最終試験の結果の要旨

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主査および副査の５名は、平成 26 年 1 月 17 日、学位申請者 Manoj Bohara 君に面接し、学位申請論文の内容について説明を求めると共に、関連事項について試問を行った。具体的には、以下のような質疑応答がなされ、いずれについても満足すべき回答を得ることができた。

Q1) In neurological field, diseases like immunological encephalitis are caused by antibodies; it is always a question how to pass the antibodies through the blood-brain barrier. So, do you have any information about CNP levels in CNS or blood in neurological disease?

A) Plasma CNP has been found to increase in clinical condition like sepsis, both in the patients and in *in-vitro* experiments. Also, CNP has been found to slightly increase in CSF at acute phase of subarachnoid hemorrhage.

Q2) Are there any evidences like viral infection or epilepsy increase CNP?

A) To my knowledge, there are no evidences of increased CNP in such conditions so far. Sepsis and chronic renal failure are the conditions where plasma CNP level has been found to be elevated.

Q3) Can you measure CNP levels in CSF in our patients?

A) Yes. CNP is present in CSF. So, it could be a good indicator in various neurological diseases in future.

Q4) Could you tell us about the location of CNP receptors, it is on the luminal side or abluminal side?

A) GC-B receptors have been found to be located on the luminal side of the BBB.

Q5) Where in the brain is CNP produced?

A) CNP has been reported to be found in pituitary, hypothalamus and olfactory epithelium.

Q6) When CNP is delivered to the blood, location of receptors on the luminal side makes sense but if CNP is somehow produced by astrocytes or parenchyma of the brain, can it also regulate BBB function?

A) We evaluated the function of CNP application from the bloodstream. But as CNP is highly expressed in brain, BBB function by injecting CNP into the brain could be interesting to evaluate.

Q7) In the method of TEER measurement, can you measure the transport of ionized molecules?

A) Yes, it measures transport of ionized molecules.

Q8) You used sodium fluorescein in in-vivo experiment. Is it ionized or non-ionized?

A) It is ionized molecule.

Q9) In your immunocytochemical study, did you use only endothelial cells or astrocytes too?

A) We used only endothelial cells for immunocytochemistry.

最終試験の結果の要旨

Q10) Is there any report showing that JunD can regulate ZO-1?

A) Yes, it has already been reported that JunD suppresses ZO-1 expression.

Q11) Through what mechanism does JunD regulate ZO-1?

A) The previous report mentioned that JunD suppresses ZO-1 by interaction with the CREB site in the proximal region of ZO-1 promoter.

Q12) As CNP is abundantly expressed in the brain, so can brain CNP affect BBB function?

A) In this research, we applied CNP from the vascular side and then evaluated the effect on BBB function. But, it is certainly a good suggestion that the effect of CNP should be checked by direct application into the brain.

Q13) In brain damage, does the concentration of CNP in CSF change?

A) CNP has been found to slightly increase in CSF at early phase of subarachnoid hemorrhage.

Q14) When you compare CNP and NECA, what is the difference between the effects of these two on BBB function?

A) According to the previous report, NECA has been found to disrupt not only ZO-1 but also other tight junction proteins; which means that the barrier opening is greater compared to CNP. This might lead to permeation of relatively higher-weight molecules into the brain as compared to CNP.

Q15) So, with CNP treatment, the morphology of BBB is maintained?

A) CNP treatment disrupted only one of the tight junction proteins, unlike NECA which disrupted other tight junction proteins also, so it relatively maintains the morphology of BBB as compared to NECA.

Q16) Is there any GC-A receptor in the endothelial cells?

A) Yes, GC-A receptor has been found to express in endothelial cells.

Q17) Does ANP change ZO-1 expression?

A) There has been no report of ANP changing ZO-1 expression so far.

Q18) Although ANP acts via GC-A receptor to produce cGMP, why it doesn't change the permeability?

A) ANP has been found to have no effect on BBB permeability but it has been reported to change the endothelial permeability in coronary and arterial cells. One explanation could be the differential expression of these receptors in various locations. GC-B receptor is highly expressed in brain but GC-A receptor is highly expressed in heart, kidney and lungs. So, the relatively less presence of GC-A receptors could be the reason of no effect of ANP on BBB permeability.

Q19) There may be 2 mechanisms of transport through BBB, via tight junctions and via the endothelial cells. Can you explain the relative importance of 2 pathways in your experiment?

A) Two pathways namely transcellular and paracellular pathways have been described in transport of molecules through the blood-brain barrier. In our research we evaluated the effect on tight junction proteins and found that CNP acts via paracellular pathway for transport of molecules into the brain.

Q20) Is CNP produced only in the brain or some other organs?

A) CNP is most abundant in brain, but it is also found in lungs, kidneys and chondrocytes.

最終試験の結果の要旨

Q21) As you suggested CNP can be a good agent to transport other drugs into central nervous system, I wonder there may be broad effect of CNP on other organs as side effects. What do you think?

A) First of all, CNP has been found to have no significant effect on pulmonary barrier and its effect on other peripheral barriers also should be explored. Regarding side effects, since CNP has weaker vasorelaxant and hypotensive properties compared to other natriuretic peptides, the cardiovascular side effects could be much lower with CNP than with ANP or BNP.

Q22) In in-vitro experiments you used up to 100 nM dose of CNP. How about the dose in in-vivo experiments?

A) We used CNP 10 nmol/kg body weight of mouse.

Q23) How about the physiological concentration?

A) Physiologically, it has very low concentration, 1.4-2.0 pmol/l. So, the dose we used was supra-physiological.

Q24) How does the decreased ZO-1 lead to disruption of tight junction and increase in permeability? ZO-1 is a cytoplasmic protein, so what happens when ZO-1 is decreased?

A) Although ZO-1 is cytoplasmic protein, it has major function to connect the integral membrane proteins to the cytoskeleton. So ZO-1 has been reported to be critical in maintaining barrier function. Previous reports also showed that decrease in ZO-1 has been well correlated with decrease in TEER values.

Q25) Even though ZO-1 is decreased, claudin and occludin are unchanged. So, why the junctional resistance is decreased?

A) ZO-1 is one of the vital proteins that affect tight junction permeability. So, disruption of ZO-1 might affect the framework i.e., the connection of membrane proteins to cytoskeleton, which can result in decreased junctional resistance.

Q26) Did you measure the transport of substances like insulin or gastrointestinal hormones with middle-size molecular weight in your system?

A) No, we did not evaluate the transport of such substances, we only used Na-fluorescein with molecular weight of 376Da and FITC-Dextran with that of 10 KDa.

Q27) In physiological conditions like strenuous exercise, BBB may be opened. Is CNP increased after such strenuous exercise?

A) CNP level has been found to increase in strenuous exercise in patients with impaired glucose tolerance but not in healthy controls.

Q28) In circumventricular organs where BBB is absent, are there any evidences that CNP may affect the process in such organs?

A) To my knowledge, there is no report of the effect of CNP on such organs.

Q29) Does CNP change the production of cerebrospinal fluid?

A) There has been no evidence so far that CNP affects the production of cerebrospinal fluid.

以上の結果から、５名の審査委員は申請者が大学院博士課程修了者としての学力・識見を有しているものと認め、博士（医学）の学位を与えるに足る資格を有するものと認定した。