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様式１０２

学位論文の内容の要旨

| 専　攻 | 分子情報制御医学専攻 | 部　門 | 生体情報学 |
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| 論文題目 | Responses of renal hemodynamics and tubular functions to acute sodium–glucose cotransporter 2 inhibitor administration in nondiabetic anesthetized rats | | |

（論文要旨）

Background

To manage type 2 diabetes mellitus, blockade of glucose reabsorption at the proximal tubule using sodium–glucose cotransporter 2 (SGLT2) inhibitors have been recently applied. SGLT2 is primarily expressed in the brush border membrane at S1 segments of the proximal convoluted tubule. It has been suggested that approximately 90% of filtered glucose is reabsorbed by SGLT2. Therefore, inhibition of this transporter leads to glycosuria, and selective SGLT2 inhibitors are considered therapeutic tools for treating type 2 diabetes. A review article reported that SGLT2 inhibition reduces blood pressure; however, the mechanism responsible for SGLT2 inhibitor-induced blood pressure reduction is unclear. We have recently reported that reductions in blood pressure using SGLT2 inhibitors are associated with natriuresis in metabolic syndrome. Similarly, a clinical study reported that treatment with canagliflozin, the SGLT2 inhibitor, significantly increased urinary sodium excretion in patients with type 2 diabetes. These findings suggest that SGLT2 inhibitor-induced blood pressure reduction is accompanied by natriuresis in patients with metabolic syndrome and diabetes. Genetic knockout of SGLT2 resulted in increased urine flow and glucosuria without affecting the glomerular filtration rate (GFR) in mice, and chronic treatment with an SGLT2 inhibitor showed sustained increases in urinary glucose excretion in wild-type mice. Additionally, in a micropuncture study, it was found that acute injection of an SGLT2 inhibitor increased urine flow, urinary glucose, and sodium excretion in a rat model of early diabetes. However, the effects of acute administration of SGLT2 inhibitors have not been examined in non-diabetic subjects. It is of particular importance that the pharmacological effects of SGLT2 inhibitors in non-diabetic subjects are studied to minimize any indirect influence induced by changes in blood glucose levels. In this regard, a study with pooled urine reported that treatment with an SGLT2 inhibitor increased the 24-h urine volume, urinary glucose, and sodium excretion in non-diabetic mice. Similarly, an SGLT2 inhibitor tended to increase 24-h urinary excretion of sodium, potassium, and chloride in dogs. However, it is difficult to exclude the possibility that urinary sodium excretion was influenced by sodium intake in these urine-storing studies. Thus, it remains unclear whether SGLT2 inhibitor-induced urinary changes are actually mediated by its direct tubular action or by other indirect mechanisms. In the current study, we investigated the direct effects of SGLT2 inhibition on renal hemodynamics and tubular functions *in vivo* by examining the acute effects of luseogliflozin, the selective SGLT2 inhibitor, in anesthetized non-diabetic rats with normal kidney function. Clinically, side effects of SGLT2 inhibitors, such as polyuria and polydipsia, have been reported during the early stages of treatment, which leads to the restriction of use of an SGLT2 inhibitor, particularly in patients with chronic kidney disease (CKD). Therefore, we also examined the effects of a SGLT2 inhibitor on renal hemodynamics and functions in 5/6 nephrectomized (Nx) rat, a model of CKD.

Methods and Findings

Male SD and Nx SD rats were anesthetized with sodium pentobarbital (50 mg $kg^{-1}$, i.p.) and isoflurane (0.5–1.5% in air) or inactin (100 mg $kg^{-1}$, i.p.). Then, animals were placed on a heated pad to maintain body temperature at 37 °C. A

polyethylene catheter was inserted into the abdominal aorta via the right femoral artery for blood pressure measurement and collection of arterial blood. We collected urinary samples for every 30 minutes. The left kidney was exposed through a retroperitoneal flank incision. The renal artery was carefully isolated from the tissue connecting the renal hilum cephalic. A Doppler flow probe was placed around the renal artery and RBF was continuously monitored. A polyethylene catheter was inserted into the left ureter for urine collection. After surgery, each rat was kept isolated for 60 min to allow for stabilization of MAP, RBF, and urine flow. All rats were given saline as a maintenance fluid at a dose of 0.7 ml $h^{-1}$. SD and Nx SD rats were anesthetized with pentobarbital and isoflurane and in Protocol 1, luseogliflozin (0.3 mg $kg^{-1}$, i.v.) or vehicle at 0.1 ml was administered, in Protocol 2, luseogliflozin (0.9 mg $kg^{-1}$, i.p.) or vehicle at 0.1 ml was administered and in Protocol 3, SD rats were anesthetized with inaction and luseogliflozin (0.9 mg $kg^{-1}$, i.p.) or vehicle was administered at 0.1 ml.

Our data showed that i.p. administration of luseogliflozin did not have any effects on mean arterial pressure (MAP), mean renal blood flow (RBF), or creatinine clearance (CrCl) in non-diabetic SD and Nx SD rats. Luseogliflozin significantly increased urine flow in both groups of SD rats. Compared to Nx SD rats, luseogliflozin significantly increased urinary sodium excretion and reduced urinary osmolality and urea concentration in SD rats. However, luseogliflozin did not alter urinary sodium excretion in Nx SD rats. Luseogliflozin resulted in a significant increase in urinary pH in SD rats which was not observed in Nx SD rats.

Discussion

The major challenge in this study was the solubility of luseogliflozin. We used dimethyl sulfoxide (DMSO) and HP-β-CD as vehicle. Luseogliflozin could be dissolved in only 100% DMSO, but acute administration of high concentration DMSO caused hematuria. Intravenous administration of HP-β-CD caused neither hematuria nor renal hemodynamic changes; however, urinary parameters were significantly affected by administration of HP-β-CD. On the other hand, we confirmed that intraperitoneal administration of HP-β-CD showed minimal effect on systemic and renal parameter. Another crucial point is the application of an adequate anesthetic. In present study, we used sodium pentobarbital and isoflurane as anesthetics in the current study.

In conclusion, the present study has demonstrated that, acute administration of the SGLT2 inhibitor induced significant increases in urinary glucose and sodium excretion without altering renal hemodynamics in non-diabetic SD rats. These renal effects of the SGLT2 inhibitor were markedly attenuated in 5/6 Nx SD rats. These findings support the hypothesis that SGLT2 inhibitors elicit direct tubular effects without changes in plasma glucose levels.


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