2024 MERIT/MERIT-WINGS Self-directed joint research

Development of BBB-crossing antibodies and evaluation in vivo using mice

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Risa Asano is engaged in the research on the antibody engineering, particularly focusing on bispecific antibodies, as well as the analysis of protein-protein interactions. In this study, she prepared antibodies and did quantitative analysis of antibodies using ELISA.

Yu Nakanishi is engaged in the research on the development of novel volume EM methods and their application to mouse brain analysis. In this study, he extracted the brain from mouse, sectioned and observed with microscopy.

Kazuki Tsujimura is engaged in exploring intracellular molecular mechanisms in neurons using molecular biological techniques and fluorescence microscopy. In this study, he performs immunostaining to evaluate antibody localization using a confocal microscope.

Kihiro Wakasa is engaged in the exploration of novel mechanisms of rare liver diseases using mice and zebrafish as a screening platform. In this study, he performed intravenous antibody injection and blood collection in mice.

1. Backgrounds

The blood brain barrier (BBB) is physical barrier that strictly controls transportation of substances between the bloodstream and brain tissue. While it protects the central nervous system by preventing the entry of pathogens and harmful substances from blood, it also poses a challenge for drug delivery into the brain. Especially, antibody delivery is highly limited (0.2 % of injected dose)[1]. To overcome

this challenge, utilization of physiological pathway has been proposed to enhance BBB permeability and facilitate drug delivery into brain. Transferrin receptor 1 (TfR1) is one of the promising targets[2].

TfR1 plays a crucial role in iron uptake and cell growth, highly expressed in brain endothelial cells[3]. Asano and colleagues have developed an anti TfR1 VHH antibody, showing BBB crossing ability in vitro model. However, whether this antibody penetrate BBB in animal models remains unclear. Thus, this study aimed to evaluate the BBB permeability of the antibody using a mouse model.

2. Results and Discussion

2.1. Preparation of antibodies

We expressed following antibodies in a mammalian cell expression system and subjected to crude purification followed by final purification by size exclusion chromatography. All of these antibodies had a HA tag added to the C-terminus for detection in ELISA.

① 8D3 IgG antibody[4] : reported to bind to mouse TfR1 and penetrate the blood-brain barrier

② anti-lysozyme HyHEL10 IgG antibody[5] : negative control

③ VHHm1 : an anti-TfR1 VHH antibody we newly obtained

④ VHH43[6] : an anti-MtsA antibody as a negative control

2.2. Evaluation of the BBB permeability of existing IgG antibodies using mice

Purified IgG antibodies were injected via tail vein injection to wild-type female C57BL/6 mice (8 weeks old) at a dose of 1 mg/kg. Blood samples were collected at 4 and 24 hours post-injection. The collected blood was centrifuged and the plasma fractions were collected. Additionally, perfusion was performed under anesthesia, replacing the whole blood with phosphate-buffered saline (PBS) before brain extraction. The extracted brains were homogenized using a homogenizer and ultrasonication, followed by centrifugation to collect the supernatant. Then, the antibody concentrations in plasma and brain tissue were quantified using ELISA. Anti-HA tag antibodies were immobilized on the ELISA plate, plasma and brain tissue samples were followingly added, and finally detection was performed using an HRP-labeled anti-mouse IgG1 antibody.

The antibody amount per brain weight against the administered dose was 0.9% for 8D3 IgG at 4 hours post-injection and 0.13% at 24 hours (Figure 1). In contrast, HyHEL10 IgG showed 1.1% at 4 hours, but the ratio was below the detection limit at 24 hours. As the experiment was done only once, the reproducibility must be confirmed in the future. However, the data suggest that 8D3 exhibits brain

penetration. However, the negative control antibody also showed a similar level of brain penetration at 4 hours post-injection. Given that an anti-lysozyme antibody is unlikely to have brain penetration ability, these results indicate some errors in this experimental system, such as insufficient perfusion or issues with quantification.

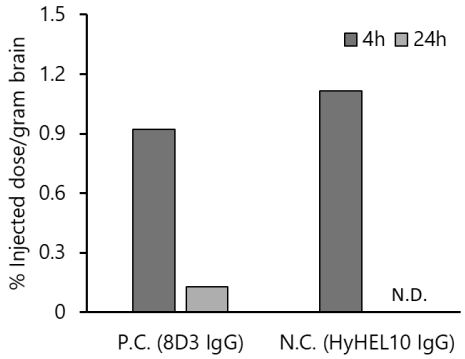

Figure 1. Brain permeability of IgG (n=1)

2.3. Evaluation of the BBB permeability of VHH using mice

Purified VHH antibodies were injected via tail vein injection, same procedure as 2.2. The dosage was 11 mg/kg. VHHm1 injected to three mice and VHH43 to two mice. Considering the short half-life of VHH antibodies in the bloodstream, blood sampling and brain tissue extraction were performed at 1 hour post-injection. In 2.2, the possibility of insufficient perfusion was suggested. To assess the success of perfusion, brain sections were stained, and the number of residual red blood cells in the brain was counted (Figure 2). In Figures 2b and 2e, red blood cells were observed within the blood vessels of the cerebral cortex, indicating incomplete perfusion

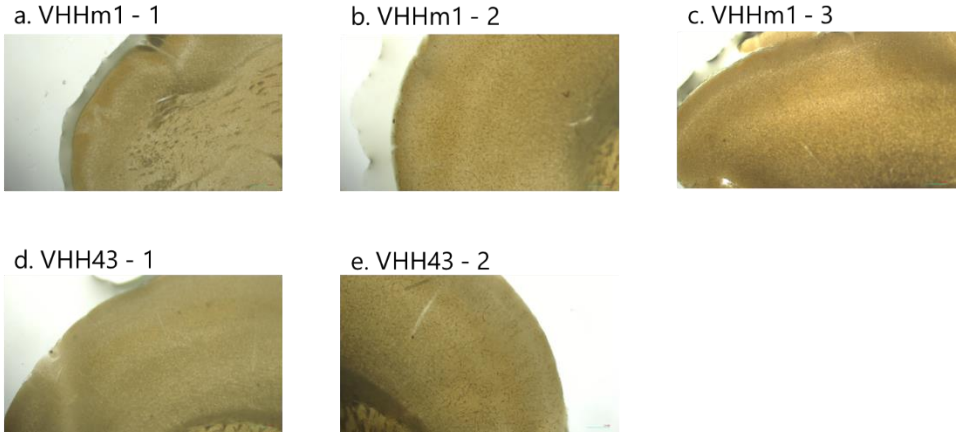

Figure 2. Brain section (a-c: VHHm1 injected mice, d,e: VHH43 injected mice)

Antibody concentrations were quantified using ELISA, for a mice in which perfusion was successfully performed. Anti-HA tag antibodies were immobilized on the plate, and after adding plasma and brain tissue samples, detection was performed using a goat anti-VHH antibody as the primary antibody and an HRP-labeled anti-goat IgG antibody as the secondary antibody.

The antibody amount per brain weight agiainst the injected dose was 0.01% for VHHm1 and 0.003% for VHH43 at 1 hour post-injection (Figure 3). Although VHHm1 showed a higher permeability than VHH43, both exhibited significantly lower values compared to existing BBB crossing antibodies. Possible reasons for the low permeability include the high administered dose and the short half-life of VHH antibodies in the bloodstream.

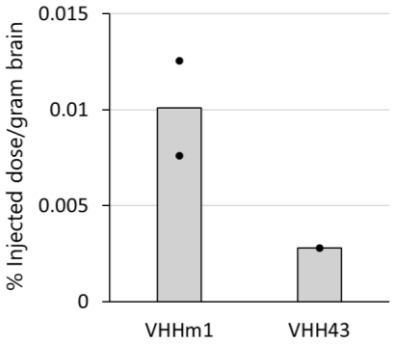

Figure 3. BBB crossing rate of VHH

3. Summary and future perspectives

This joint research aimed to evaluate the blood-brain barrier (BBB) permeability of VHHm1, an anti-TfR1 antibody using mice. First, we successfully established the evaluation system. The results suggested that VHHm1 exhibited higher permeability than the negative control. In the future, we confirm the reproducibility, optimize the antibody dosage, and try antibody engineering to extend the half-life of VHH antibodies for the development of optimized BBB-permeable antibodies.

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