179. 脳マラリアと CNS 疾患に対する薬剤標的としての嗅球

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緒　言

Malaria is an important infectious disease risking around 3.5 billion people's life every day. According to the WHO report, 212 million cases and 429,000 deaths from malaria were recorded in 2015. Cerebral malaria is one of the severe complications of malaria infection in humans caused by *P. falciparum* parasites and characterized by sudden clinical symptoms such as convulsions and coma with high rates of death or long-term disabilities. Cerebral malaria affects ～3 million people, mostly children and travelers each year with fatality rates around 15% to 20%. Sub-Saharan African children account 90% of cerebral malaria-associated deaths and life-long cognitive impairments while causing huge economic burden to society. Therefore, early diagnoses and treatment of malarial disease should be given priority to prevent deaths from this preventable disease. However, pathophysiology of cerebral malaria is not understood well, probably has many features similar to other CNS disorders.

We have recently made a great progress on the understanding of spatiotemporal regulation of pathophysiological and immunological mechanisms of cerebral malaria by using experimental mouse cerebral malaria model. We found that the olfactory bulb is the weakest brain location during cerebral malaria[1)]. We showed by using cutting-edge technologies, ultrahigh-field MRI and multiphoton microscopy, that the olfactory bulb is physically (bleedings occur) and functionally (loss of smell) damaged by *Plasmodium* parasites. We found that the complex small capillary architecture of olfactory bulb supports the parasite accumulation and occlusion following by microbleeding, events associated with high fever and cytokine/chemokine storm. These findings are particularly important that, early detection of olfaction loss may offer new innovation for ideal medical treatment of cerebral malaria.

Due to the non-permissive blood-brain-barrier, it has been very challenging to deliver drugs into the CNS. However, recent evidences suggest that intranasal delivery might be a promising route for the administration of small compounds [2)]. It is thought that olfactory bulb plays important role for this. Therefore, we aimed at treating cerebral malaria by using olfactory as a delivery route.

方法および結果

1.　Time of intervention

Our previous findings have suggested that blood-brain-barrier (BBB) is altered from the olfactory bulb far earlier (～day 4 after infection) than previously known development of cerebral malaria (～day 6). This has suggested the possibility of the early interventions at the onset of early symptoms (day 4) to prevent coma development.

2.　Proof-of-concept that chemokine receptor CXCR3 blockage can help mice to survive from cerebral malaria

The CD8 T cell accumulation into brain tissue, a hallmark of cerebral malaria, partly occurs *via* the activation of chemokines from astrocytes around olfactory glomeruli and vessels during early stages of infection. Activated astrocytes release CCL21 which in turn attract CD8 T cells by using CXCR3-CCR7 axis[1)]. We therefore performed initial proof-of-concept treatment by using antibody raised against chemokine receptor CXCR3 (Figure 1). We found

that while one-time intravenous administration of anti-CXCR3 Ab on day4 after infection had limited success on survival (Fig. 1A), combination with antimalarial-drug chloroquine (CQ) on day5 and day6 have helped to improve mice survival significantly (Fig. 1B).

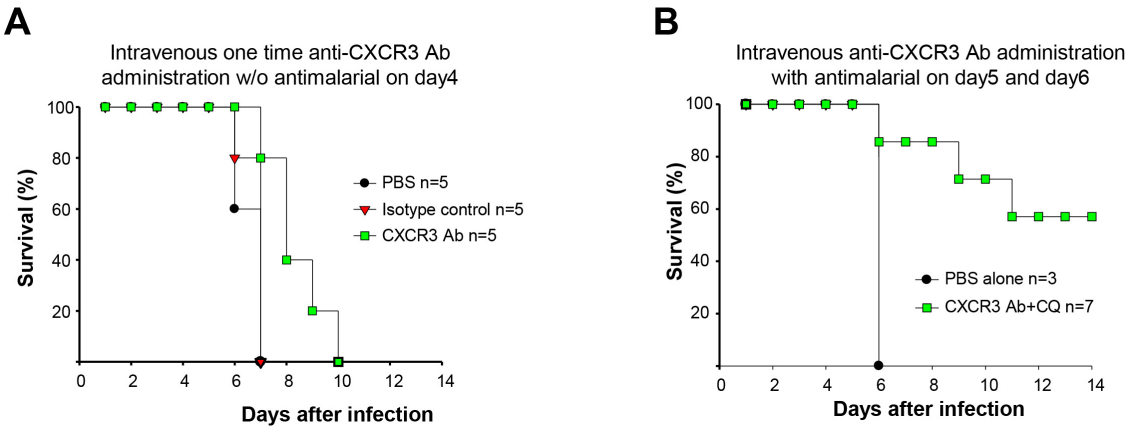

Fig. 1. Proof-of-concept study by using anti-CXCR3 Ab intravenously for the treatment of cerebral malaria

Survival curves of A, recombinant anti-CXCR3 and isotype control antibody treated groups of mice after infection with 106 *Plasmodium berghei* -ANKA infection. Mice were i.v. injected antibodies once 100 μg on day4 after infection. B, Mice were i.v. injected antibody twice 100 μg per day per mouse together with antimalarial drug CQ on day5 and 6 after infection.

3. Search for small compounds which can block chemokine receptor CXCR3

We have next searched candidate compounds which can directly block chemokine/chemokine receptor (i.e. CXCR3) especially expressing on CD8 T cells which cause pathology in the brain during cerebral malaria. Because the BBB is an obstacle for the drug delivery into brain, small compounds are needed. Chemokines are generally small molecules with approximately 10 kDa molecular mass, but still larger than the small molecule which binds to designated chemokine receptors which are trans-membrane spanning, G protein- coupled receptors (GCPR). Our screen of small compounds currently available which may penetrate *via* olfactory have resulted a new compound, NBI-74330. It has been reported that the highly specific CXCR3 antagonist NBI-74330 can inhibit cell migration during atherosclerosis (Fig. 2)[3)].

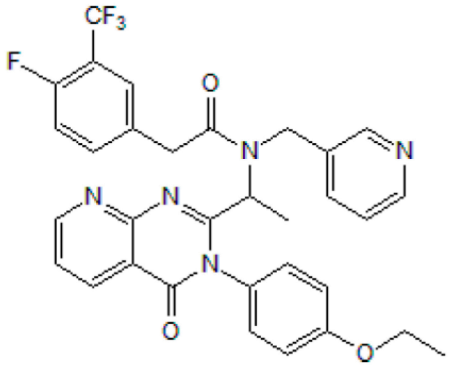

Fig. 2. Structure of compound NBI-74330
This compound is available *via* Tocris Bioscience.

4. Intranasal administration of CXCR3 antagonist during cerebral malaria

We performed studies by CXCR3 antagonist, compound NBI-74330, to treat cerebral malaria. Unexpectedly, we realized that it was not easy to solubilize this compound in a solutions biologically compatible. We have tried few different solutions, but was not successful to solubilize enough for the intranasal administration. Finally, compound solubility has been achieved in an unexpected solution which allowed us to perform intravenous injections into mice (Fig. 3). However, the compound NBI-74330 was not better than antibody administration for blocking CXCR3 during cerebral malaria.

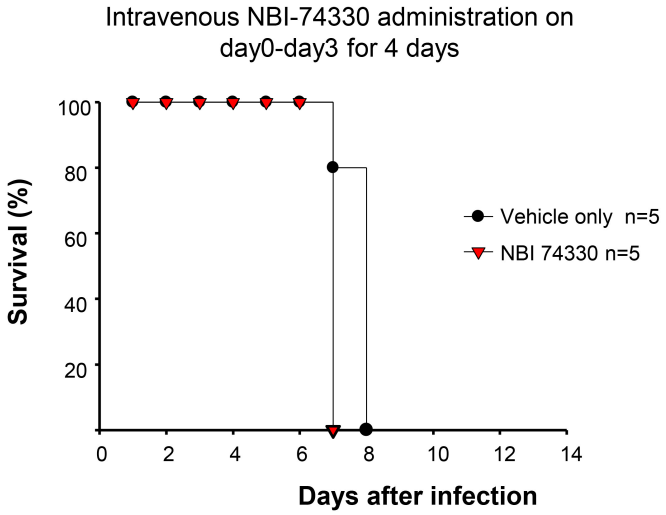

Fig. 3. Administration of NBI-74330 did not improve the cerebral malaria outcome
Survival curve of NBI-74330, a chemical antagonist of CXCR3 and vehicle treated groups of mice after infection with 106 *Plasmodium berghei* -ANKA infection. Mice were i.v. injected on day0-day3 after infection daily for total 4 days.

考　察

In this research, we have explored small compound delivery methods *via* olfactory to treat cerebral malaria. The compound NBI-74330 has been studied as an attractive strategy to treat other CXCR3-mediated disorders such as atherosclerosis. However, as our findings suggested, its use for blocking the development of cerebral malaria both *via* intravenous or intranasal administrations (data not shown) is limited. Further studies are needed to improve the usage of this compound and other additional compounds for the treatment of cerebral malaria.

共同研究者

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