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Low dose of sodium-glucose transporter 2 inhibitor ipragliflozin attenuated renal dysfunction and interstitial fibrosis in adenine-induced chronic kidney disease in mice without diabetes

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ABSTRACT

Background: Sodium−glucose co-transporter 2 (SGLT2) inhibitor, a new class of glucose lowering agents, has been shown to be reno-protective in diabetes.
Objective: We aimed to explore whether SGLT2 inhibitor ipragliflozin has a direct reno-protective effect on non-diabetic chronic kidney disease (CKD) in mice.
Methods: CKD mice was induced by feeding of 0.25% w/w adenine containing diet. Low dose ipragliflozin (0.03 or 0.1 mg/kg/day) was orally administered to CKD mice for 4 weeks, concomitantly with adenine containing diet.
Results: CKD mice exhibited increases in kidney weight/body weight ratio, plasma creatinine levels, urinary fatty acid binding protein 1 excretion and plasma interleukin-6 levels, and a decrease in hematocrit, accompanied by morphological changes such as crystal deposits in the tubules, tubular dilatation, interstitial fibrosis, and increased 8-hydroxy-2′-deoxyguanosine staining. Low dose ipragliflozin (0.03 or 0.1 mg/kg/day) did not affect either plasma glucose levels or urinary glucose excretion, while it improved levels in plasma creatinine ($P < 0.05$ for 0.03 mg/kg/day, $P < 0.001$ for 0.1 mg/kg/day), interleukin-6 ($P < 0.05$ for 0.1 mg/kg/day) and hematocrit ($P < 0.05$ for 0.1 mg/kg/day), and morphological changes dose-dependently except crystal deposit formation in the CKD mice.
Conclusions: Low-dose ipragliflozin has a reno-protective effect in non-diabetic adenine-induced CKD mice, independently of plasma glucose levels and urinary glucose excretion. Low dose SGLT2 inhibitor may be a useful therapeutic option for non-diabetic CKD with the advantage of fewer adverse effects.

1. Introduction

Sodium−glucose co-transporter 2 (SGLT2) inhibitors were developed as anti-diabetes drugs that block the reabsorption of glucose and sodium in the S1 segment of the proximal tubules. Recently, SGLT2 inhibitors were found to elicit reno-protective effects in patients with diabetes [1,2]. The reno-protective effect is explained by multiple beneficial effects in plasma glucose control, blood pressure, body weight and lipid profiles, and an attenuation of glomerular hyperfiltration [3,4]. All these mechanisms are mediated by an inhibition of tubular SGLT2. In contrast, we recently demonstrated that low dose SGLT2 inhibitor exerts the reno-protective effect independently of plasma glucose levels and urinary glucose excretion in Type 2 diabetes model, db/db mice [5]. This finding provides a rationale for exploring the reno-protective effect of this class of drugs in chronic kidney disease (CKD) without diabetes.

In this study, we examined the protective effects of low dose of SGLT2 inhibitor ipragliflozin on the progression of renal dysfunction in adenine-induced CKD mice, which exhibit pathological features of CKD characterized by chronic renal interstitial inflammation and fibrosis [6,7].

Abbreviations: CKD, chronic kidney disease; DHA, 2, 8-dihydroxyadenine; FABP1, fatty acid binding protein 1; IL, interleukin; NF-kB, nuclear factor kappa B; SGLT2, sodium−glucose co-transporter 2; 8-OHdG, 8-hydroxy-2′-deoxyguanosine.

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2. Materials and methods

2.1. Animals

Five-week-old male C57BL/6JJcl mice were purchased from Clea Japan (Tokyo, Japan). CKD mice were induced by feeding of a 0.25% w/w adenine-containing diet [8]. The mice were randomly divided into normal diet-fed mice (n = 8), non-treated CKD mice (n = 8), and ipragliflozin-treated CKD mice (n = 8 for both 0.03 and 0.1 mg/kg/day). Ipragliflozin was orally given to the CKD mice once daily for 4 weeks concomitantly with adenine-containing diet. Ipragliflozin was kindly provided by Astellas Pharma Inc. (Tokyo, Japan). At 4 weeks after the start of adenine diet and ipragliflozin treatment, mice were placed in metabolic cages for 24 h to collect the urine, and then all mice were anesthetized with a solution of hydrochloric acid medetomidine (Kyoritsu Seiyaku Corporation, Tokyo, Japan), midazolam (Sandoz K.K., Tokyo, Japan), and butorphanol (Meiji Seika Pharma Co., Ltd, Tokyo, Japan). A blood sample was obtained by cardiac puncture for the determination of plasma concentration of creatinine, uric acid and IL-6, and then mice were killed by exsanguination. The kidney was immediately excised in formalin for the following experiments. All procedures and animal care were approved by the Committee on Ethics of Animal Experiments, Graduate School of Pharmaceutical Sciences, Kyushu University and were conducted according to the Guidelines for Animal Experiments of the Graduate School of Pharmaceutical Sciences, Kyushu University.

2.2. Blood and urine analysis

Plasma and urinary glucose (glucose CII-test), plasma uric acid (uric acid C-test), plasma creatinine (Lab assay Creatinine), urinary protein (Protein Assay Rapid) were determined using an assay kit (Wako Pure Chemical Industries, Osaka, Japan). Plasma interleukin (IL)-6 concentration was measured using a Mouse Quantikine ELISA Kit (R&D Systems, Inc., Minneapolis, MN, USA). Urinary fatty acid binding protein 1 (FABP1) concentration was measured using a Mouse/Rat FABP1/L-FABP Quantikine ELISA Kit (R&D Systems, Inc.). The results of these urinary studies are expressed as values corrected to the level of urinary creatinine.

2.3. Morphological study

Paraffin sections of 3 μm were stained with hematoxylin and eosin, periodic acid-Schiff reagent and Masson's trichrome. Immunostaining for 8-hydroxy-2′-deoxyguanosine (8-OHdG), an oxidative stress marker, in the kidney was performed as described previously [9]. The area of tubular dilatation and interstitial fibrosis were evaluated using ImageJ software (the US National Institutes of Health) as previously described [10,11].

2.4. Statistical analysis

All data are shown as the mean ± SEM. Statistical analyses were performed by one-way analysis of variance with the Tukey–Kramer test. A probability value of 0.05 was set as the minimum level of statistical significance.

3. Results

Treatment of ipragliflozin for 4 weeks increased urinary glucose excretion and decreased plasma glucose levels significantly in a dose-dependent manner at 1 and 10 mg/kg/day. At dose of 0.1 mg/kg/day, ipragliflozin had no significant effect on either plasma glucose levels or urinary glucose excretion.

Then, we explored the reno-protective effect of ipragliflozin in adenine-induced CKD mice at doses of 0.03 and 0.1 mg/kg/day to clarify whether the reno-protective effects are independent of plasma glucose lowering and urinary glucose excreting effect. The CKD mice exhibited loss of body weight, less food intake, and increased ratio of kidney weight/body weight in agreement with previous reports [6,7]. As for renal function, plasma creatine levels were significantly increased in the CKD mice comparing with control mice, accompanied by increased levels of urinary FABP1 excretion. Hematocrit values were significantly decreased, and plasma levels of IL-6, an inflammation marker, were significantly increased in CKD mice (Table 1).

Treatment of ipragliflozin significantly decreased plasma creatinine levels dose-dependently at doses of 0.03 and 0.1 mg/kg. It significantly decreased the ratio of kidney weight/body weight, plasma IL-6 levels, and significantly increased hematocrit levels at dose of 0.1 mg/kg. It did not significantly affect either non-fasting

Table 1
Characteristics of adenine-induced CKD mice and control mice after 4 weeks of treatment with the ipragliflozin.

	ND (n = 8)	AD		
		0 mg/kg (n = 8)	0.03 mg/kg (n = 7)	0.1 mg/kg (n = 8)
Body weight (g)	23.0 ± 0.5	15.3 ± 0.3 *	15.1 ± 0.3 *	15.4 ± 0.2 *
Kidney weight/Body weight (%)	0.67 ± 0.01	0.87 ± 0.03 ***	0.87 ± 0.02 ***	0.79 ± 0.01 ***,#
Food intake (g/day)	2.9 ± 0.1	1.7 ± 0.06 ***	1.2 ± 0.06 ***	1.4 ± 0.09 ***
Water intake (g/day)	4.7 ± 0.1	6.7 ± 0.5	6.1 ± 0.6	6.5 ± 0.7
Hematocrit (%)	54.9 ± 0.6	40.1 ± 1.8 ***	44.0 ± 0.5 ***	44.8 ± 0.9 ***,#
Non-fasting plasma glucose (mg/dL)	138.8 ± 6.5	75.5 ± 4.6 ***	73.3 ± 4.8 ***	74.9 ± 3.9***
Plasma Creatinine (mg/dL)	0.78 ± 0.3	1.27 ± 0.07 ***	1.03 ± 0.05 *,#	0.89 ± 0.07###
Plasma uric acid (mg/dL)	0.52 ± 0.09	0.43 ± 0.08	0.80 ± 0.18	0.58 ± 0.07
Plasma IL-6 (pg/mL)	4.6 ± 1.4	108.9 ± 12.9 ***	91.6 ± 11.0	69.8 ± 6.4#
Urine output (mL/day)	0.99 ± 0.21	2.32 ± 0.36	2.50 ± 0.45 *	2.55 ± 0.40 *
Urinary sodium excretion (mmol/mg cre)	0.11 ± 0.01	0.33 ± 0.02 ***	0.34 ± 0.03 ***	0.35 ± 0.03 ***
Urinary glucose excretion (mg/mg cre)	0.17 ± 0.05	0.24 ± 0.13	0.66 ± 0.5	0.56 ± 0.16
Urinary protein excretion (mg/mg cre)	0.046 ± 0.017	0.018 ± 0.002	0.028 ± 0.05	0.027 ± 0.008
Urinary FABP1 (ng/mg cre)	0.11 ± 0.05	37.2 ± 5.1 ***	37.2 ± 1.7 ***	35.3 ± 3.7 ***

ND, normal diet-fed mice, AD, adenine containing diet-fed mice; IL-6, interleukin-6; cre, creatinine; FABP1, fatty acid binding protein 1. Data are the means ± SEM. Statistical analysis was performed by one-way analysis of variance with Tukey-Kramer test. *p < 0.05 and ***p < 0.001 versus normal diet-fed mice. #p < 0.05 and ###p < 0.001 versus adenine-fed mice.

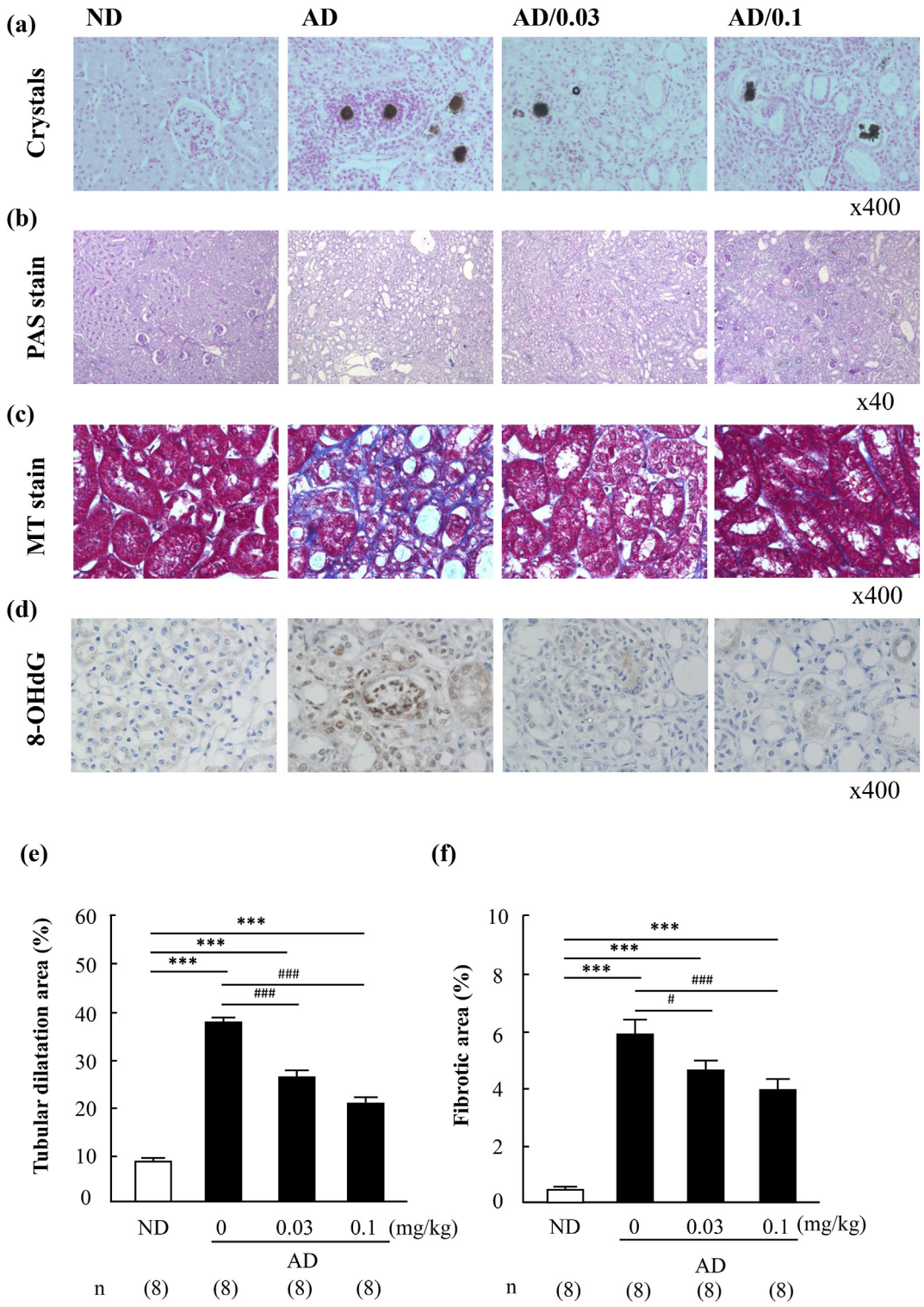

Fig. 1. Effect of ipragliflozin on morphological changes in real tissue of mice with adenine-induced chronic kidney disease. Representative photomicrographs of renal sections showing (a) crystal deposits in tubules (hematoxylin and eosin staining), (b) tubular dilatation (PAS, periodic acid-Schiff staining) (c) Masson's trichrome staining (d) 8-hydroxy-2′-deoxyguanosine immunostaining. ND, normal diet-fed mice; AD, adenine-fed mice; AD/0.03 or 0.1, adenine-fed mice treated with ipragliflozin at dose of 0.03 or 0.1 mg/kg/day, respectively. Quantitative analysis of (e) tubular dilatation area and (f) fibrotic area. AD, adenine-fed mice; 0, 0.03 or 0.1, mice treated with ipragliflozin at dose of 0, 0.03 or 0.1 mg/kg/day. n, experimental mice number. All data are expressed as the mean ± SEM. Statistical analysis was performed by one-way analysis of variance with Tukey-Kramer test. *P < 0.05, ***P < 0.001 vs. normal diet-fed mice. #p < 0.05 and ###p < 0.001 vs. adenine-fed mice.

blood glucose levels or urinary glucose excretion levels, and also did not affect body weight, urinary output, urinary sodium excretion, urinary FABP1, or plasma uric acid levels (Table 1).

Fig. 1 shows that crystal deposits, tubular dilatation, fibrotic areas stained in blue by Masson's trichrome, and 8-OHdG staining were more prominent in the renal tissue of CKD mice than control mice.

Low doses of ipragliflozin did not affect crystal deposit formation, while they significantly attenuated both renal tubular dilatation and fibrotic area dose-dependently, and appeared to normalize 8-OHdG staining in the CKD mice (Fig. 1).

4. Discussion

To our knowledge, this is the first report to show that low-doses of ipragliflozin, which did not affect either plasma glycemic control or urinary glucose excretion, attenuated renal dysfunction and interstitial fibrosis in adenine-induced CKD mice.

Although the mechanism by which adenine induces CKD is not fully understood, crystal deposits of 2, 8-dihydroxyadenine in the tubules are thought to lead to kidney injury via the induction of interstitial inflammation and oxidative stress [7]. In the present study, ipragliflozin treatment reduced the circulating levels of IL-6 and 8-OHdG staining dose-dependently in adenine-induced CKD mice, suggesting the anti-inflammatory and antioxidant effect of ipragliflozin. Therefore, it is very likely that low doses of ipragliflozin attenuated renal dysfunction and interstitial fibrosis in adenine-induced CKD via these effects. Previous studies have shown that SGLT2 inhibitors attenuated renal inflammation and oxidative stress in various diabetic animal models [12,13]. However, it has been unclear that these were the direct effects of SGLT2 or not, because glycemic control and other systemic changes induced by increased urinary glucose excretion were improved in these experimental diabetic models. In our model, low doses of ipragliflozin did not affect the levels of plasma glucose, urine output, urinary glucose and sodium, plasma uric acid, or body weight. These findings indicated that ipragliflozin may attenuate renal inflammation and oxidative stress independently of these systemic changes in non-diabetic CKD model, although the mechanism underlying such direct effects remained to be elucidated. Several recent studies have shown that SGLT2 inhibitors directly act on podocytes, mesangial cells, and inflammatory cell such as macrophage other than proximal tubular cells [5,14–16]. Together, the present results further supported the possible existence of new targets for the reno-protective effect of SGLT2 inhibitors other than tubular SGLT2-dependent mechanisms.

Currently, several clinical studies to elucidate the reno-protective on CKD are going on, but it is still controversial [17]. In addition to ours, several studies have shown its effectiveness [14,18], but no effect was observed in other kinds of CKD models. Dapagliflozin failed to exert the reno-protective effect in 5/6 nephrectomy model [19], and empagliflozin had no reno-protective effect on an oxalate-induced CKD model that develops nephrocalcinosis-related tubular atrophy and interstitial fibrosis [20]. Long-term clinical studies should be done to confirm the reno-protective effects of SGLT2 inhibitors in non-diabetic CKD patients.

There were several limitations in this study. First, since we used only ipragliflozin, it was unclear whether the reno-protective effect is drug-specific or not. Second, we could not demonstrate the new target and mechanism that explain how low dose ipragliflozin exerted the direct reno-protective effect. The mechanism should be clarified in future studies.

In conclusion, we showed that low-dose ipragliflozin have a reno-protective effect in non-diabetic adenine-induced CKD mice, independently of plasma glucose levels and urinary glucose excretion. Low dose SGLT2 inhibitor may be a useful therapeutic option for non-diabetic CKD, with the advantage of fewer adverse effects.

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CRediT authorship contribution statement

Mayumi Yamato: Conceptualization, Methodology, Investigation, Writing - original draft. **Nao Kato:** Investigation, Writing - review & editing. **Ai Kakino:** Investigation, Writing - review & editing. **Ken-ichi Yamada:** Supervision, Writing - review & editing, Funding acquisition. **Toyoshi Inoguchi:** Conceptualization, Supervision, Writing - review & editing, Funding acquisition.

Declaration of competing interest

The authors have no conflict of interest.

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