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Scientific Correspondence

The fine anatomy of the perivascular compartment in the human brain: relevance to dilated perivascular spaces in cerebral amyloid angiopathy

Cerebral white matter hyperintensities (WMH) observed on magnetic resonance imaging (MRI), or low attenuation on computed tomographic scanning (CT), are the most frequent brain imaging finding in patients with small vessel disease or dementia. It has been assumed that WMH are due to arteriosclerosis or blood-brain barrier breakdown, though recently it was demonstrated that WMH have distinct molecular signatures in Alzheimer's disease (AD) where markers of Wallerian degeneration are present, compared to normal ageing [1]. Dilated perivascular spaces (PVS) are of particular interest for the study of interstitial fluid (ISF) dynamics because they are related to the intramural periarterial drainage (IPAD) of ISF and solutes along arterial basement membranes and can potentially be detected by MRI. MRI-visible dilated PVS have been described in the white matter of patients with AD or cerebral amyloid angiopathy (CAA) [2,3]. There are no detectable PVS around arteries in the cerebral cortex, even in pathological conditions, whereas PVS can dilate in the white matter and may indicate failure of drainage of ISF and solutes [4]. Recently we demonstrated in an elderly dog that arterioles are enveloped by one complete layer of leptomeninges in both grey and white matter, often with a second incomplete layer in the white matter, whereas venules have incomplete layers of leptomeningeal cells [5]. This suggests that arterioles in the white matter in young brains may have a distinct perivascular compartment formed between (i) the leptomeningeal layer adjacent to the side of tunica media facing side the parenchyma and (ii) the leptomeningeal layer adjacent to the glia limitans (astrocyte end feet) that potentially could be expanded to allow the formation of a dilated PVS. One limitation of establishing the underlying anatomy of the dilated PVS, both in CAA and more generally, has been the lack of fixed post-mortem material from large mammals, suitable for ultrastructural studies. Here, using biopsy tissue from the white matter of a patient with CAA and a young control patient with no signs of CAA or dementia, we identify by transmission electron microscopy (TEM) the exact anatomical location of the dilated PVS to further clarify IPAD mechanisms. TEM was performed following our standard protocols [6]. Briefly, fresh tissue was submersion fixed overnight in 3.4% formaldehyde plus 3% glutaraldehyde in 0.1M PIPES buffer, cut into 1 mm cube sections and processed for TEM [6]. Tissue blocks were trimmed and ultrathin 80-nm sections cut. The ultrathin sections were collected onto copper grids and viewed using a FEI Technai T12 electron microscope operating a EMSIS MegaView III digital camera and EMSIS image capture software (formerly iTEM software, Universal TEM Imaging platform, Soft Imaging System, Münster, Germany). A series of high power images of arterial walls (from n = 3 arteries/case) was acquired and then reconstructed as a montage using Adobe Photoshop CS6 with an automated photomerge script set to autolayout with image blend, vignette removal and geometric Distortion Correction.

Control tissue was obtained from a 26-year-old female patient undergoing frontal lobectomy for seizures due to an underlying cavernoma. Tissue was obtained after patient consent under ethical approval REC 12/NW/0794. Clinical neuropathology in Southampton General Hospital reported that the cortical samples from the white subcortical region showed evidence of reactive gliosis, with no other abnormality observed. Analysis of subcortical white matter at the posterior resection margin most distant from the cavernoma by TEM revealed arteries in the white matter with closely apposed layers of cells and their basement membranes (Figure 1A). A complete layer of endothelial cells with tight junctions and an intact basement membrane was observed. Layers of smooth muscle cells were separated by basement membranes from the endothelium and from the pial glial basement membrane. There were no spaces observed between the basement membranes of the smooth muscle and the pial glial basement membranes. Two layers of intact

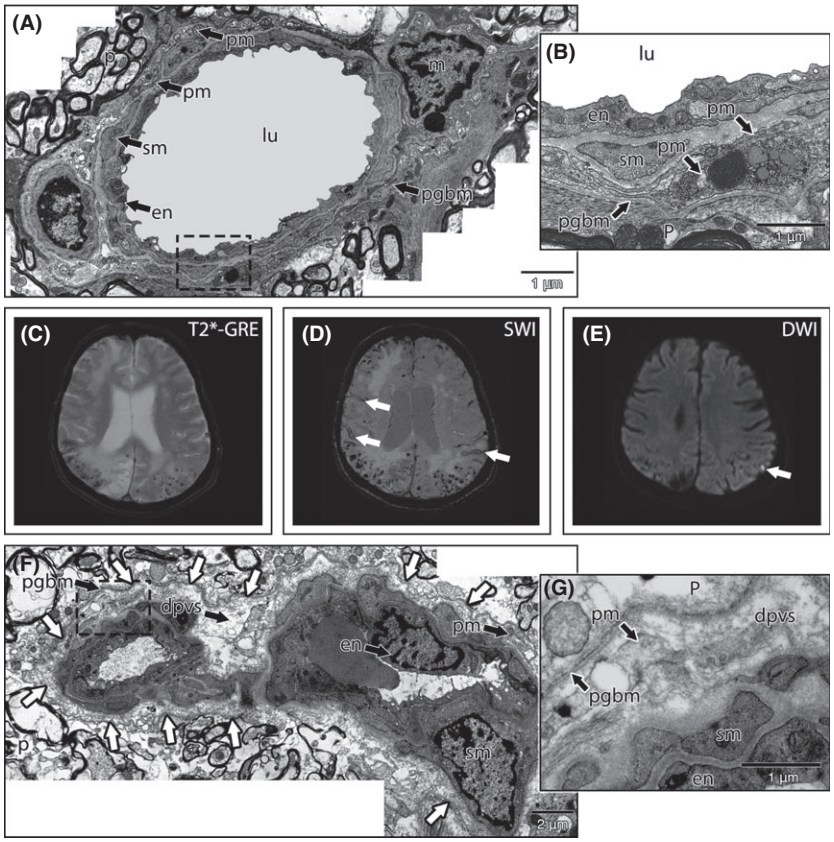

Figure 1. (**A**) Reconstructed montage of an artery from the posterior frontal subcortical white matter of a control patient. The endothelium (en), smooth muscle cells (sm) and pia mater (pm) appear normal. The pial-glial basement membrane (pgbm) is only expanded in the presence of macrophages (m). **(B)** High power of the vessel wall highlighted by the box seen in **(A)**. Note the two layers of pia mater (pm) with no dilated perivascular space. **(C–E)** MRI of a 68-year-old with rapidly progressing dementia. The ventricles are dilated **(C)**. Susceptibility-weighted imaging revealed multiple strictly lobar cerebral microbleeds (arrows) and widespread cortical superficial siderosis **(D)**. Diffusion-weighted imaging showed a small cortical hyperintense lesion in the left occipital region **(E)**. **(F)** Reconstructed montage of an artery from the right frontal subcortical white matter of a CAA patient. The endothelium (en) appears enlarged. There is a dilated perivascular space (dpvs) (white arrows) and expansion of the pial glial basement membrane (pgbm). The pia mater (pm) appears disrupted with only fragments visible. **(E)** High power of the vessel wall highlighted by the box seen in **(F)**. Note only one layer of pia mater (PM) surrounded by a dilated perivascular space (dpvs) is visible. Other abbreviations; smooth muscle (sm); parenchyma (p); lumen (lu).

pia mater were observed either side of the pial glial basement membranes (Figure 1**B**).

CAA tissue was obtained from a 68-year-old man who initially presented with rapidly progressive dementia, over several months. A brain MRI including T2*-weighted gradient echo (Figure 1**C**) and susceptibility-weighted imaging sequences (Figure 1**D**) showed multiple strictly lobar cerebral microbleeds and widespread cortical superficial siderosis. Diffusion-weighted imaging showed a small cortical hyperintense lesion in the left occipital region, suggesting an active occlusive vasculopathy (Figure 1**E**). As part of his investigation for treatable causes of dementia, he underwent a non--dominant frontal lobe brain biopsy. The right frontal lobe specimen was divided for diagnostic histology and TEM. Histological assessment showed severe CAA and frequent diffuse and mature amyloid beta (Aβ) plaques in the neural parenchyma. There was no evidence of vasculitis. TEM revealed arteries in the white matter with thick and distorted walls, with an enlarged PVS. The PVS had an intact pial-glial basement membrane separating it from the surrounding parenchyma (Figure 1**F**). One layer of pia mater was observed adjacent to the pial-glial basement membrane. This layer appeared highly disrupted with only fragments visible (Figure 1**G**).

Dilatation of PVS occurs in the white matter of the cerebral hemispheres, in the midbrain and in the grey

matter of the basal ganglia [7]. The potential space between the outer aspect of an artery wall and the brain parenchyma, can be expanded experimentally by the intracerebral injection of particles [8]. As cortical arteries enter the surface of the cerebral cortex, the pia mater is reflected from the surface of the brain on to the vessels in the subarachnoid space, thus separating the subarachnoid space from the cerebral cortex [8]. In the present study we confirmed that there is no PVS around cortical arteries in the normal brain as layers of smooth muscle, basement membrane and one layer of leptomeninges are all compressed together. The CAA patient is not age-matched to the control, so therefore both ageing and CAA may have destroyed the pia mater adjacent to the pial-glial basement membrane resulting in a dilated PVS. This arrangement may provide the anatomical substrate for fluid to accumulate as MRI-visible PVS, possibly contributing to WMH. Furthermore, the control tissue was from a patient with a vascular lesion, although care was taken to use tissue that had normal appearance, from the wide resection. Based on our observations we propose a mechanism by which the IPAD pathways become compromised in the cortical arteries, such that fluid from the white matter is unable to drain efficiently with increasing age, or after immunization against Aβ, resulting in axonal destruction and dilated PVS [1,9,10].

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Author contributions

ROC, AV and DJW designed the study. MM-S performed the electron microscopy. DB provided the control tissue. SB and JH performed the histopathology. All authors contributed to the interpretation of data and writing the manuscript.

Conflict of interest

The authors confirm that this article content has no conflicts of interest.

Ethical Approval

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M. MacGregor Sharp*
D. Bulters*
S. Brandner†
J. Holton†
A. Verma‡
D. J. Werring†[1]
R. O. Carare*[1]

**Faculty of Medicine, University of Southampton, Southampton, UK, †Department of Brain Repair and Rehabilitation, Stroke Research Centre, UCL Institute of Neurology and the National Hospital for Neurology and Neurosurgery, London, UK and ‡Biogen, Cambridge, MA, USA*
[1]Contributed equally to the study.

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