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様式１０７

学位論文審査の結果の要旨

令和　3　年　11月　2　日

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論文題目	Luseogliflozin, a SGLT2 Inhibitor, Does Not Affect Glucose Uptake Kinetics in Renal Proximal Tubules of Live Mice			
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〕

1. Why did you focus on luseogliflozin, do you have been used other SGLT2i?
Luseogliflozin, a SGLT2 inhibitor, is currently a very popular anti-diabetic drug. In addition to controlling blood glucose level, it also exhibits surprising effects of protecting the kidneys and heart. But the mechanism of renal protection is still unclear. This makes us have a strong interest in it. We also have been used dapagliflozin and canagliflozin.

2. Do you check the urine glucose?
We did not check urine glucose in this study, we have been checked it in previous study and luseogliflozin treatment significantly increased the urinary glucose excretion.

3. Is SGLT2 only specifically expressed in the kidney?
Yes. SGLT2 was localized to the brush-border membrane (BBM) of the renal proximal tubule convoluted segments.

4. What time point you checked blood glucose level?
We check blood glucose level just before 2NBDG injection.

5. How did you decide on the protocol of luseogliflozin? Do you check efficacy of luseogliflozin on all time point?
In previous experiments, we found that luseogliflozin reached its maximum effect 90 minutes after injection. At the same time, studies have shown that the insulin we use reaches its maximum effect 30 minutes after injection. Based on the above evidence, we have formulated this protocol.

6. How to distinguish proximal tubules and distal tubules in your images?
First, the appearance of 2NBDG in the lumen of the distal tubule is much later than that of the proximal tubule. Secondly, the residence time of 2NBDG in the lumen of the distal tubule (more than 10 minutes) is much longer than that of the proximal tubule (less than 10 seconds). In addition, the cell morphology is also different.

7. How did you distinguish brush border and basement membrane?
The brush border membrane is on the inside of the tubule (facing the lumen), and the basement membrane is on the outside of the tubule.

8. In cell experiments, you did not find functional expression of SGLT2, did you check the expression of SGLT2 (in animal experiment)?
We did not check it in this study. Short-term treatment will not affect. In addition, the sodium content in the body is very stable.

9. Has luseogliflozin been used in clinical treatment?
Yes, it is used in the treatment of type 2 diabetes.

10. Are there any side effects of luseogliflozin?
Common side effects include nausea/vomiting, decreased appetite, abdominal pain, severe thirst, malaise, dyspnea or disturbance of consciousness is present.

11. Why luseogliflozin did not lower the blood glucose in normal/hypo group?
SGLT2 inhibitors only lower plasma glucose levels by blocking reabsorption of filtered glucose, which falls as plasma levels fall. Thus, they do not usually cause hypoglycemia in the absence of therapies that otherwise cause hypoglycemia. The body also has some gluconeogenesis and glycogen decomposition to maintain plasma glucose levels.

12. How many times can you repeat this measurement in same mice?
To ensure the accuracy of the data, we usually perform a 2NBDG injection on a mouse and observe it under a microscope. Because it may take more than 30 minutes to wait for 2NBDG to be completely empty, and we may cause heat damage to the kidneys over time during the continuous recording process. Therefore, it is not recommended to perform multiple experiments on the same mouse.

13. Is 2NBDG quickly clean in one flow?
Yes. It only takes a few seconds to clear in the lumen, and it takes longer to detect in the cell.

14. How many transporters expressed in proximal tubule cells? Only SGLT2 and GLUT2?
It is currently known that only SGLT2 and GLUT2 are present in the curved proximal tubules, while the straight proximal tubules express SGLT1 and GLUT1. The distal renal tubules and collecting ducts also express different transporters, but they have no significant effect on glucose reabsorption.

15. You cannot explore the environment of SGLT1?
Yes, the depth of the area we explored is about 30 microns from the surface of the kidney. At this depth, only the curved proximal tubules can be seen. Usually, the depth of SGLT1 expression is greater than 100 microns.

16. How much percentage does luseogliflozin block the SGLT2?
According to reports, it can block 100% of SGLT2.

17. Did you check the dose depended effect of luseogliflozin? Can we use a low dose of luseogliflozin?
We checked it in our previous study. Low dose of luseogliflozin is not effective.

18. What is the significance of this experiment?
SGLT2 inhibitor is believed to block glucose uptake into the cells. This believes without evidence disrupts our understanding of SGLT2i pharmacology. We demonstrated that SGLT2 inhibitor alone does not shut down glucose uptake.

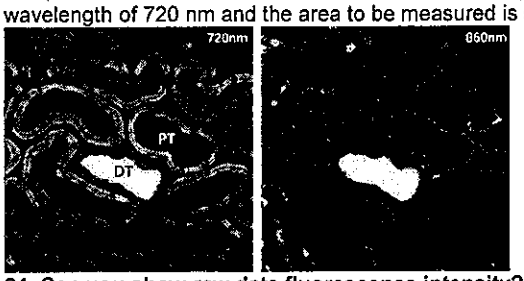
19. What drives GLUT2 to transport glucose when you block SGLT2?
First of all, it has been proved to be bidirectional transport in the liver. Secondly, it is a kind of serendipity. We saw glucose uptake in SGLT2-inhibited animals and found no changes, then guess why and remember what is known in the liver. GLUT2 transports glucose along the concentration gradient in a way of facilitating diffusion, and its transport process does not consume energy.

20. What was your expectation before the experiment? what about uptake process?
We believe that luseogliflozin treatment does not change the glucose kinetics in the normal proximal renal tubules. When SGLT2 is inhibited, proximal tubule cells can uptake glucose from tissue fluid through GLUT2.

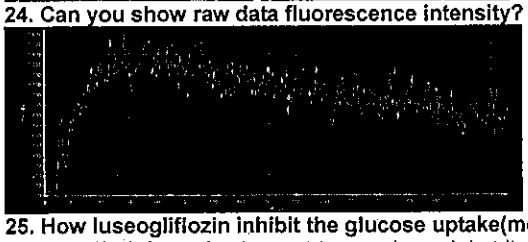
21. How you explain luseogliflozin lower the blood glucose level at 90min?
SGLT2 inhibitors only reduce plasma glucose levels by blocking the reabsorption of filtered glucose. When the blood glucose level is high, the amount of glucose loss caused by SGLT2 inhibition is very large, and gluconeogenesis and glycogen decomposition are not enough to compensate for the loss of glucose.

22. After luseogliflozin treatment, did you see fluorescence increase in distal tubule cells?
We did not see a difference between the groups because of the fluorescence saturation. This is the limitation of the current technology.

23. How did you determine the measurement size of the cell.
First, we can locate the cell based on the nucleus (clear black dot). Then, the cell boundary is determined under a laser with a wavelength of 720 nm and the area to be measured is selected.



24. Can you show raw data fluorescence intensity?



25. How luseogliflozin inhibit the glucose uptake(mechanism)?
The specific information has not been released, but it can be determined that it is not a competitive inhibition, but the inhibition of the reabsorption of glucose by inhibiting the reabsorption of sodium.

26. Can 2NBDG pass through the SGLT2?
Yes, it has been confirmed in cell experiments, but in vivo experiments are impossible due to technical limitations.

27. How SGLT2i improve the CKD?
It is currently being explored. One possibility is that when the patient is in CKD, the proximal tubule cells are usually damaged and cause a decrease in GLUT2 expression. In this case, inhibiting SGLT2 will reduce the glucose level in the proximal tubule cells, which in turn stimulates the expression of VEGF which Stimulate the formation of blood vessels. This will increase the nutrient supply to the damaged proximal tubule cells, thereby improving the cell condition.

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