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MINI-SYMPOSIUM: White matter damage in dementia

Endothelial Cells and Human Cerebral Small Vessel Disease

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Abstract

Brain endothelial cells have unique properties in terms of barrier function, local molecular signaling, regulation of local cerebral blood flow (CBF) and interactions with other members of the neurovascular unit. In cerebral small vessel disease (arteriolosclerosis; SVD), the endothelial cells in small arteries survive, even when mural pathology is advanced and myocytes are severely depleted. Here, we review aspects of altered endothelial functions that have been implicated in SVD: local CBF dysregulation, endothelial activation and blood–brain barrier (BBB) dysfunction. Reduced CBF is reported in the diffuse white matter lesions that are a neuroradiological signature of SVD. This may reflect an underlying deficit in local CBF regulation (possibly via the nitric oxide/cGMP signaling pathway). While many laboratories have observed an association of symptomatic SVD with serum markers of endothelial activation, it is apparent that the origin of these circulating markers need not be brain endothelium. Our own neuropathology studies did not confirm local endothelial activation in small vessels exhibiting SVD. Local BBB failure has been proposed as a cause of SVD and associated parenchymal lesions. Some groups find that computational analyses of magnetic resonance imaging (MRI) scans, following systemic injection of a gadolinium-based contrast agent, suggest that extravasation into brain parenchyma is heightened in people with SVD. Our recent histochemical studies of donated brain tissue, using immunolabeling for large plasma proteins [fibrinogen, immunoglobulin G (IgG)], do not support an association of SVD with recent plasma protein extravasation. It is possible that a trigger leakage episode, or a size-selective loosening of the BBB, participates in SVD pathology.

INTRODUCTION TO CEREBRAL ENDOTHELIAL CELLS

The endothelium that lines the entire vascular tree is a thin, flat cellular monolayer. Endothelial cells are phenotypically plastic, sensitive to injury and central to several major vascular pathologies (32, 40, 56). All vascular endothelial layers share the following functions.

- To allow ready diffusion of oxygen and CO_2 across capillary walls.
- To form a physical barrier, preventing escape of cells, platelets and plasma proteins from an intact vessel.
- To prevent thrombus formation in healthy tissue and to promote thrombosis following tissue injury.
- To act as a gatekeeper in the inflammatory process, signaling blood-borne leukocytes to adhere, migrate and invade a localized region of tissue injury.
- To lead vessel sprouting and revascularization, in rapid response to angiogenic stimuli, under the influence of cell signaling molecules, such as vascular endothelial growth factor (VEGF)-A and angiopoietin.
- In small contractile vessels, endothelial-derived nitric oxide reduces myocyte contractile tone and hence augments vessel diameter and distal blood flow.
- Other paracrine roles include release of the vasoconstrictor peptide endothelin-1.

In addition to these properties common to all endothelia, brain endothelial cells have the following specialized functions.

- The blood–brain barrier (BBB). This is a combination of exceptionally tight junctions between adjacent endothelial cells, combined with an array of transporter proteins in endothelial plasma membrane and other cellular players (notably astrocytic foot processes, pericytes and microglia) (1, 103). These mechanisms together exclude potentially harmful solutes from brain tissue and from the endothelial cytoplasm.
- As a corollary of the BBB, metabolic fuel substrates for brain metabolism—most notably glucose—have to enter brain tissue entirely via transport proteins in the luminal membrane of endothelial cells.
- Brain endothelial cells have a distinctive expression profile, relative to endothelia in other vascular beds (18, 66, 69).

• Endothelia in brain small arteries (and to some degree capillaries) participate in the autoregulation of local cerebral blood flow (CBF), which is a special feature of the brain vasculature (6, 8).
• Brain endothelial cells regulate, and interact with, other mural components (eg, basal lamina) (15).

WHAT IS SMALL VESSEL DISEASE (SVD)?

Cerebrovascular SVD comprises a number of distinct histopathological entities, including (i) "arteriolosclerosis"; (ii) "lipohyalinosis" (and the related condition of "fibrinoid necrosis"); and (iii) "small vessel atherosclerosis" (25, 49, 50, 68). There is an overlap in their clinical, radiological and etiopathogenic features, and the distinction (particularly in studies without autopsy follow-up) is not always clear-cut. In this review, we will focus on "arteriolosclerosis," the form of SVD that correlates best with the diffuse radiological changes in white matter known as "leukoaraiosis" (48, 50, 68, 80). In the old literature, this was sometimes referred to, in its severe form, as Binswanger's disease (22, 101). Arteriolosclerosis is also associated with lacunar infarcts (17, 49, 68) and with vascular contributions to cognitive impairment, gait disturbance and dementia (19, 30, 82).

Arteriolosclerosis (also referred to as "hyaline arteriolosclerosis" or "simple small vessel disease") is the most common form of SVD and has well-known associations with aging, hypertension and diabetes (49, 50, 68). Genotyping studies have thus far revealed no strong genetic basis for sporadic SVD (44), including prospective tests for linkage with the genes encoding endothelin-1 (ET-1) and endothelial nitric oxide synthase (eNOS) (31, 36). For review, see (11). By contrast, CADASIL (cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a monogenetic form of SVD (much less common than sporadic SVD). The causal gene for CADASIL is *NOTCH3* and SVD pathology manifests in early adulthood. In adults, *NOTCH3* expression is primarily within myocytes. Clinical studies of CADASIL support the notion of a relatively intact endothelium in the presence of SVD vasculopathy (62, 71, 72, 78).

Arteriolosclerosis is characterized by smooth muscle loss and collagenization of small arteries and arterioles (approximately 40–150 μm outer diameter) (7, 49–51, 61). It can be considered the "smallest" of common cerebral small vessel diseases, in that somewhat larger-diameter vessels are affected by lipohyalinosis (40–300 μm) and small vessel atherosclerosis (200–800 μm) (50).

In arteriolosclerosis, the vessels become "an elongated, tortuous and pauci-cellular hyaline tube with a variable degree of stenosis" (49). The degree of stenosis can be measured by the sclerotic index [defined as (OD-ID)/OD, where ID and OD represent vessel inner and outer diameters, respectively], which is considered an established measure of single vessel SVD vasculopathy (13, 29, 34, 63, 64). Although the endothelium appears microscopically intact in arteriolosclerosis, a number of studies suggest that endothelial function is disturbed, and indeed, that this disturbance may be primary in the pathogenesis of SVD.

ENDOTHELIAL CELLS IN SVD

A long-standing observation is that even in severe SVD, with almost complete loss of myocytes and other mural cells, the endothelial layer remains intact (13, 49) (see Figure 1). We and others have noted robust endothelial labeling with histological markers in cases with severe SVD. This includes endothelial

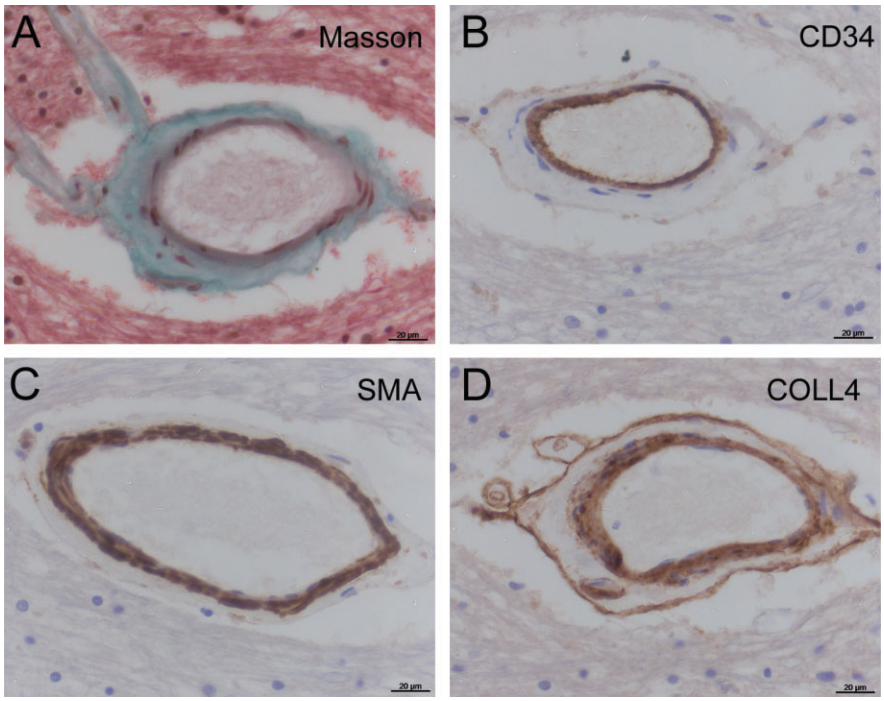

Figure 1. Small vessel disease vasculopathy (arteriolosclerosis) in a small artery of deep subcortical white matter. A. With Masson trichrome stain, fibrous connective tissue (green) is clearly seen in medial layer of the vessel wall. Counterstains show cytoplasm and parenchymal tissue (pink) and nuclei (black). B. Neighboring section, immunolabeled with endothelial marker CD34 (brown). Despite medial fibrosis and depletion of myocytes from the vessel wall, endothelial layer is intact. C. Myocytes within the medial layer are labeled with smooth muscle actin (SMA). D. "Double layer" labeling with collagen-4 (COLL4). In B–D, the chromogen is diaminobenzidine (brown) and nuclear chromatin is counterstained with hematoxylin (blue). Deep frontal white matter, male aged 77 years; SVD severity score 6, see Ref. (82). Scale bars: 20 μm.

labeling for CD34, CD31, factor VIII, glucose transporter 1 and *Ulex* lectin (13, 27, 29, 65, 100, 101). This paradoxical survival of the endothelium is also evident in patients with CADASIL (13).

DYSREGULATION OF CBF OCCURS IN SVD

Measurements of CBF in living subjects suggest a reduction in baseline CBF within deep white matter, and impaired CBF autoregulation, in people with symptomatic SVD (57, 67). Endothelial nitric oxide signaling is an important factor in local CBF regulation and has been used as a marker to demonstrate endothelial dysfunction and decreased vasodilation in response to external stimuli such as hypercapnia or salbutamol in patients with lacunar infarction, compared with controls [eg (16, 33)]. For a detailed discussion, see a recent systematic review (59). As endothelial nitric oxide signaling is an important factor in local CBF regulation, this further suggests that endothelial dysfunction is a pathogenic factor in SVD.

Immunohistological labeling of a large population cohort of donated brains showed upregulation of hypoxia-related markers within white matter lesions (24). These markers included HIF-1α, VEGFR2 and neuroglobin. Evidence of vessel wall thickening was also apparent in these white matter lesions (24). This is an important evidence of the association between SVD vasculopathy and diffuse ischemic white matter lesions seen on magnetic resonance imaging (MRI) scans.

Several studies have used measurements in other vascular beds to report on endothelial changes in cerebral SVD. These include the skin (77), the kidney (29, 94, 95) and the sublingual vessel network (60). If cerebral SVD is indeed a manifestation of a generalized systemic change (87), then these approaches may well be revealing.

SVD MAY BE ASSOCIATED WITH ENDOTHELIAL ACTIVATION—BUT WHERE?

Endothelial activation refers to the change in the expression of surface markers within endothelia in response to a noxious stimulus or other challenges (28). Numerous laboratories worldwide have assayed serum biomarkers of endothelial activation in patients with SVD, in a range of clinical settings [eg (14, 21, 26, 36–38, 53, 58, 74, 90, 96)]. These circulating markers of endothelial activation include intercellular adhesion molecule-1 (ICAM-1), soluble thrombomodulin (sTM), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor and others. In a large prospective population study of type 2 diabetic patients, serum ICAM-1 was positively associated with white matter lesion progression (a surrogate for SVD) over a 6-year study period (58). This association survived correction for other risk factors and for baseline lesion load, and was considered strongly supportive of a role for endothelial activation in SVD. Serum biomarker studies have been recently reviewed (46, 47, 84).

With these serum markers in mind, we tested for local expression of three markers (ICAM-1, thrombomodulin and IL-6) in vascular endothelium of small arteries of human deep gray matter, relevant to SVD (29). We examined donated brains from older people (age 65 years or older) with neuropathologically defined SVD (19) and compared these with aged control subjects where SVD was graded as "absent" or mild" (based on criteria related to vascular pathology and parenchymal effects of SVD) (19, 29). All sections were examined by at least two observers, blinded to clinical details and SVD grading, and sclerotic index was also measured in small arterial vessels within the size range of interest (20–200 μm outer diameter). We hypothesized that endothelial activation would be more evident in small arteries of SVD cases than in aged controls, and that this would be manifest as greater prevalence of endothelial ICAM-1 and IL-6, with depletion of thrombomodulin. In fact, we saw no association of ICAM-1 or IL-6 with SVD grade or sclerotic index, and far from being depleted, endothelial thrombomodulin of small arteries was augmented in SVD cases relative to aged controls (29).

These results indicated to us that cerebral endothelial activation is not a feature of SVD (29). Further, as thrombomodulin is a potent anticoagulant, it seemed unlikely that local thrombotic events are primary in SVD-related stroke events. Thrombotic occlusion is not a recognized common feature of arteriolosclerosis (50).

Histopathological data indicating that local arterial endothelium is not activated in SVD (29) can be reconciled with serum biomarker data, suggesting an association with endothelial activation (listed previously). As concluded in a recent meta-analysis (84), the recorded serum levels of endothelial markers may derive from any vascular bed (ie, not necessarily brain endothelium). The notion of heightened circulating markers of endothelial activation in patients with SVD is consistent with the hypothesis that cerebral SVD is part of a systemic vasculopathy in which the profile of endothelial activation differs between organs (87).

A related theme is the suggestion that SVD is associated with "neuroinflammation" (29, 74, 75). While cerebral tissue does not undergo conventional inflammation in SVD (85), activation of microglia is clearly a feature of the diffuse white matter parenchymal lesions associated with SVD (23, 24). We and others have used CD68-labeled cells (clone PGM1) with ameboid morphology as an indicator of cerebral parenchymal injury. Clearly, there are subfamilies of microglial cells, differing in phenotype and pathophysiological role (70, 79, 85). These may provide useful clues to the pathogenic process of SVD vasculopathy and its parenchymal impact.

BBB impairment occurs in SVD—true or false?

The notion that local BBB impairment and arterial SVD are associated—and even causally linked—has been raised in many histopathology studies (2, 3, 55, 88) and was well-articulated in an influential review in 2003 (97). This is an attractive concept to explain the SVD-related diffuse white matter lesions seen on clinical T_2-weighted MRI scans that resemble patterns of diffusion. Defective endothelial cells are central to this idea, with an imperfect BBB allowing harmful plasma components to escape the lumen, leading to myocyte damage in the artery wall and diffuse tissue damage in the surrounding parenchyma (with edema, ischemia and cytotoxicity all playing a role). This notion has been addressed in biochemical analyses of serum proteins in cerebrospinal fluid and is supported in some studies but not others [see a

recent meta-analysis (20)]. Likewise, several radiological studies have examined partition of intravascular contrast agent into brain tissue, as a marker of BBB dysfunction, in patient groups with SVD, lacunar stroke and/or VCID (20, 86, 89, 98, 99). These radiological mapping methods require dedicated software algorithms, developed in several expert centers, to process the MRI scans before and after contrast injection. Some of these studies suggested an association of contrast agent extravasation with disease (86, 89), while other studies did not (20, 98, 99). Among neuropathology reports on brain tissue, labeled for extravasation of vascular markers, some have found a positive association between SVD severity and extravascular plasma proteins (eg, fibrinogen, albumin or immunoglobulin G (IgG)] (88, 91), while others found no such association (93, 100). We have recently examined a large collection of donated brains of older people (age 65 years or older) with minimal Alzheimer's disease pathology (Braak stage II or less) (12, 82). Within subcortical white matter and putaminal gray matter (two SVD-prone anatomical regions), extravascular labeling of fibrinogen (in cells and extracellular tissue) was estimated in a blinded fashion, using machine-based morphometry in parallel with categorical scoring by a neuropathologist. We found no association of fibrinogen with SVD severity, in either anatomical location (12).

Observations that are difficult to reconcile with a BBB-SVD causal link are the findings that other pathological states where plasma extravasation clearly is a feature, such as multiple sclerosis and neuromyelitis optica, do not involve SVD-like arteriopathy. In addition, examination of histopathological material suggests that many of the small vessels exhibiting extravascular labeling of cells and surrounding tissue are veins. In two well-powered studies where quantitation was carried out in a blinded fashion, labeling of extravascular plasma proteins did not support an association of BBB dysfunction with SVD (12, 100) . A possible "trigger" event, where brief, local BBB "loosening" has a role in SVD pathogenesis, is difficult to exclude and to test.

BBB formation is not intrinsic to cerebral endothelial cells but is controlled by local cell signaling molecules, such as VEGF (4, 5) and transforming growth factor-β (TGF-β) (54). Recent elegant studies of transgenic mice that lack effective pericyte coverage of capillaries suggest an influence of pericyte-endothelial cross talk on BBB function (9, 10). This influence of pericyte signaling on the BBB is borne out in a small study of human tissue comparing Alzheimer's disease cases with aged controls (81).

DIETARY FACTORS, ENDOTHELIA AND SVD

Several clinical studies hint at the influence of dietary factors in SVD. These include diabetic status (92), plasma glycemic state (33, 104) and plasma levels of the non-essential amino acid homocysteine (37, 38, 43, 53). Moderately elevated plasma homocysteine concentrations (10–20 μmol/L) were associated with increasing leukoaraiosis severity (38, 43). This statistical association was lost after correction for circulating endothelial markers (ICAM-1 and thrombomodulin), suggesting that the interaction between plasma homocysteine and white matter lesions may be endothelium-dependent (38). How these nutritional factors relate to SVD vasculopathy, and its effects on brain parenchymal tissue, seem an area that may be fruitful for future research.

CONCLUSION

The endothelial cells that line the small arteries deep within our brains are integral to the SVD process, although much molecular detail remains to be understood. They may also be an important first point of contact for any preventive or therapeutic strategy.

SEARCH STRATEGY

For this review, we incorporated a PubMed search for publications in English whose title or abstract contained the following terms: endotheli* AND (brain or cerebr*) AND (small_vessel_disease or arteriolosclerosis or lipohyalinosis or fibrohyaline or hyalin*) (search date: August 13, 2014). This search retrieved 103 publications. Abstracts were viewed by two authors (AHH and ATO) and reviews, animal studies and irrelevant diseases were excluded, leaving n = 37 primary publications (14, 16, 21, 27, 29, 31, 33, 35–39, 41–43, 45, 52, 53, 58, 60, 62, 65, 71–78, 83, 89, 90, 94–96, 102, 104). Bibliographies of these papers, and of related reviews, were also searched.

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