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Pathogenesis of Leukoaraiosis: A Review

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<http://dx.doi.org/10.5772/63655>

Abstract

Leukoaraiosis (LA) represents the most common phenotype of cerebral small vessel disease. It is of undoubted significance regarding its vast prevalence and neuropsychiatric consequences, such as cognitive impairment, higher risk for ischaemic stroke and death. It has been associated with increasing age and conventional vascular risk factors (VRF). Despite huge efforts, LA pathogenesis is still incompletely understood. The hypotheses of ischaemia and malfunctioning blood-brain barrier seem to oppose each other. Hence, the focus has turned to endothelial dysfunction, through which both aforementioned mechanisms could be coupled. The VRF, which are almost universally present in patients with LA, have a detrimental impact on endothelium on their own. However, in LA there may be an additional or even primary endothelial dysfunction at play. This seems to be at the core of LA pathogenesis, leading to chronic ischaemia in cerebral white matter and blood-brain barrier dysfunction culminating in LA. The genetic susceptibility to harmful effects of VRF on endothelial function seems to play an important role. Regarding the burden of LA, interventional approaches should be aimed at decelerating or even halting the progression of the disease. These should focus on strict management of VRF and strategies to enhance endothelial function.

Keywords: cerebral small vessel disease, endothelial dysfunction, leukoaraiosis, pathogenesis, L-arginine

1. Introduction

Leukoaraiosis (LA) represents the most common phenotype of cerebral small vessel disease (CSVD) [1]. It is of undoubted clinical significance regarding its vast prevalence (10–27% in otherwise healthy subjects between 50 and 75 years of age) and neuropsychiatric consequences, such as cognitive impairment, higher risk for ischaemic stroke and death [2]. It has been associated with increasing age and conventional vascular risk factors (VRF), such as arterial hypertension

(AH). Despite huge efforts, LA pathogenesis is still incompletely understood. The hypotheses of ischaemia [3] and malfunctioning blood-brain barrier (BBB) [4] seem to oppose each other. Hence, the focus has turned to endothelial dysfunction, which may explain both aforementioned mechanisms [5]. The VRF, which are almost universally present in patients with LA, have a detrimental impact on endothelium on their own. LA pathogenesis mirrors the interplay between various factors. Endothelial dysfunction seems to be at the core of LA pathogenesis, leading to chronic ischaemia in cerebral white matter (WM) and BBB dysfunction culminating in LA. The genetic susceptibility to harmful effects of VRF on endothelial function seems to play an important role besides the presence and the extent of VRF [6]. Endothelial dysfunction could even be the primary event in LA pathogenesis [7]. Regarding the clinical and socioeconomic burden of LA, interventional approaches should aim at decelerating or even halting the progression of the disease. In the author's view, these should focus on strict management of VRF and strategies to enhance endothelial function in patients with LA.

This chapter outlines the different concepts of LA pathogenesis. The LA pathophysiology will be thoroughly presented, covering basic principles, such as cerebral microcirculation, autoregulation and blood pressure regulation, the interplay between VRF and LA, the role of endothelial function, various functions of nitric oxide (NO) and L-arginine. The differences in LA pathogenesis in different WM regions will be presented. The alternative hypotheses of LA pathogenesis will also be covered. The proposed genetic mechanisms putatively involved in LA pathogenesis will also be mentioned. To conclude, the interventional approaches with the aim of actively influencing the natural path of the disease will be outlined.

2. Definition of cerebral small vessel disease

2.1. Leukoaraiosis

Cerebral small vessel disease is a frequent finding on neuroradiological imaging in elderly population. Leukoaraiosis is a common term denoting diffuse confluent changes of WM with often irregular margins in elderly population with VRF [8]. The changes are hypodense on computer tomographic images (CT) and hyperintensive on T2-weighted and flow-attenuated inversion recovery (FLAIR) magnetic resonance images (MRI). Their appearance directly reflects a higher proton density and water content in the affected WM. The term was used for the first time in 1987 to describe changes in subcortical WM on CT [9]. The WM changes were bilateral, symmetric, diffuse and restricted to periventricular regions extending to semioval centre. The first recognition of CSWD dates back to the late nineteenth century (Binswanger and Alzheimer), but from today's perspective their patients probably had diseased cerebral vessels due to neurosyphilis [10]. With the advent of modern imaging techniques in the 1970s, it was possible for the first time to show WM lesions *in vivo*. The scientific community had thought for a long time that LA is merely a coincidental finding without any significant clinical consequences. Especially during the recent years, we have seen many new discoveries showing that LA is associated with cognitive impairment and higher risk for stroke and death [2]. It is known that LA is a risk factor for dementia and myocardial infarction [11]. In patients older

than 60 years, one-sixth of the population develops dementia and another sixth stroke. Taken together, one-third of the population is affected. Leukoaraiosis is more prevalent in patients who have already sustained a stroke. It is also known that the presence of LA represents a risk factor for the first and consequent strokes. The association between LA and stroke can be regarded in two different ways. Stroke and LA share the same VRF. In LA there is a chronic WM perfusion impairment, which is more prone to the development of a frank ischaemic infarction. There is a strong association between LA and lacunar infarctions (LI), which are both part of the wide spectrum of CSWD and frequently coexist in the same patient. Leukoaraiosis is also associated with cerebral atrophy. The progression of LA is associated with declining WM and cerebral grey matter (GM) mass; consequentially, the cerebral ventricles enlarge. Atrophy of GM could be regarded as the consequence of deafferentation, because the loss of subcortical WM leads to the interruption of corticosubcortical connections.

2.2. Other manifestations of cerebral small vessel disease

Lacunar infarctions develop due to the occlusion of small perforate arteries and represent the second manifestation of CSWD, albeit with different pathophysiology. Enlarged Virchow-Robin perivascular spaces are a frequent finding in CSWD and normally appear in the vicinity of the affected vessels. On CT they can easily be confused with LI, but one can definitely distinguish them from LI on MRI. Cerebral microbleeds are small intraparenchymal bleeds in perivascular spaces and represent the leakage of blood constituents through the affected vessel wall and can frequently be encountered in LA, especially in cerebral amyloid angiopathy and also in patients with untreated AH. They may harbour a greater risk for frank cerebral intraparenchymal bleed, especially if patients are prescribed anticoagulants.

3. Risk factors for developing leukoaraiosis

Although some studies have not revealed the association between LA and VRF, the former is more frequently found in patients with a history of stroke and putative vascular cognitive impairment. The most significant risk factors for LA are cerebrovascular and cardiovascular diseases [12]. The prevalence of LA typically rises with age. There is a close, albeit not exclusive, association with AH and antihypertensive therapy. A very significant risk factor for LA is advancing age. Although LA should be regarded as a pathological entity, it could at least partly be a process of normal ageing. It is not clear at what age LA begins to develop. There are no conclusive data on the “normal” extent of LA for any given age. According to the majority of studies, at least scant WM changes could be expected in subjects older than 50 or 65 years. Systematic review of studies has not revealed significant differences in LA prevalence among sexes. Possibly, LA is more prevalent in blacks compared to Caucasians mainly due to higher prevalence of AH in blacks, whereby it is usually also less well treated with resultant higher absolute values of arterial blood pressure (ABP). Blacks could even be more prone to the harmful effects of AH. Arterial hypertension is the strongest modifiable risk factor for LA [12]. According to different studies, it is present in 24.6 to 54.9% of LA patients [13]. Both higher systolic and diastolic pressures contribute. There is no threshold level of ABP above which LA

starts to emerge, but merely the association represents a continuum. Diurnal swaying of ABP is also important. Recently published data from the United States revealed that higher pulse pressure has a significant influence on progression of cognitive impairment in people over 45 years of age [14].

Diabetes mellitus (DM) has been implicated especially in the formation of periventricular LA. Higher blood glucose fasting levels are associated with LA. The finding that higher levels of insulin were found in patients with DM and LA may suggest that insulin resistance could be a risk factor for developing LA. However, the pathophysiological mechanisms are presently unknown. On the other hand, many studies have not found a significant correlation between LA and DM. Dyslipidemia (especially elevated LDL level) is an important risk factor for atherosclerosis of large vessels, whereas its influence on developing LA is less well known. In some studies, lower HDL values and hypertriglyceridemia were associated with higher risk for developing LA, in others obviously not. Smoking tobacco has been associated with LA in some studies only.

The association between LA and atherosclerosis of large vessels is controversial. Some studies have failed to demonstrate any significant association, whereas in others the association between the two existed [15, 16]. The possible common denominator might be VRF, meaning that atherosclerosis and LA occur independently but contemporarily. On the other hand, it is known that narrowing of lumina of large vessels leads to higher risk for chronic ischaemia and LA [1]. The association between ischaemic heart disease (IHD) and LA could not be regarded as causative but merely as the consequence of the fact that the two share the same VRF. Some studies have succeeded in showing the association between LA and IHD, whereas others have not [17, 18]. The association between lower values of vitamin B₁₂ and especially periventricular LA has been shown in some studies. It is known that lower values of vitamin B₁₂ and hyperhomocysteinaemia, which can be the consequence of vitamin B₁₂ depletion, could be associated with LA [19]. However, there are no relevant data, which suggest that supplementing vitamin B₁₂ and/or lowering homocysteine levels would improve LA or decelerate its progression.

Cerebral autosomal dominant arteriopathy with subcortical infarctions and leukoencephalopathy (CADASIL) manifests with LA as well, although it is a very infrequent cause of LA in population [20]. Small arteries in brain, skin and peripheral nerves show granular osmiophilic deposits in tunica media, with arterial lumen being narrowed due to high electronic density deposits [21]. Normal autoregulatory mechanisms are disturbed due to structural changes in smooth muscle cells leading to WM malfunction. Genetic factors may play an important role in LA according to studies where the association between LA and polymorphisms of numerous genes, for instance for angiotensin convertase and apolipoprotein(a) have been found [22]. Such factors are not necessarily directly associated with the presence of LA but could determine subject's proneness to the development of risk factors for LA or his/her susceptibility to develop end organ damage as the consequence of that risk factor. The susceptibility to develop AH and LA as the consequence of AH is partially genetically determined. Far less commonly, LA could be brought about by many different pathological mechanisms such as neurodegenerative diseases (Alzheimer's, inherited amyloid angiopathies), infections (HIV), inflammations (multiple sclerosis, neurolupus), traumatic head injuries, brain irradiation, cerebrospinal fluid

disturbances, chemotherapeutics and metabolic disease, including mitochondriopathies, leukodystrophies and others (e.g., Fabry's disease).

4. Pathophysiology

4.1. Cerebral blood flow and autoregulation

Brain is a highly metabolic organ with autonomous autoregulation, which enables constant cerebral blood flow (CBF) despite fluctuations in mean arterial pressure (MAP). Cerebral autoregulation is in the domain of small cerebral arteries and arterioles. Cerebral autoregulation is divided into mechanoregulation and chemoregulation. Mechanoregulation depends on transmural pressure and endothelial vasodilatation, whereas chemoregulation depends on serum CO₂ level [23]. Endothelial vasodilatation in cerebral vascular bed is of much greater amplitude compared to other regions [24]. Mechanoregulation seems to be the main regulatory mechanism of CBF. On the other hand, chemoregulation has an important influence on CBF during metabolic disturbances and is, unlikely to mechanoregulation, independent of fluctuations in MAP [23]. Both autoregulatory mechanisms function independent of each other. Although their cellular mechanisms are not completely understood, NO seems to play the crucial role, since it is needed for maintaining chemoregulation of CBF [25]. A significant mechanism of regulation of majority of vascular beds is preserved endothelial function. Disturbed function of cerebral endothelium results in diminished release of endothelial NO, culminating in attenuated relaxation of vascular smooth muscle cells of small arteries. Animal and human studies have revealed that mechanoregulation is not diminished despite ageing and other diseases affecting endothelium [26]. Contrary to this, many studies have shown that chemoregulation of CBF depends on the integrity of endothelium. Attenuated chemoregulation has been found in patients with cerebral endothelial dysfunction [27].

4.2. Arterial blood pressure deregulation

It is known that all symptomatic patients with LA do not have AH. Arterial blood pressure deregulation is very complicated, putatively adding to the pathogenesis of LA [12]. Patients with LA had higher levels of ABP and distinct circadian rhythm of ABP with large daily fluctuations or absence of nocturnal physiological fall of ABP [28]. Frequent periods of hypotension in symptomatic patients with LA speak in favour of disturbed cerebral autoregulation in patients with AH and higher burden of periventricular LA. Cerebral autoregulatory mechanisms maintain constant CBF in the MAP interval between 60 and 150 mmHg despite swaying of systemic ABP. Unlike other bodily regions, great intracranial arteries as well as extracranial parts of carotid arteries play an important role in regulation of vascular resistance of cerebral circulation. We should not overlook physiological responses of small cerebral vessels, which are crucial for autoregulation. Their response to ABP depends on their diameter. In cats, fluctuations in MAP between 110 and 160 mmHg provoke only pial arteries wider than 200 µm to respond. Arterioles narrower than 100 µm only dilate at MAP less than 90 mmHg [29]. In MAP less than 70 mmHg, the rate of their widening is larger than in wider vessels.

We presume similar mechanisms are at play in humans. In patients with AH and arteriosclerotic arteries, fall of ABP brought about by cardiac arrhythmia or disturbed autoregulation can lead to fall of CBF due to the inability of sclerotic vessels to dilate [30]. In patients with AH, autoregulatory boundaries are shifted upwards [31]. Quick fall of ABP in physiological boundaries can significantly lower CBF in WM of patients with chronic AH [32]. In this way, WM of AH patient develops ischaemia at ABP levels which could still be regarded as normal in normotensives [32]. Furthermore, autoregulatory responses of vessels in WM of experimental animals are less efficient as in GM vessels, so at lower values of ABP, falls of CBF are more pronounced in WM compared to GM [33].

4.3. Pathological hallmarks of leukoaraiosis

Oedema, ischaemia and degenerative changes of subcortical WM are principal pathologic characteristics reflecting LA on CT or MRI. Ischaemia probably involved in LA formation includes transitory events characterised by falls of regional CBF culminating in incomplete infarction, a scenario which can be tested in experimental models. Histopathologic studies on rat brain show that oligodendrocytes and myelinated axons are very prone to ischaemic damage. Chronic cerebral hypoperfusion leads to progressive rarefaction and glial activation of WM. Occlusion of rat middle cerebral artery lasting more than 24 hours leads to swelling of oligodendrocytes in subcortical WM after 30 minutes [34]. After 3-hour duration of the occlusion, oligodendrocytes already show irreversible signs of injury like pyknosis and rupture of plasmalemma. Vacuolisation of WM is the consequence of spaces which emerge by the separation of internal myelin sheath from axolemma and also from an increase of extracellular space and swelling of astrocytic processes. All changes described appear prior to irreversible neuronal injury with eosinophilia which implies that early damage of WM is independent of injury to the neuronal perikaryon. In rat models with bilateral occlusion of internal carotid arteries, two consistent types of WM changes have been noted, namely reactive astrogliosis and unspecific rarefaction of WM [35]. It is very important that increased accumulation of extracellular fluid and astrogliosis are also two main pathohistological changes in those areas where CT and MRI reveal LA in humans.

4.4. Leukoaraiosis in different regions of cerebral white matter

Progression of LA follows a uniformed pattern. In the beginning, periventricular lesions on top of horns of lateral ventricles develop (capping) progressing around the ventricles. The LA changes in deep WM initially appear in frontal lobes, then parietooccipital lobes, far less frequently in brainstem and basal ganglia. They very seldom affect temporal lobes, which are typically affected in CADASIL. At first the changes are punctiform, single, but in time they merge and become confluent affecting the whole area. The mechanisms of LA development depend on local blood circulation of a particular subcortical WM region.

4.4.1. Blood supply of different parts of cerebral white matter

The majority of cerebral hemispheric WM is supplied by long penetrant arteries stemming perpendicularly from subarachnoid arteries of pial network on the brain surface. They

travel through cortical layers perpendicularly relative to brain surface and enter WM together with myelinated fibres [12]. Penetrant arteries are 20–50 mm long and 100–200 µm wide [12]. They give rise to tiny branches supplying a cylindrical section of WM, known as metabolic unit [12]. Juxtaventricular WM is supplied by ventriculofugal branches of subependymal arteries, which are 15 mm in length, stemming from choroid arteries or end branches of striatal arteries.

4.4.2. *Juxtaventricular leukoaraiosis*

This form of LA is up to 3 mm away from lateral ventricles and starts ventricularly where there is redundant blood supply, so it is not primarily caused by ischaemia, but demyelination with resultant subependymal gliosis and disruption of ependyma, less frequently granular ependymitis or venous congestion due to collagenosis of veins. Studies support the hypothesis of “leaking” of ventricular walls. Comparable type of LA around horns of lateral ventricles develops in patients with hydrocephalus.

4.4.3. *Periventricular leukoaraiosis*

Ventriculofugal branches supplying parts of basal ganglia, internal capsule and thalamus stem from arteries of circle of Willis [36]. Ventriculofugal branches travel towards penetrant centripetal arteries of the pial system but seldom, if ever, form anastomoses with them [37]. Leukoaraiosis more than 3 mm away from lateral ventricles is normally ischaemic in origin and emerges as the consequence of microcystic infarctions and local myelin rarefactions [38]. Periventricular WM 3–13 mm away from lateral ventricles represents the border zone between ventricular and cortical blood supply, which is prone to ischaemic impairment due to local or systemic lowering of CBF [39]. Arteriolosclerosis, tortuosity and arterial elongation in elderly people with AH are a probable cause for decrease of CBF in WM [40]. Periventricular WM is prone to ischaemia already at moderate falls of CBF due to the scarcity of anastomoses between the branches of long medullary penetrant arteries [37]. Such periventricular LA is often associated with large vessel disease, atherosclerosis of the aorta or internal carotid artery, smoking, hypercholesterolemia, myocardial infarction or peripheral artery disease [41].

4.4.4. *Leukoaraiosis in deep white matter and juxtacortical leukoaraiosis*

Deep WM more than 13 mm away from lateral ventricles is supplied by medullary arteries stemming without collaterals from cortical branches of middle cerebral arteries and are substrate of CSWD pathologically associated with fibrohyalinosis and arteriolosclerosis due to hyperhomocysteinaemia and AH [42]. Lacunar infarctions are more frequent in this part of WM than elsewhere. White matter directly beneath cerebral cortex and up to 3 to 4 mm away (U fibres) is supplied by medullary as well as corticomedullary arterioles and arteries showing juxtacortical LA [43]. Mechanisms of its development are far more diverse (demyelination) and not always of ischaemic origin. In ischaemic LA, the U fibres are typically spared [12].

4.5. Ischaemic hypothesis

A strong epidemiologic association between LA and many cerebrovascular diseases speaks in favour of ischaemia playing a significant role in LA emergence and progression [12]. Ageing, chronic AH and DM share a common substrate for the type of changes these conditions trigger in small penetrant WM arteries and arterioles. These changes include substitution of smooth muscle cells with fibrohyalinous material together with thickening of vessel wall and narrowing of vessel lumen (arteriolosclerosis) [44]. Arteriolosclerosis found almost universally in LA areas is probably one of the reasons for altered blood flow in WM leading to localised ischaemic regions of necrosis and cavitations—lacunar infarctions—or diffuse rarefaction of WM-LA. In LA, diminished CBF in WM has been found. Some authors describe changes of CBF in the whole brain or only in the GM of patients with LA [12]. There are, however, only few studies comparing regional CBF in areas with and without LA. One of these found lowered CBF in regions of LA compared to normal WM [45]. Similarly, diminished regional CBF has been found applying SPECT and xenon CT imaging [46]. Decreased regional CBF in LA regions needs to be proven at first. This leaves an open question whether fall of CBF is the cause of LA or just reflects lower metabolic needs of WM, which has atrophied due to other reasons. Therefore, it is difficult to claim whether lower CBF is the cause or just a consequence of tissue damage. Decreased CBF in non-demented patients with LA has been found in frontal and parietal WM but not in occipital lobes [47]. This may reflect the fact that LA pathogenesis probably depends on its topographic localisation in the brain. In patients with less extensive, localised LA, the changes of CBF have not been shown; probably due to the fact that pathogenesis of initial LA stages differs from that of extensive diffuse lesions. It is interesting that CBF in WM measured by MRI perfusion was decreased even in the regions where there are no changes in T2-sequences [3]. Hence, the whole picture is much more complex than one might think at the first glance. Applying new imaging techniques, significant alterations of WM integrity have been discovered in regions appearing normal on T2-weighted sequences [48]. Structurally normal WM does not necessarily imply it functions properly, so it is possible that CBF is decreased secondarily.

4.6. Hypothesis of a leaky blood-brain barrier

The second hypothesis states that malfunctioning BBB leads to injury of WM due to the toxic effects of serum proteins [4]. The diseased endothelium enables serum proteins to enter into the vessel wall causing its swelling leading to hyaline degeneration and fibrosis. This further leads to thickening of vessel wall, narrowing of vessel lumen, decreased blood flow and chronic ischaemia of WM. On the other hand, endothelial dysfunction leads to decomposition of BBB [49]. The plasma constituents to which BBB is normally impermeable can now pass through BBB and enter cerebral interstitium and brain parenchyma, harming neurones and glial cells. These regions manifest as LA on CT/MRI and in pathologic specimens. In regions of LA, WM was full of extravasated serum proteins like IgG, complement and fibrinogen [50]. This may show that the diseased WM can be the place where BBB is leaking. Magnetic resonance imaging with contrast medium showed diminished integrity of BBB which is associated with the stage of LA expression [51]. What is more, BBB permeability is enhanced even in those regions not

showing frank LA on imaging [50]. In longitudinal studies, new LA areas have been found in the regions with abnormal blood perfusion or altered BBB permeability [52].

4.7. Endothelial hypothesis

4.7.1. Endothelium

Vasomotor role of endothelium has already been proven. Mediators of cerebral endothelium have been determined functioning through endothelial G-protein coupled receptors (acetylcholine, bradykinin, ATP), intermediary mediators, like NO, some prostanoids and endothelially derived hyperpolarising factor (EDHF). Endothelium releases not only vasodilator but also vasoconstrictive substances, such as endothelin-1. It is not surprising that in light of the complexity of endothelial vasomotor activity, one can conclude that any endothelial injury may lead to its aberrant function. The preserved endothelial function is crucial for undisturbed function of cerebrovascular circulation. Endothelial dysfunction is best researched in patients with VRF such as AH and DM. Both conditions have detrimental impact on endothelially derived NO [53]. It is clear that defective endothelial release of NO is the main indicator of endothelial dysfunction. The same holds true for the influence of ageing on endothelial dysfunction, which could represent an important part of LA pathogenesis. Recently, focus has turned to immune response playing a significant role in LA (neuroinflammation) [52]. It has been shown that cerebral tissue in the vicinity of LA regions includes many foamy macrophages, activated astrocytes and microglia, which speaks in favour of vivid communication between astroglia, pericytes and endothelium [54]. Increased expression of inflammatory indicators in these areas like apolipoprotein E, alfa2-microglobuline and IgG possibly adds to pathophysiological processes leading to LA [55].

4.7.2. Endothelial dysfunction

Recently, the role of endothelial dysfunction in different vascular diseases has been highly debated. It is known that endothelial dysfunction can be brought about by different VRF, metabolic diseases, systemic and local inflammation [7]. Even in LA, there are more and more data showing that endothelium is implicated in its beginning and progression. Hypotheses of decreased blood flow in WM and diseased BBB are mutually exclusionary but could be coupled through endothelial dysfunction. Endothelial dysfunction might play an important role and can be one of the first steps in developing ischaemia due to CSWD [56]. Probably endothelial dysfunction is not specific to LA and occurs in other cerebrovascular diseases as well [57]. Endothelial dysfunction leads to BBB malfunction, diseased autoregulation of CBF and prothrombotic changes. During endothelial activation, some molecules are released to the blood in higher quantities. These can be determined laboratorially, such as intercellular adhesion molecule 1, thrombomodulin and tissue factor and tissue factor pathway inhibitor [58]. Indicators of endothelial dysfunction are lipoprotein-associated phospholipase A2, myeloperoxidase and high-sensitivity C-reactive protein, as well as tumour necrosis factor alpha and interleukin-6 [59]. Inflammation in vessel wall obviously plays an important role in formation and progression of LA [60]. Asymmetric dimethylarginine is a circulating endoge-

nous inhibitor of NO and as such implicated in endothelial dysfunction, especially together with hyperhomocysteinaemia [61]. Homocysteine concentrations correlate with the degree of LA. Hyperhomocysteinaemia is an independent risk factor for LA, since homocysteine is toxic to endothelium [19]. Some studies mention the role of endothelial germ cells implicated in repairing endothelium. A special type of haptoglobin (phenotype 1-1) was associated with decreased ability of endothelium to repair its damage [62].

In a preliminary study conducted by the author of this review and his co-workers, it was found that cerebrovascular reactivity to L-arginine (CVR), an estimate of cerebral endothelial function, and flow-mediated dilatation (FMD) of the right brachial artery, an estimate of systemic endothelial function, were significantly diminished in patients with LA compared to control subjects with identical VRF without LA [7]. Moreover, CVR and FMD correlated positively in LA patients and the degree of cerebrovascular as well as systemic endothelial dysfunctions correlated with the degree of LA [7]. Overall, these results suggest that in patients with LA, both cerebral and systemic endothelial functions are impaired to the degrees that are much higher than could be expected based on present VRF. These results seem to reveal a so far unreported, more than expected additional impairment of cerebral endothelial function alongside systemic endothelial function, which is probably involved in LA pathophysiology.

4.7.3. *L-arginine*

Up until now, some studies have been performed about the effects of L-arginine on cerebral vasculature, in majority of cases on animal models [63]. L-arginine serves as a donor of NO through NO synthetase (NOS). L-arginine is also known as the most potent vasodilator and is the primary determinant of vascular tone, especially in cerebral vasculature [63]. Among the three isoforms of NOS, the endothelial (eNOS) seems to be of utmost importance in NO-mediated dilatation of cerebral arteries and arterioles [63]. NO forms endogenously as well as exogenously *in vivo*. L-arginine is the main source of endogenous NO. Contrary to endogenous L-arginine, exogenously derived L-arginine not only releases NO in this way but also through releasing other vasoactive substances [64]. The main exogenous sources of NO are NO donors releasing NO through NOS-independent mechanisms. Examples are organic nitrates and sodium nitroprusside. Endothelially derived NO is released constantly in basic quantities (tonically), whereas diverse stimuli dynamically increase its formation. After forming, NO merges with the nearby vascular smooth muscle and other cells. NO effect is short-lived due to quick inactivation. In target cells, NO activates guanylate cyclase with resultant rise of intracellular concentration of cyclic guanosine monophosphate (cGMP). Higher concentration of cGMP leads to lower intracellular calcium concentration in vascular smooth muscle cells and resultant vasodilatation.

L-arginine influences vascular endothelial cells and consequentially blood flow. L-arginine infusion causes vasodilatation and enhanced blood flow in many vascular beds [49]. Animal and clinical studies have shown that L-arginine not only leads to vasodilatation through endothelially derived NO but also decelerates thrombotic activity, cell proliferation, inflammation and other processes, which culminate in cardiovascular diseases. L-arginine prevents and diminishes the consequences of already present atherosclerosis, decelerating adhesion of

monocytes on endothelium, lowers ABP in some patients with AH and returns normal endothelial function in hypercholesterolemia [65]. Its application seems to be safe [66].

4.8. Alternative hypotheses of leukoaraiosis pathophysiology

Patients with normotensive hydrocephalus (NTH) have a high prevalence of WM changes. Experimental hydrocephalus in dogs leads to reversible WM changes after shunting has been performed [12]. This was the base for hypothesising that disturbances in cerebrospinal fluid (CSF) circulation may play an important role in pathogenesis of LA, especially of extensive periventricular changes [67]. The increased accumulation of CSF in ventricles leads to higher interstitial pressure in periventricular parenchyma and resultant ischaemia of WM. This is supported by observations that in NTH, blood flow in WM returns to normal values after CSF shunting with resultant diminishing of intraventricular pressure. Leaking of CSF into adjacent brain parenchyma may be the consequence of structural changes of ependymal cells.

White matter changes similar to those in LA (myelin pallor sparing U fibres with reactive astrogliosis and thickened small vessels) have been described in circumstances where brain oedema represents a precursor of LA [12]. In this way, transitory cerebral oedema might be additional cause of WM changes. Higher content of interstitial fluid in WM of patients with LA giving the appearance of hypodensity on CT images can be the consequence of AH and resultant changes of BBB becoming more permeable. In AH patients, capillary permeability to proteins may also be increased. Simultaneously, apart from long-term effects of AH, even short-lived hypertensive outbursts may provoke transudation of fluid and transfer of proteins into brain interstitium [12].

It is known that a substantial proportion of patients with Alzheimer's disease (AD) has radiological and pathohistological changes of WM resembling LA, albeit to a lesser degree compared to patients with cerebrovascular diseases [68]. It is highly unlikely that LA in AD only reflects Wallerian degeneration due to cortical neuronal loss. It is known that histological markers of Wallerian degeneration such as lipid-laden macrophages are missing in the majority of LA lesions. The mismatch between the degree of changes in adjacent cortical GM and WM speaks against this hypothesis. In AD patients, LA can be the consequence of ischaemia due to structural changes in small cerebral vessels in the scope of amyloid angiopathy present in 90% of AD patients [69]. The hypothesis of amyloid angiopathy in AD being causally associated with LA is further supported by the fact that LA has been found in patients with cerebral amyloid angiopathy without changes typical of AD.

5. Intervention studies: a possibility to influence the natural path of leukoaraiosis

In the light of undoubted clinical consequences, understanding LA pathophysiology is important from the viewpoint of its prevention and decelerating its progression [70]. Patients with risk factors for LA could be treated with medications to prevent its occurrence. It is

presently thought that LA changes already present seem to be irreversible. It is believed that in order to prevent LA occurrence and decelerate its progression, optimal control of all VRF in a given person is crucial [71]. There is at present relatively scarce evidence that any type of intervention would decelerate or halt the progression of LA. These data come mostly from observational studies and far less frequently from controlled randomised studies. Antihypertensives turned out to postpone the occurrence of LA and decelerate its progression regarding the results of the EVA MRI study [72]. In the PROGRESS study, treatment with diuretic and ACE inhibitor was efficient in halting or decelerating the rate of LA progression in patients with baseline extensive LA [73]. For the time being, there is no compelling evidence that lowering ABP triggers ischaemia in WM alongside disturbed autoregulation.

Regarding the association between stroke and LA, it seems reasonable to lower the risk for stroke by standard medications in its secondary prevention, namely antiaggregation agents and statins. Statins have long been known for their enhancement of endothelial function and cerebral vasomotor reactivity, but there is little clinical evidence for their efficacy in LA. Statins turned out to be associated with deceleration of LA progression at already progressed LA but not in cases with mild initial stages of LA [74]. On the other hand, in the PROSPER study, beneficial effects of pravastatin on LA progression in elderly patients with high risk for vascular complications were not found [75]. Some recent studies report beneficial effects of low doses of fluvastatin and valsartan on the compliance of large arteries or arterial aging [76–78]. In these studies, the research focused on large arteries, whereas in LA small cerebral vessels are affected. Despite this, there is a growing body of evidence that large vessel disease characterised by decreased compliance of vessel wall reflects in pathological changes of cerebral small vessels. This is summed up in the concept of pulse-wave encephalopathy [79]. One could hypothesise that enhancing the compliance of a large artery may lead to halting effects on LA progression. This may offer sound basis for future intervention studies in LA.

The use of acetylsalicylic acid (ASA) inhibiting cyclooxygenase has potential benefits in LA. Cyclooxygenase catalyses biochemical reactions in which superoxide free radicals form inside endothelial cells. In this way, ASA may decrease endothelial impairment and simultaneously inhibit matrix metalloproteinases which are probably involved in progression of LA changes [21]. Dipyridamole, an antiaggregation agent together with its vasodilator effect, may be involved in decelerating LA progression since it lowers ABP [80]. However, at present, relevant data showing undoubted efficiency of antiaggregation agents on LA progression or improved clinical outcomes in LA patients are missing. Substituting L-arginine has been safe and efficient in many other vascular diseases. On the basis of L-arginine's known effects, it is an interesting presumption that long-term oral administration of high doses of L-arginine could result in decelerating LA progression as well as having a positive influence on clinical consequences [81–83].

6. Conclusion

Leukoaraiosis represents the most common phenotype of CSWD of undoubted clinical significance. It has been associated with increasing age and conventional VRF. Despite huge

efforts, the LA pathogenesis is still incompletely understood. The hypotheses of ischaemia and malfunctioning BBB seem to oppose each other. Endothelial dysfunction seems to be at the core of LA pathogenesis, leading to chronic ischaemia in WM and BBB dysfunction. The genetic susceptibility to harmful effects of VRF on endothelial function seems to play an important role. Interventional approaches should aim at decelerating or even halting the progression of the disease.

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