronic liver diseases, including hepatic inflammation, liver fibrosis, and hepatic carcinogenesis (34). Among them, the role of IR on the development of liver fibrosis has been widely examined. Both hyperglycemia and hyperinsulinemia, the main manifestations of IR, directly augment the proliferation of HSCs and enhance the production of connective tissue growth factor and extracellular matrix (34). We also have reported that a combination of hyperglycemia-mimic and hyperinsulinemia-mimic condition cumulatively increased the proliferation of human HSC line LX-2 compared with that in each single condition (34). In addition, it has been reported that insulin receptor is upregulated by activated HSCs (14). Among several signaling pathways involved in IR-induced development of liver fibrosis, insulin receptor substrate (IRS) 1/2 plays a central role (35). The activation of phosphatidyl inositol 3 kinase (PI3K)–Akt pathway followed by the tyrosine phosphorylation of IRS1/2 is crucial for proliferation of HSCs (35). In the current study, we also confirmed that high glucose and insulin states accelerated cell proliferation among HSCs by the activation of the IRS1–PI3K–Akt pathway. Therefore, the presence of IR strongly and directly interacts with the development of liver fibrosis in chronic liver disease.

 In our experimental rodent liver fibrosis model, immune response against porcine serum antigens activates HSCs via hepatic macrophages and leads to fibrogenesis (37). Of note, non-diabetic littermate LETO rats did not exhibit the development of fibrosis despite repeated fibrogenic stimuli by porcine serum injection. This finding may

provide evidence that IR is a potent promoter of liver fibrosis following a certain initiating condition for liver fibrogenesis.

In this experimental model, we found that treatment with ipragliflozin successfully alleviated IR states in OLETF rats injected with porcine serum, resulting in amelioration of the development of liver fibrosis. Ipragliflozin is categorized as an SGLT2 inhibitor, which inhibits urine glucose reabsorption from the proximal tubular cells (21). We showed that ipragliflozin could lower plasma glucose and insulin levels, especially in diabetic OLETF rats, resulting in improved IR. In contrast, injection with porcine serum into non-diabetic LETO rats caused increasing plasma insulin levels and an exacerbation of IR; however, liver fibrosis did not occur in this group. According to a previous report, these observations may have been caused by high postprandial glucose and insulin levels that resulted from insulin hyposecretion in OLETF rats (38).

The expression of SGLT2, a sodium–glucose cotransporter, is limited in the renal proximal tubules, but not in the liver. Because of this localization, we postulated that an SGLT2 inhibitor would have no direct effect on HSC. In this study, we showed that ipragliflozin could not inhibit LX-2 proliferation induced under mimicked IR conditions. In addition, ipragliflozin altered neither the gene expression of fibrotic markers (i.e. TGF-β and collagen 1A1) nor the IRS1–PI3K–Akt signaling induced by insulin *in vitro*. These data suggested that the SGLT2 inhibitor had no direct effect on liver fibrogenesis, but that it might inhibit hepatic fibrosis indirectly by improving systemic IR.

Noteworthy, a clinically comparable low dose usage (3–6 mg/kg/day) of ipragliflozin was sufficient for the amelioration of the development of liver fibrosis. In recent years, SGLT2 inhibitors have been proposed as new promising agents for type 2 DM treatment (21). Several therapeutic attempts of using other anti-diabetic agents against the development of liver fibrosis focused on IR and demonstrated certain effects (15, 16). We have previously reported that DPPIV inhibitor suppressed HSC proliferation and

fibrogenic gene expression through the inhibition of ERK1/2, p38, and Smad2/3 phosphorylation (33). In addition, synthetic PPARγ ligands and thiazolidinediones (TZD) reduced extracellular matrix deposition and HSC activation both *in vitro* and *in vivo* (39). Our findings support previous evidence that anti-diabetic treatment may contribute in preventing the progression of chronic liver disease in diabetes.

There are several limitations of the current experiment. First, our rat liver fibrosis model did not exhibit steatosis. As commonly known, chronic liver diseases with coexistent IR will probably present with steatosis (40). In addition, there were several evidences of interaction between IR and steatosis (40). Both IR and steatosis are related to and may enhance hepatic inflammation and fibrosis (41). In this experiment, however, neither OLETF nor LETO rats had hepatic steatosis or steatosis-generated reactive oxidative stress (data not shown). Therefore, our rodent model lacks these interaction effect on the pathogenesis of liver fibrosis. Second, distinct from other anti-diabetic agents, ipragliflozin did not have a direct effect on HSCs; the anti-fibrotic effect of ipragliflozin was exclusively attributed to the alleviation of IR in our experimental model. However, on the basis of clinical evidence, SGLT2 inhibitors reduced body weight of patients with type 2 DM (42). Similar to these reports, the body weights of obese diabetic OLETF rats were reduced by ipragliflozin in our current experiment. Because the reduction of excess body weight is the most effective way to reduce IR (43, 44), SGLT2 inhibitors are expected to possess additional indirect effects on ameliorating liver fibrosis via the reduction of body weight. Further experiments using different rodent models are required.

In conclusion, we first report that low-dose ipragliflozin, a clinically available anti-diabetic SGLT2 inhibitor, successfully ameliorated porcine-induced liver fibrosis in diabetic OLETF rats. Because SGLT2 inhibitors have already been proven to be

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clinically useful for type 2 DM, are safe, and are widely used, this drug may represent a

potential therapeutic strategy against liver fibrosis in the near future.

Conflict of interest

The authors declare that they have no conflict of interest.

<Figure>

Figure 1 Average body weight (A) and liver weight (B) of OLETF (G1–G4) and LETO (G5–G8) rats at the end of the experimental period. The data presented are mean ± SD values. *p < 0.05 represents significant difference between groups.

Table 1 Characteristic serological features of G1–G8 rat groups. The data presented are mean ± SD values. *p < 0.05 represents significant differences vs. negative control groups in the same rat. †P < 0.05 represents significant difference vs. positive control groups in the same rat.

Figure 2 Representative Sirius Red staining photomicrographs of the development of liver fibrosis in OLETF rats and LETO rats (original magnification, ×40) A-H; G1 to G8, respectively. I; Semi-quantitative analysis of the development of liver fibrosis by an image analyzer system. Data represent mean ± SD values. *p < 0.05 represents significant difference between groups.

Figure 3 (A-E) Representative αSMA immunohistochemistry in OLETF rats (original magnification, ×40). A-D; representative images of each treatment group (A-D, G1-G4, respectively). E; semi-quantitative analysis of the αSMA positive HSCs. (F-H) Hepatic mRNA expressions of αSMA (F), collagen 1A1 (G), and TGF-β (H) in OLETF rats. Data represent mean ± SD values. *p < 0.05 represents significant difference between groups.

Figure 4 (A) Cell proliferation assay of LX-2 cells treated with different concentrations of ipragliflozin *in vitro* by WST-1 assay. There were no significant differences between the treated and untreated group (negative control). (B) Cell proliferation assay. (C) Number

of LX-2 cells treated under normal glucose conditions, IR-mimicking conditions, and IR-mimicking conditions plus ipragliflozin. The IR-mimicking condition increased the proliferation of HSCs, but ipragliflozin did not. (D-E) Expressions of gene markers for fibrosis in LX-2 cells treated with normal glucose, high glucose plus insulin, and high glucose plus insulin plus ipragliflozin. The mRNA expressions for TGF-β (D) and collagen 1A1 (E) were not changed by any condition, indicating that IR and SGLT2-I had no influence on the fibrotic ability of HSCs. (F) The effects of IR and ipragliflozin on the IRS1–PI3K–Akt signaling pathway are shown by western blotting. IR was shown to upregulate the phosphorylation of IRS-1 and Akt, while ipragliflozin did not.

Data represent mean ± SD values. $*p < 0.05$ represents significant difference between groups.

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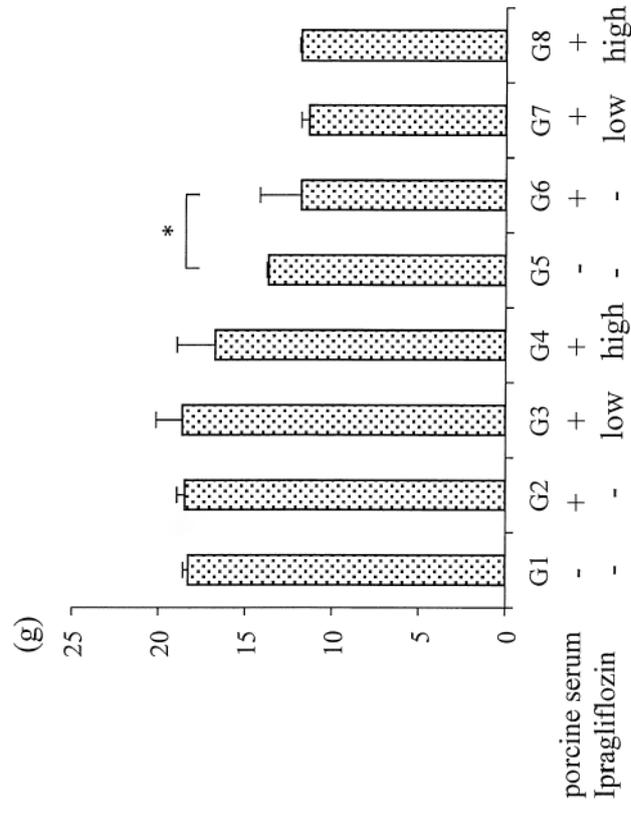
A

(g)

porcine serum
Ipragliflozin

B

(g)

porcine serum
Ipragliflozin
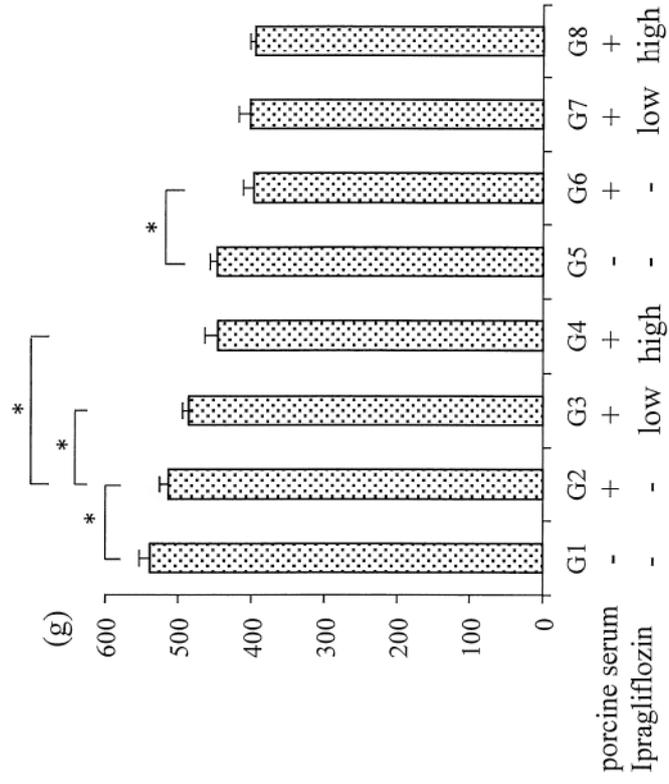
Table.1 Serological and urinary characteristic features of G1-G8 rat groups.

	G1	G2	G3	G4	G5	G6	G7	G8
Alb(mg/dL)	4.0±0.1	3.7±0.5	3.9±0.1	3.9±0.1	4.1±0.1	4.0±0.2	4.0±0.1	4.0±0.2
T-Bil(mg/dL)	0.07±0.01	0.10±0.007*	0.10±0.017	0.12±0.037	0.05±0.01	0.06±0.02¶	0.04±0.01	0.04±0.01
ALT(IU/L)	34±2	221±12*	168±78	224±53	41.3±7.6	39.0±2.6¶	37.0±4.7	49.5±7.8
Glu(mg/dL)	169±5	174±7	158±14	148±13†	140±19	154±5¶	153±6	147±16
Insulin(ng/µL)	1.7±0.1	1.7±0.1	1.4±0.2†	1.0±0.2†	1.2±0.3	1.5±0.1*¶	1.2±0.3	1.1±0.1†
HOMA-R	18.4±1.8	18.9±1.5	13.8±1.4†	9.4±1.4†	10.2±2.5	15.0±0.2*¶	11.9±2.6	10.4±2.2†
QUICKI	0.258±0.003	0.258±0.002	0.267±0.003†	0.280±0.005†	0.277±0.008	0.264±0.000*¶	0.272±0.008	0.276±0.007†
Urinary glucose level	0	0	171±209†	461±75†	0	0	144±240†	376±215†

A

Absorbance(O.D.)

SGLT2-I

NT 0.1 1 5 10 20 50 PDGF-BB

N.S.

(μM)

B

Absorbance(O.D.)

N.S.

*

Glucose	100	280	280	mg/dl
Insulin	0	200	200	nM
SGLT2-I	0	0	10	μM

C

Cell number of LX-2(x10⁴cells/HPF)

N.S.

* * * *

Glucose	100	280	100	280	280	mg/dl
Insulin	0	0	200	200	200	nM
SGLT2-I	0	0	0	0	10	μM

D

Gene expression ratio

N.S.

Glucose	100	280	280	mg/dl
Insulin	0	200	200	nM
SGLT2-I	0	0	10	μM

E

Gene expression ratio

N.S.

Glucose	100	280	280	mg/dl
Insulin	0	200	200	nM
SGLT2-I	0	0	10	μM

F

p-IRS1   - 180kDa
IRS1   - 180kDa
p-Akt   - 60kDa
Akt   - 60kDa
β-actin   - 45kDa

Glucose	100	280	280	mg/dl
Insulin	0	200	200	nM
SGLT2-I	0	0	10	μM