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Chronic cerebral hypoperfusion and dementia

Kenichiro Yata, MD, Hidekazu Tomimoto, MD

Department of Neurology, Mie University Graduate School of Medicine, Tsu, Mie, Japan

Address all correspondence to:

Kenichiro Yata, M.D.

Department of Neurology, Mie University Graduate School of Medicine

2-174 Edobashi, Tsu, Mie 514-8507, Japan

Tel: +81-59-232-1111; Fax: +81-59-231-5212

E-mail: ken-yata@mbk.nifty.com

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Short running title: Small vessel disease and neurovascular unit

Abstract

"Cerebral small vessel disease" is a general term featuring a group of disease conditions with characteristic lesions affecting mainly small vessels in the brain, such as Binswanger's disease, leukoaraiosis (LA), and lacunar infarctions. Cerebral small vessels consist of a series of blood vessels, which originate from the pial arteries on the surface of the brain, and branch into arterioles, capillaries and postcapillary venules. Each of the blood vessels has a distinct structure and function. Blood-brain barrier (BBB), which does not exist in the other organs, functions in the brain. Dysfunction of BBB is thought to be a major cause of cerebral small vessel diseases. Recent findings have shown that maintenance of BBB is kept by various types of cells with different nature, such as vascular endothelial cells, astrocytes, and pericytes which work collaboratively as a neurovascular unit. Currently, larger vessels at the arteriolar level have been studied intensively; however, the pathologic condition of the neurovascular unit at the capillary level still needs to be elucidated.

The bilateral carotid artery stenosis (BCAS) model simulates chronic cerebral hypoperfusion, formation of white matter lesions, and cognitive impairments seen in humans. Using this model, we found microcirculation disturbance especially in the postcapillary venule, and postulated it as a final step leading to white matter lesions and cognitive impairment. Taken together, we suggest that chronic cerebral hypoperfusion plays a pivotal role in the pathogenesis of cerebral small vessel diseases.

Key Words: Chronic cerebral hypoperfusion, neurovascular unit, small vessel disease, two-photon laser scan microscopy, vascular dementia,

Introduction

Binswanger's disease, white matter lesions, and lacunar infarctions are mainly due to small vessel pathologies, and their causative mechanism is considered as markedly different from that of cerebral thrombosis and embolisms affecting larger vessels. In recent years, those diseases have been collectively referred to as cerebral small vessel diseases (SVD), and microcirculation and small blood vessels in the brain have been the focus of related research.[1] Cerebral SVD exhibits a wide range of clinical

symptoms, such as vascular dementia and parkinsonism. Moreover, they have also been linked to Alzheimer's disease in recent years.

Blood vessels affected in cerebral SVD include pial arteries, arterioles, capillaries and postcapillary venules, encompassing the vessels originating on the brain surface and penetrating into the brain parenchyma. Each of the blood vessels has a distinct structure and function, but commonly constitutes blood-brain barrier (BBB). The maintenance of BBB is kept by various types of cells which work collaboratively as a neurovascular unit (NVU). Dysfunction of the BBB is thought to be one of the major contributors to the pathogenesis of cerebral SVD.[2] However, little is known about how NVU maintains and manages the BBB. SVD pathology is induced by long-lasting and multi-step cascade including BBB disintegration and chronic cerebral hypoperfusion. In animal models, white matter lesions can be directly induced by chronic cerebral hypoperfusion, and therefore, it is thought to be one of the last step of this cascade.

1. Structural specificities of cerebral blood vessels

Arteries all over the body are generally composed of a three-layer structure, the intima (vascular endothelial cells), the tunica media (smooth muscle cell layer), and the

adventitia. The intima and the tunica media are separated by the internal elastic lamina, and the tunica media and the adventitia by the external elastic lamina. Arteries in the brain are characterized by lack of the external elastic lamina which disappears after the artery penetrate through the dura, and is covered by a thin layer of leptomeningeal cells.[3] The specific property of cerebral blood vessels is that they are part of the BBB, and BBB dysfunction is thought to be involved in the pathogenetic mechanism of SVD.

Pial arteries and leptomeningeal arteries

Large blood vessels at the surface of the brain branch into small vessels of 40 to 900 μm in diameter towards the brain parenchyma . These blood vessels have a similar three-layer structure with the adventitia which are devoid of connective tissue, but supported gently by the arachnoid membrane inside the cerebrospinal fluid. The small blood vessels (100 to 200 μm) at the surface of the brain are innervated by a large number of perivascular nerves.[4,5]

Arterioles and penetrating arteries

Pial arteries at the surface of the brain give out branches perpendicularly to the brain surface, penetrate into the brain parenchyma, and are converted to arterioles of

approximately 50 μm in size.[6,7] This group of blood vessels, directed towards the brain parenchyma, are divided into a group of blood vessels heading from the cortical pial arteries towards the area around the lateral ventricle (superficial perforators) and a group of blood vessels heading from the large blood vessels at the base of the brain towards the basal ganglia (deep perforators). In arterioles, the internal elastic lamina becomes discontinuous; endothelial cells and smooth muscle cells are in direct contact with each other to form the myoendothelial gap junction

In addition, the outermost layer of blood vessels is covered by a layer of leptomeningeal cells, and the Virchow-Robin space (VRS) is formed between this layer and the glia limitans of the cerebral parenchyma.[4,8]

Capillaries

Capillaries have a diameter of 10 μm or less. They are composed of non-fenestrated endothelial cells, basal membrane and pericytes, and are surrounded by glial cells without a leptomeningeal cell layer.[9] Tight junction is present at the margin between the endothelial cells and surrounded by pericytes. These pericytes express α-smooth muscle actin, which have a potential for contraction. They are involved in formation and function of the BBB as well as regulation of blood flow by contraction of

α-smooth muscle actin, and have been the focus of attention as part of the neurovascular unit. Pericytes are covered by the glial cell endfeet from the outside to form the BBB, through which substances are exchanged between blood and brain tissue.

Previous findings have revealed that in order to manage and maintain the BBB, various types of cells work in coordination as an NVU. The concept of NVU was proposed recently to allow the understanding of brain function from a broader perspective.[10] Until then, neurons had been the focus of attention. However, based on this concept, different types of cells other than neurons, such as vascular endothelial cells, astrocytes, and also the extracellular matrix (ECM) work in a coordinated manner, and thereby, the ensemble formed is regarded as a single unit to maintain brain function.

Postcapillary venules

Capillaries connect to each other and form postcapillary venules. Those postcapillary venules, which are the beginning of veins, are believed to be the sites where white blood cells infiltrate into the brain parenchyma.[8]

2. Vascular disorders in small vessel diseases

Pathological findings associated with SVD include degenerative changes in the walls of small arteries and arterioles. The degenerative changes associated with hypertension have 3 different subtypes, namely small vessel arteriosclerosis/atherosclerosis, lipohyalinosis and arteriolosclerosis.[11-13] Pathological findings showing a leakage of blood components resulting from repetitive damage in the BBB have been attributed to the pathogenesis of such vascular disorders. The lesions initially appear as small vessel arteriosclerosis and lipohyalinosis, spreading to the relatively large, penetrating branch arteries, which encompass the area of the basal ganglia. In the next stage, arteriolosclerosis and lipohyalinosis are found in arterioles within the white matter. In parallel, small vessel arteriosclerosis are found for leptomeningeal arteries. Large blood vessels at the surface of the brain do not usually present abnormalities.[14]

Small vessel arteriosclerosis/atherosclerosis

This is found at the bifurcation (junctional atheroma) of large blood vessels (200-800 μm) on the brain surface, as well as at the proximal portions of the

penetrating branches (microatheroma). The initial changes consist of thickening of the intima or accumulation of lipid inside. This causes subsequently proliferation of the intima, rupture of the internal elastic lamina and accumulation of lipid-laden macrophages. Rupture of the atheroma may causes occlusion of the vessels and distal embolism. These findings are no different from those found in large blood vessels.[11,15]

Fibrinoid necrosis/ lipohyalinosis

This is found in some parts of vascular walls of arteries measuring 40 to 300 μm in size inside the brain parenchyma. As the first step in the pathogenesis of fibrinoid necrosis/ lipohyalinosis, a long-term high arterial pressure causes damages of the BBB and leakage of plasma proteins, which lead to accumulation of a hyaline-like substance composed of fibrin and plasma proteins (fibrinoid necrosis).[16] In the initial stages, fibrinoid necrosis is not accompanied by inflammation. In the repair process, the hyaline-like substance is replaced by collagen, the vascular wall becomes uniform and unstructured, and in rare occasion, lipid-containing cells may be found (lipohyalinosis).

Arteriolosclerosis

This is the most frequent vascular pathological finding in SVD, and it consists of degenerative alterations that characterize this disease.[11,16] This finding can be observed in small blood vessels (40 to 150 μm). The aforementioned fibrinoid necrosis/ lipohyalinosis causes a partial degeneration of the vessel wall, but in arteriolosclerosis, the vessel wall shows a concentric changes which may affect its entire circumference. It is accompanied by hyaline thickening of the vascular wall (fibrohyalinosis), degeneration of smooth muscle cells, and collagenous fibrosis of the internal elastic lamina that causes a concentric narrowing of vessel lumens. However, it rarely leads to a complete vascular occlusion.

Capillary loss and string vessel formation

When blood flow in the capillary is interrupted, capillary degeneration may occur immediately.[17-19] Apoptosis of the vascular endothelial cells and incorporation by macrophages have been reported previously.[20] String vessel is thought to be a remaining basal membrane after loss of endothelial cells during the regression process. Conversely, capillary growth has been reported to occur during hypoxia.[21,22]

NVU is thought to play a major role in the management and maintenance of capillaries; however, little is known about how the regression mechanism occurs, or whether there is capillary growth in NVU

3. Chronic cerebral hypoperfusion and vascular dementia

Vascular dementia is a heterogeneous syndrome, mainly consisting of SVD with dementia, multi-infarct dementia (MID), and strategic single infarct dementia. SVD with dementia is the most prevalent form of vascular dementia, and consists of multiple lacunar infarctions and white matter lesions.[23] It has been hypothesized that chronic cerebral hypoperfusion owing to arteriosclerosis causes these pathological changes. This view is strengthened by the terminal blood supply and by the lack of anastomosis between vessels in these deep subcortical white matters.[24] However, the relation between chronic cerebral hypoperfusion and dementia has not been completely elucidated. Moreover, improvement of cognitive deficits after resolution of chronic cerebral hypoperfusion has been inconclusive in the patients with arteriovenous malformation[25] or carotid stenosis/occlusion[26-28]

In recent years, accumulating epidemiological evidence has indicated that vascular risk factors, such as hypertension, diabetes, dyslipidemia, and adiposity at midlife, enhance the risk of Alzheimer disease (AD), [29] and coexistence of brain vascular pathology accelerates cognitive decline in AD. Moreover, heart disease such as valvular disease, atrial fibrillation, and congestive heart failure, all of which seem to induce chronic cerebral hypoperfusion, may also increase the risk for AD.[30] In animal studies, chronic cerebral hypoperfusion accelerates AD pathology, including amyloid beta aggregation and cognitive dysfunction.[31, 32] Amyloid-beta aggregation in turn may enhance inefficient microcirculation and cause BBB disruption, indicating a vicious cycle between chronic cerebral hypoperfusion and AD pathology.

Efforts aimed to elucidate the pathological mechanisms of SVD has been hampered by lack of an appropriate animal model that can fully recapitulate the pathological condition associated with SVD.[33] Stroke-prone spontaneously hypertensive rat models simulate arteriolar changes, however, they only exhibit mild and variable degrees of white matter lesions. Shibata et al. have developed a mouse model of chronic cerebral hypoperfusion (bilateral common carotid artery stenosis model: BCAS), which is induced by using external microcoils with varying inner diameters from 0.16 mm to

0.22 mm (Fig 1A).[34] The appropriate strain is limited to C57Bl/6, because this strain has the most poorly developed posterior communicating arteries. The degree of chronic cerebral hypoperfusion depends on the inner diameter of the microcoils (Fig 1B), with a diameter of 0.16 mm or less to be the threshold for mortality, as well as damage the gray matter such as the hippocampus(Fig 1C) . In this model, where hypoperfusion is induced by a 0.18 mm micorcoil, white matter lesions, similar to those found in humans, could be reproduced after cerebral hypoperfusion for 1month (Fig 1C, D). [34]At this point, the hippocampus or other gray matter is not damaged. However, the damage of hippocampus is revealed after cerebral hypoperfusion for 5-6 month.[35] Furthermore, this mouse shows the selective memory impairment. [35,36] Working memory, which may be attributable to damage of the frontal-subcortical circuits (white matter lesions) is selectively impaired without reference memory disturbance after cerebral hypoperfusion for 1month.[36] However, reference memory which is relied on the integrity of the hippocampus is also impaired after cerebral hypoperfusion for 5-6 month. [35]

This model clearly indicates that chronic cerebral hypoperfusion is the major causative mechanism of white matter lesions and cognitive abnormalities. However, it is apparent that this model does not cause direct damage to small blood vessels, and remains elusive

whether microcirculations is altered or not. Therefore, we further assessed microcirculation under chronic cerebral hypoperfusion using two-photon laser scan microscopy (Fig 2). This method allowed a detailed observation of arterioles on the brain surface to a depth of 700 μm (Fig 2A). Furthermore, an overall picture of the NVU allowed us to understand the roles of various types of cells at the capillary level (Fig 2B). For example, astrocytic foot process could be observed stretching towards the vascular wall, suggesting a close relationship to microcirculation (Fig 2C). This observation further underscores the fact that astrocytes release arachidonic acid products into the perivascular space, which is believed to cause an influx of calcium into the foot process, and consequently could lead to vasoconstriction.[37] After inducing BCAS, we could not detect any morphological changes in any of the cellular component s of the NVU. However, interestingly, blood flow velocity was markedly decreased over a prolonged period (Fig 2D), and in the pial arteries and veins, rolling and adhesion of leukocytes was observed (Fig 2E). Furthermore, capillary plugging due to leukocytes was frequently found in deep cortical capillaries (Fig 2F). Taken together, our data indicate that leukocyte activation may play a critical role in microcirculation impairment under chronic cerebral hypoperfusion.

4. Conclusion

In cerebral SVD, damage at the arteriolar level has been extensively studied. However, to the best of our knowledge, little is known about microcirculatory changes at the capillary level. In future studies, it is required to focus on the mechanism of damage to the BBB at the capillary level, and the NVU function which ensures the management, maintenance, and repair of the BBB. Moreover, the BCAS model has a microcirculation disturbance at the capillary level as well as white matter lesions and cognitive impairment, suggesting that chronic cerebral hypoperfusion is the major causative mechanism of white matter lesions and cognitive decline. Furthermore, the restoration of microcirculation failure may be an effective therapeutic intervention for cognitive dysfunction.

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Figure legends

Figure 1 Bilateral carotid artery stenosis (BCAS) model

Fig 1A. Stenosis was induced by applying a microcoil outside the common carotid artery. The severity of stenosis was controlled by changing the diameter of the microcoil.

Fig 1B. Rt graph showing the mortality rate in mice with varying inner diameter-microcoils. The threshold for mortality rate was 0.16 mm in diameter. Lt graph showing cerebral blood flow changes with varying inner diameter of microcoils.

Fig 1C. A microcoil, with an inner diameter of 0.18 mm, induced white matter (WM) lesions; however, it did not cause any damage to the hippocampus. A microcoil with an inner diameter of 0.16 mm was found to cause changes in the hippocampus. H&E: Hematoxylin and eosin

Fig 1D. Graph showing the relationship between the diameter of the microcoil and white matter (WM) lesions (green circle 0.16 mm, blue circle 0.18 mm, red circle 0.20 mm, and yellow circle 0.22 mm). White matter lesions appeared 2 weeks after surgery.

Fig 2 Cerebral vascular structure and neurovascular unit (NVU) observed using two-photon laser scan microscopy

Fig 2A. Images showing overall distribution of blood vessels, from the brain surface to the deeper regions (seen from above and laterally). The images clearly show blood vessels entering the brain parenchyma vertically from the brain surface.

Fig 2B. Image showing branches of arterioles in deep regions of the brain. It shows the presence of a large number of astrocytes and pericytes, and the formation of the neurovascular unit (NVU).

Fig 2C. Magnified image of the bifurcation. The image shows astrocytes stretching their foot processes to the vascular wall.

Fig 2D. Repetitive line scans were performed along the central longitudinal axis (the white line in a reference image) of the vessel. Linear shadows produced by non-fluorescent erythrocytes within the plasma stream permitted computation of vessel flow velocity, which was proportional to the slope $\Delta x/\Delta t$. White lines in line scan images indicates the flow velocity, respectively.

Fig 2E. Microcirculation in a pial small vein. Arrows indicate the rolling and adhesion of leukocytes on the vessel wall.

Fig 2F. Microcirculation in deep cortical capillaries. Arrow indicate the site of capillary plugging by a leukocyte.

Figure 1. Bilateral common carotid artery stenosis (BCAS) model

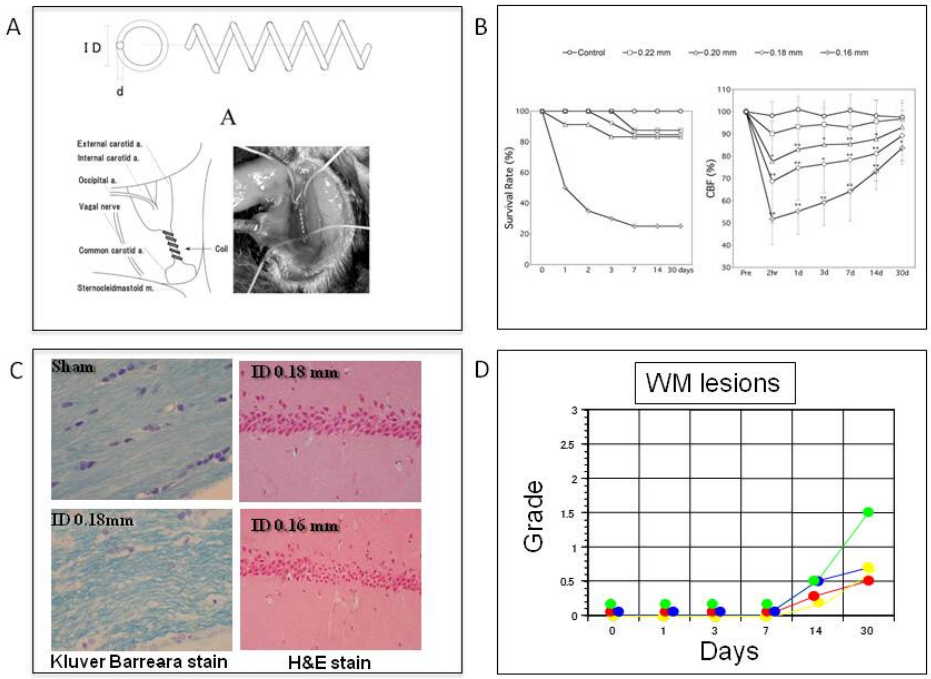

Figure 2. Cerebral vascular structure and neurovascular unit (NVU) observed using two-photon laser scan microscopy