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様式２１３

学位論文審査の結果の要旨

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論文題目		Effects of diuretics on SGLT2 inhibitor–induced changes in blood pressure in obese rats suffering from the metabolic syndrome
学位論文の審査結果		(合格) ・ 不合格　（該当するものを○で囲むこと。）

〔 要 旨 〕

Objective: Experiments were carried out to investigate whether diuretics (hydrochlorothiazide + furosemide) impact on the effects of a sodium–dependent glucose co–transporter 2 (SGLT2) inhibitor on glucose metabolism and blood pressure in metabolic syndrome SHR/NDmcr–cp(+/+) rats (SHRcp). Methods: Male 13–week–old SHRcp were treated with: (i) vehicle; (ii) the SGLT2 inhibitor luseogliflozin (10 mg/kg/day); (iii) diuretics (hydrochlorothiazide; 10 mg/kg/day + furosemide; 5 mg/kg/day); or (iv) luseogliflozin + diuretics (n = 5–8 for each group) by oral gavage for 5 weeks. Blood pressure and glucose metabolism were evaluated by a telemetry system and oral glucose tolerance test, respectively. Results: Vehicle-treated SHRcp developed non-dipper type hypertension (dark– vs. light–period mean arterial pressure (MAP): 148.6±0.7 and 148.0±0.7 mmHg, respectively, P=0.2) and insulin resistance. Compared with vehicle–treated animals, luseogliflozin–treated rats showed an approximately 4000–fold increase in urinary excretion of glucose and improved glucose metabolism. Luseogliflozin also significantly decreased blood pressure and turned the circadian rhythm of blood pressure from a non–dipper to dipper pattern (dark– vs. light–period MAP: 138.0±1.6 and 132.0±1.3 mmHg, respectively, P<0.01), which were associated with a significant increase in urinary excretion of sodium. Addition of diuretics did not influence luseogliflozin-induced improvement of glucose metabolism and circadian rhythm of blood pressure in SHRcp. Conclusion: These data suggest that a SGLT2 inhibitor elicits its beneficial effects on glucose metabolism and hypertension in subjects with metabolic syndrome undergoing treatment with diuretics.

Question 1: How did you estimate the numbers of animals?
Answer: Usually for animal experiments, animal number more than 5 is accepted. We have used 5–8 animals in different groups.
Question 2: Why food intake was increased following SGLT2 inhibitor treatment?
Answer: SGLT2 inhibitor treatment caused a significant increase in urinary glucose excretion. Perhaps animals may feel hungry and food intake is increased.
Question 3: What kind of mechanism caused SGLT2 protein downregulation following treatment with a

SGLT2 inhibitor?
Answer: It has been shown that SGLT2 expression is increased in subjects with obese, metabolic syndrome and diabetes, suggesting that increase in glucose reabsorption by high glucose delivery at the proximal tubules augments SGLT2 expression. Since SGLT2 inhibitor decreases the reabsorption of glucose at the proximal tubules, SGLT2 expression may be decreased.
Question 4: What is the advantage of using combination of a SGLT2 inhibitor and diuretics?
Answer: It has been shown that SGLT2 inhibitor alone could decrease blood pressure only 5–7 mmHg in clinics. However, in case of hypertensive subjects with metabolic syndrome, it is necessary to reduce blood pressure more. From this perspective, combination therapy would be a good therapeutic choice.
Question 5: How did you decide the dosage of Luseogliflozin?
Answer: We decided the dose of luseogliflozin by following previous study in the rat.
Question 6: How dipping pattern changed following treatment with a SGLT2 inhibitor?
Answer: There are multiple possible mechanisms underlying the impairment of dipping pattern of blood pressure. In this study, we found that plasma insulin level was declined as well as urinary excretion of sodium was increased by treatment with a SGLT2 inhibitor. So, we think these changes may turn the dipping pattern of blood pressure from non-dipper to a dipper type in these rats following treatment with a SGLT2 inhibitor.
Question 7: What is the determinants of arterial blood pressure? Did you observe any changes?
Answer: The determinants of arterial blood pressure are plasma volume, overall compliance, cardiac output and peripheral resistance. In this study, we found that SGLT2 inhibitor caused a significant increase in urinary sodium excretion, suggesting sodium depletion and reduction of plasma volume.
Question 8: How does SGLT2 inhibitor improve insulin resistance?
Answer: Hyperglycemia itself makes an important contribution to insulin resistance. Euglycemia following treatment with a SGLT2 inhibitor could improve glucose tolerance and insulin secretion.
Question 9: Clinicians worried about dehydration when they use SGLT2 inhibitor.
Answer: We have calculated the water balance and our data indicated that there was no significant interaction among the treatment groups, suggesting water balance were well regulated.
Question 10: How is about kidney function following treatment with a SGLT2 inhibitor and diuretics?
Answer: We have calculated creatinine clearance and we didn't observe any significant difference in creatinine clearance following treatment with a SGLT2 inhibitor or its combination with diuretics.
Question 11: The circadian rhythm of rodents is different from human. How you will implement these data to human?
Answer: Activity is one of the important factor that regulates circadian rhythm. Rodents are active in the night, therefore their night time blood pressure is high in comparison to day time (sleeping time) blood pressure. Conversely, human are active in the day time, and day time blood pressure is high in comparison to night time or sleeping time. So, we think the information of the rat model may be important for human as well. However, clinical study would be conducted in this regard.
Question 12: What are the recommended future experiments?
Answer: Although we have measured different kinds of sodium transporters expression, it is not clear yet how urinary sodium excretion increased following treatment with a SGLT2 inhibitor. To explore further, future experiments will be undertaken to determine the activity of these transporters and relationship with sodium balance following treatment with a SGLT2 inhibitor.

本論文は、メタボリックシンドロームに対するSGLT2阻害薬の腎作用を詳細に検討したものであり、ナトリウム利尿が生じ、体液量が減少することにより、血圧パターンを正常化することを実験的に証明した。SGLT2阻害薬の新しい作用を証明したという点で意義があり、本審査委員会では、審査員全員一致して博士（医学）論文に相応しいものと判断し、合格とした。

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