ORAL PRESENTATIONS

Mapping meningeal vasculature in non-human primates

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Background

The blood-brain barrier has been the focus of most prior work examining intracranial vasculature in the context of various brain diseases.[1] Recently, meningeal vasculature has become more widely recognized as a key contributor to brain clearance and its immune function.[2] Meninges are highly vascularized and complex tissue. Vessels of the outer dural layer comprise an extensive, parallel intracranial vascular bed, which lies outside the brain and subarachnoid space. In addition to the blood vasculature, meninges harbor lymphatic channels that potentially provide extra capacity for clearance of proteinaceous fluid and immune cell trafficking. Most of our knowledge of the meningeal vasculature, including lymphatics, comes from rodent models. Rodent meninges are readily available, small, thin and optically transparent. These characteristics permit imaging in whole-mount flat preparations.[3] Technical barriers, however, remain high for imaging studies of the meninges of larger mammals. This is especially true for primates, and ultimately humans. Non-human primate (NHP) and human dura is large, thick and opaque, with a high content of connective tissue. These characteristics limit options for routine high-resolution imaging and leave unanswered questions about the architecture of blood and lymphatic vessels in primate dura. So far, the presence of lymphatic vessels in primates has been demonstrated by non-invasive techniques like magnetic resonance imaging (MRI) or on sections of paraffin-embedded specimens. Neither of the techniques fully addresses spatial and phenotypical features of the vascular networks. In our work, we provide solutions for these technical barriers using new clearing and imaging protocols to successfully visualize blood and lymphatic vessels in NPH dura in their entirety.

Methods

Here we used novel approaches to tissue clearing and resonance scanning confocal imaging of large areas with sickness over 1000 μM.

Results

Our approach revealed extensive and dense vascular networks in NHP dura probed with vascular marker CD31 (Figure 1). Image clarity and resolution is sufficient for visualization of the smallest vessels. In

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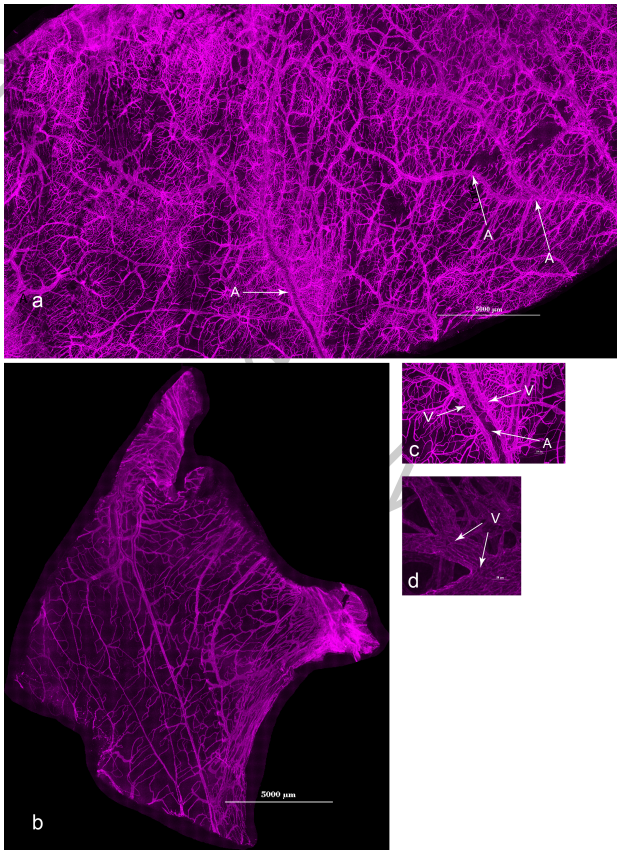

Figure 1. Dura is highly vascularized in lateral areas and tentorium cerebelli. Panoramic view of monkey dura probed with CD31 antibody to visualize blood vessels. (a) Lateral area of the dura cut along falx cerebri and a portion of tentorium cerebelli (b). A panel of the representative close up fields of the vasculature in the lateral area showing arteries, veins and capillaries (c, d). V – veins, A – arteries, C – capillaries. Scale bars are 5000 μm on (a) and (b); 250 μm on c, 50 μm on d.

the dura, blood vessels are mostly represented by veins. Vascular networks can be further analyzed with semi-automated tracing and quantitative metrics in 3D space. We showed that lymphatic vessels in NPH dura are located similarly to that in rodents: in the area of the superior sagittal sinus (SSS) and along the middle meningeal artery (MMA). They are also present in the major dural fold, tentorium cerebelli, which is underdeveloped in rodents. Unlike previously described in mice, these vessels are negative for LYVE-1 lymphatic marker but strongly positive for podoplanin. In the area of SSS, there is a large plexus of branching irregular blind-ended sacs with a wide range of diameters. Vessels in the MMA region have a different appearance. Two vessels always run along the veins flanking MMA that follow the artery branching. Our protocol also permits imaging of the extracellular matrix and the cells that reside in the dural environment.

Conclusions

We developed clearing, mounting and imaging protocols that permitted panoramic fluorescence-based microscopy of NPH dura. These new techniques are directly applicable to primate models of neurodegenerative diseases with a focus on the complex interplay between meningeal arteries, veins, and lymphatics.

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