A sodium-glucose transporter 2 inhibitor attenuates renal capillary injury and fibrosis by a vascular endothelial growth factor–dependent pathway after renal injury in mice

Yifan Zhang[1,2,6], Daisuke Nakano[1,6], Yu Guan[1,6], Hirofumi Hitomi[1], Akiyoshi Uemura[3], Tsutomu Masaki[4], Hideki Kobara[4], Takeshi Sugaya[5] and Akira Nishiyama[1]

[1]*Department of Pharmacology, Kagawa University Medical School, Kagawa, Japan;* [2]*Department of No. 2 Orthopedics, Shijiazhuang City No. 1 Hospital, Shijiazhuang, Hebei, China;* [3]*Department of Retinal Vascular Biology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan;* [4]*Department of Gastroenterology & Neurology, Kagawa University, Kagawa, Japan; and* [5]*Department of Internal Medicine, St. Marianna University School of Medicine, Kanagawa, Japan*

Multiple large clinical trials have shown that sodium-glucose cotransporter (SGLT) 2 inhibitors reduce the risk of renal events. However, the mechanism responsible for this outcome remains unknown. Here we investigated the effects of the SGLT2 inhibitor luseogliflozin on the development of renal fibrosis after renal ischemia/reperfusion injury in non-diabetic mice. Luseogliflozin significantly suppressed development of renal fibrosis, prevented peritubular capillary congestion/hemorrhage, attenuated CD31-positive cell loss, suppressed hypoxia, and increased vascular endothelial growth factor (VEGF)-A expression in the kidney after ischemia/reperfusion injury. Luseogliflozin failed to induce the above-mentioned protection in animals co-treated with sunitinib, a VEGF receptor inhibitor. Additionally, luseogliflozin reduced glucose uptake and increased VEGF-A expression in the kidneys of glucose transporter 2 (GLUT2)-downregulated mice following ischemia/reperfusion and in GLUT2-knock-down cells compared with those in normal controls. Withdrawal of glucose from cultured medium, to halt glucose uptake, remarkably increased VEGF-A expression and reversed the luseogliflozin-induced increase in VEGF-A expression in the proximal tubular cells. Thus, luseogliflozin prevented endothelial rarefaction and subsequent renal fibrosis after renal ischemia/reperfusion injury through a VEGF-dependent pathway induced by the dysfunction of proximal tubular glucose uptake in tubules with injury-induced GLUT2 downregulation.

Kidney International (2018) ■, ■–■; https://doi.org/10.1016/j.kint.2018.05.002

KEYWORDS: glucose uptake; renal fibrosis; sodium glucose co-transporter 2; vascular endothelial growth factor

Correspondence: *Daisuke Nakano, Department of Pharmacology, Faculty of Medicine, Kagawa University, 1750-1 Ikenobe, Miki, Kita, Kagawa 761-0793, Japan. E-mail: dnakano@med.kagawa-u.ac.jp*

[6]These authors contributed equally to this work.

Received 15 September 2017; revised 25 April 2018; accepted 3 May 2018

Sodium-glucose cotransporter 2 (SGLT2), which is expressed in the convoluted segments of renal proximal tubules, transports sodium ion (Na^+) and glucose at a 1:1 stoichiometry from the tubule lumen into the cells, and it mediates approximately 90% of glucose reabsorption under physiological conditions.[1] Regardless of its extensive role in glucose transport, little attention was initially paid to the pathophysiological role of SGLT2 in the proximal tubules and surrounding renal environment because patients with familial renal glucosuria due to congenital SGLT2 mutation did not show apparent renal function changes.[2] However, the sudden inhibition of glucose transport via an SGLT2 inhibitor may observably influence renal pathophysiology due to its acute nature, in contrast with that of a congenital SGLT2 mutation. Notably, the EMPA-REG OUTCOME trial reported that empagliflozin, an SGLT2 inhibitor, reduced the risk of major adverse cardiovascular events[3] as well as the risks of serum creatinine doubling and renal replacement therapy initiation[4] in patients with type 2 diabetes at high risk for cardiovascular events. Similar renal protection was observed recently by the CANVAS Program for canagliflozin, another SGLT2 inhibitor.[5]

Acute kidney injury (AKI) is a common clinical syndrome defined as a sudden onset of reduced renal function,[6] which could occur during and/or after heart failure, and the AKI incidence has steadily increased over the last decade. Furthermore, AKI survivors have an increased risk of developing chronic kidney disease (CKD),[7] heart failure, and myocardial infarction.[8] Numerous studies have revealed the multiple pathways, such as autophagy,[9] endothelial rarefaction,[10] cell cycle arrest, and cell senescence,[11,12] involved in the mechanism of AKI and subsequent development of CKD.

We recently reported that an acute SGLT2 knockdown in renal proximal tubular cells prevented high-glucose–induced tubular cell senescence.[13] Furthermore, the occurrence of AKI tended to be reduced, or at least not increased, in SGLT2

inhibitor-treated subjects in both the EMPA-REG OUTCOME[4] and CANVAS Program,[5] and a recent study using mice reported the protective effects of dapagliflozin against ischemia-reperfusion (IR) injury,[14] despite its presumed risk of dehydration. These findings led us to hypothesize that SGLT2 inhibition might prevent AKI after IR injury in mice by accelerating tubular recovery and might prevent renal interstitial fibrosis development, a common outcome of CKD. Therefore, we assessed the ability of luseogliflozin treatment initiated after IR in nondiabetic mice to prevent AKI or to accelerate recovery from AKI, and, furthermore, observed its effects on renal fibrosis development.

We also investigated the potential mechanism for this effect. Proximal tubules express 2 glucose transporters, SGLT2 and glucose transporter 2 (GLUT2). Because GLUT2 is a facilitated diffusion transporter, inhibition of SGLT2 alone should not influence the net glucose uptake in proximal tubules *in vivo*; GLUT2 can take up glucose from the interstitial spaces. Importantly, IR injury changes proximal tubule polarity and influences protein expression levels.[15] Thus, we also hypothesized that AKI disrupted the cell membrane GLUT2 expression and, thus, that luseogliflozin induced a depletion of glucose uptake. This loss of glucose uptake could change the phenotype of proximal tubules and the long-term outcome of kidney injury.

RESULTS

Effect of luseogliflozin on post-IR renal function and morphology

This study employed nondiabetic animals because AKI severity and recovery could be affected by blood glucose levels. Throughout the experimental period, the blood glucose level did not significantly change following luseogliflozin treatment in either the sham-operated or IR groups (Supplementary Figure S1A). Luseogliflozin increased the urine volume significantly in the sham-operated groups at days 1, 3, and 7 (Supplementary Figure S1B), but the similar trend in the IR groups did not reach statistical significance. Additionally, luseogliflozin markedly increased urinary glucose excretion levels in both the sham-operated and IR groups, although the response was greater in the sham-operated groups (Supplementary Figure S1C). IR induced an increase in blood urea nitrogen (BUN) and a decline in creatinine clearance, which both reflect the severity of renal malfunction, at day 1 after reperfusion ($n = 5$–8) (Figure 1a and b). The BUN and creatinine clearance levels were relatively recovered in the following days (days 3 and 7), but these levels remained different from those in the sham-operated groups. Luseogliflozin did not affect the creatinine clearance and BUN levels at day 1 or the recovery of BUN and creatinine clearance from IR-induced injury compared with vehicle treatment (Figure 1a and b). There was no statistically significant difference in the AKI-induced histological damage between the luseogliflozin-treated and vehicle-treated groups at days 1 and 3 (Figure 1c); however, treatment with luseogliflozin attenuated the histological damage at day 7.

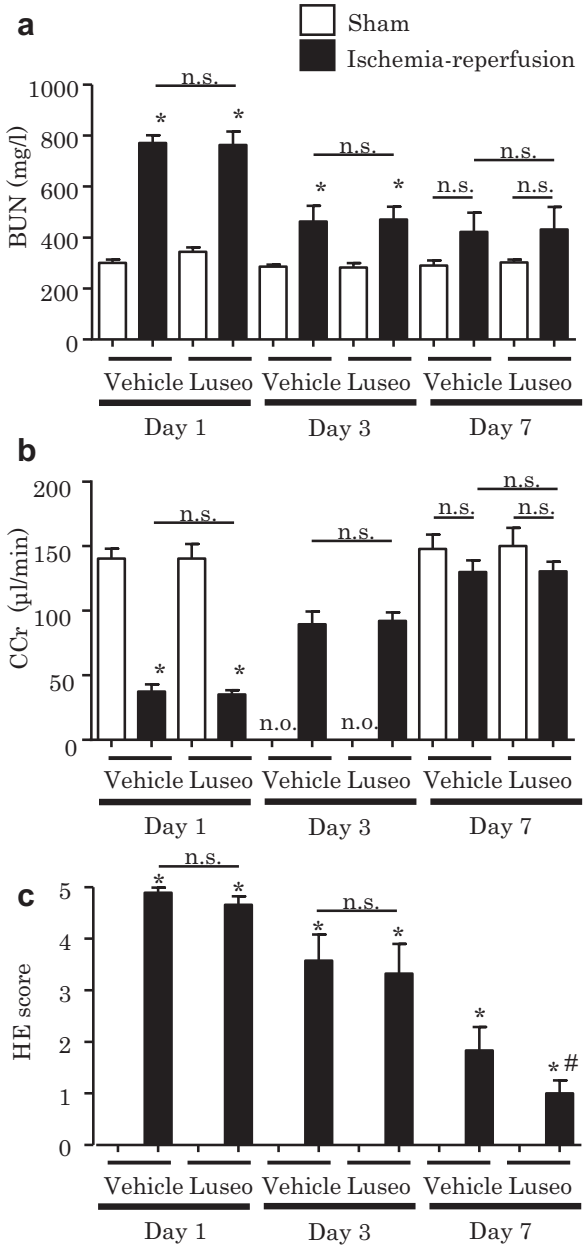

Figure 1 | Time course of changes in blood urea nitrogen (BUN), creatinine clearance, and histological damage score in the kidney after ischemia-reperfusion. The (**a**) blood urea nitrogen levels, (**b**) creatinine clearance, and (**c**) histological damage kidney scores were measured on days 1, 3, and 7 in luseogliflozin (luseo)- or vehicle-treated groups of mice that underwent a sham-operation or ischemia-reperfusion ($n = 5$–8). n.o., not observed; n.s., not significant. *$P < 0.05$ versus sham-operated group, #$P < 0.05$ versus vehicle-treated group.

AKI is characterized by an increase in tubular cell death, proliferation,[11] and autophagy.[9] Luseogliflozin did not affect the numbers of propidium iodide-positive dead cells at day 1, of LC3-positive puncta in GFP-LC3#53 reporter mice at day 3, or of bromodeoxyuridine- and Ki67-positive cells at day 3 ($n = 3$–4) (Supplementary Figure S2), supporting the observation that luseogliflozin treatment, when started at 6 hours after reperfusion, did not alleviate AKI.