

# Vascular cognitive impairment: pathophysiological mechanisms, insights into structural basis, and perspectives in specific treatments

This article was published in the following Dove Press journal:  
*Neuropsychiatric Disease and Treatment*

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**Abstract:** Vascular cognitive impairment (VCI) and vascular dementia are the most common forms of cognitive disorder associated with cerebrovascular disease and related to increased morbidity and mortality among the older population. Growing evidence suggests the contribution of blood-pressure variability, cardiac arrhythmia, hyperactivation of the renin–angiotensin–aldosterone system, endothelial dysfunction, vascular remodeling and stiffness, different angiopathies, neural tissue homeostasis, and systemic metabolic disorders to the pathophysiology of VCI. In this review, we focus on factors contributing to cerebrovascular disease, neurovascular unit alterations, and novel approaches to cognitive improvement in patients with cognitive decline. One of the important factors associated with the neuronal causes of VCI is the S100B protein, which can affect the expression of cytokines in the brain, support homeostasis, and regulate processes of differentiation, repair, and apoptosis of the nervous tissue. Since the pathological basis of VCI is complex and diverse, treatment affecting the mechanisms of cognitive disorders should be developed. The prospective role of a novel complex drug consisting of released–active antibodies to S100 and to endothelial NO synthase in VCI treatment is highlighted.

**Keywords:** vascular cognitive impairment, cerebrovascular disease, neurovascular unit, endothelial dysfunction, S100 protein

Cerebrovascular disease (CBVD) is a major cause of morbidity and mortality among the older population.<sup>1</sup> There is growing evidence suggesting the contribution of blood-pressure instability, cardiac dysrhythmia (atrial fibrillation), hyperactivation of the renin–angiotensin–aldosterone system, endothelial dysfunction, vascular remodeling and stiffness, angiopathy of different etiology, patient lifestyle (including smoking and drinking), nervous tissue disturbances (eg, amyloid  $\beta$  and  $\tau$  protein in Alzheimer's disease, which have a negative impact on neuronal functional processes), and systemic metabolic disorders (particularly diabetes mellitus and dyslipidemia) to CBVD development. Vascular cognitive impairment (VCI) refers to a cognitive disorder form associated with CBVD. Treatment of VCI is currently focused on vascular risk factors. Understanding the pathogenesis of VCI will pave the way for the development of treatments targeting disease-underlying processes. In this review, we focus on factors contributing to CBVD, neurovascular unit alterations, and novel approaches to cognitive improvement in patients with VCI.

## Introduction

With increasing life expectancy, cognitive impairment and dementia are becoming an important public health problem. In the Canadian Study of Health and Aging,<sup>2</sup> prevalence of mild vascular cognitive impairment (VCI) among respondents aged 65–84 years was higher than that of vascular dementia. Patients with VCI have higher mortality<sup>2,3</sup> and institutionalization rates.<sup>2</sup>

VCI refers to all forms of cognitive deficits of vascular origin, ranging from mild cognitive impairment to dementia. VCI can be classified as vascular mild cognitive impairment (amnestic, amnesic plus other domains, non-amnesic single domain, and nonamnesic multiple domain) and vascular dementia.

In this article we have analyzed VCI concepts focusing on pathophysiological mechanisms and possible treatment options.

## Vascular cognitive impairment and cerebral small-vessel disease

The diagnostic criteria for probable VCI according to a statement from the American Heart Association–American Stroke Association include: neuroimaging evidence of cerebrovascular disease (CBVD) and either a temporal relationship between CBVD and cognitive deterioration or a relationship between the severity of cognitive deficits and presence of subcortical vascular lesions, and cognitive impairment should not progress gradually (to exclude neurodegeneration).<sup>4</sup>

CBVD is the most common cause of VCI<sup>5</sup> and dementia.<sup>6</sup> The term CBVD includes a spectrum of disorders causing large-artery and small-vessel disease (SVD). Cerebral SVD is the main cause of cognitive impairment progression in older people,<sup>7</sup> and increases the risk of dementia and stroke.<sup>8–10</sup>

Cerebral SVD includes a variety of conditions with various etiologies that affect the small arteries, arterioles, venules, and capillaries of the brain.<sup>11</sup>

Hypertensive vasculopathy (HV) and cerebral amyloid angiopathy (CAA) are the two most common forms of cerebral SVD.<sup>12</sup>

HV includes a great variety of functional and structural changes in small arteries that occur due to hypertension. It involves vascular remodeling, inflammation, endothelial dysfunction, and increased contractility.<sup>13</sup> Long-standing hypertension can lead to lipofibrohyalinosis, arteriosclerosis, and arteriolosclerosis of deeply penetrating vessels in the brain,

causing diffuse white-matter lesions<sup>14</sup> and cerebral microbleeds.<sup>15</sup> Some consider that blood–brain barrier (BBB) dysfunction may impact HV-associated cerebral SVD.<sup>16</sup>

HV can also affect the venous system of the brain. In several studies, it was associated with venous collagenosis.<sup>17,18</sup> Thickening of the walls of periventricular veins and venules with collagen subtypes I and III is more frequent in brains with leukoaraiosis and increases with age.<sup>19</sup>

CAA indicates amyloid  $\beta$  (A $\beta$ ) accumulation in vessel walls, and is known to be a major cause of lobar intracerebral hemorrhage.<sup>20</sup> CAA prevalence is age-dependent. In the population-based Vantaa 85+ study (253 women, 53 men, mean age at death 92.3 years), 69.6% of participants had CAA, with the highest prevalence in the parietal lobe.<sup>21</sup>

In pathological studies, CAA has been associated with cortical watershed microinfarcts in both Alzheimer's disease (AD) and vascular dementia.<sup>22,23</sup> These multiple microinfarctions may cause cerebral blood flow (CBF) disturbances, due to capillary occlusion,<sup>24</sup> which can lead to progression of white matter hyperintensity (WMH).<sup>25</sup>

Cortical superficial siderosis can also be found in patients with CAA,<sup>26</sup> which is thought to be more indicative of CAA than HV, as in CAA mainly superficial cortical and leptomeningeal vessels are involved.<sup>27</sup> Cortical superficial siderosis is more frequently observed in patients with cognitive impairment and AD.<sup>28,29</sup>

CAA is also one of the major causes of cerebral microbleeds.<sup>30</sup> At 7 T magnetic resonance imaging (MRI), cerebral microbleeds were observed in 78% of patients with AD or mild cognitive impairment in one study.<sup>31</sup> In the Rotterdam Study, the presence of microbleeds was associated with cognitive decline and increased risk of dementia (HR 2.02, 95% CI 1.25–3.24).<sup>32</sup> It is hypothesized that cerebral microbleeds in strategic areas of the brain may damage cortical and subcortical tracts<sup>33</sup> or signify microvascular damage that leads to VCI.<sup>34</sup> Increasing evidence suggests that CAA can also contribute to VCI, even in the absence of AD.<sup>35</sup> In a prospective cohort study, 79% of CAA participants had mild cognitive impairment, and their scores for executive function and processing speed were lower than those of ischemic stroke controls.<sup>36</sup> Higher MRI WMH volume was associated with lower processing-speed scores<sup>36,37</sup> and executive function<sup>37</sup> in CAA. Small-vessel microstructural damage in CAA can make an independent contribution to cognitive impairment, as CAA is also

associated with cognitive decline before symptomatic intracerebral hemorrhage.<sup>38</sup>

## Vascular cognitive impairment pathophysiological mechanisms

### Cardiovascular risk factors

A large number of publications have been published on the role of cardiovascular risk factors in the occurrence and progression of cognitive impairment. Cardiovascular disease is a well-known risk factor for cognitive impairment and dementia.<sup>39</sup> In particular, conditions that increase cardiovascular risk, including diabetes, essential hypertension (EH), hyperuricemia, and smoking, also increase risk of VCI, which is a common cause of cognitive impairment.<sup>40</sup> EH-induced cerebral SVD promotes arterial and arteriolar lesions in subcortical white matter surrounding basal ganglia. Recent evidence suggests that subcortical vessels are vulnerable to the damaging effect of increased blood pressure, due to their specific anatomical structure: a short straight-flow section after branching out from the brain base arteries.<sup>41</sup> WMH detected using MRI is highly predictive of cerebral SVD.<sup>8</sup> EH-associated cerebral SVD manifests with arteriosclerosis, characterized by smooth muscle-cell death, deposition of hyaline material in vessel walls, and lipohyalinosis. In more severe cases, fibrinoid necrosis of vascular walls leads to their rupture and hemorrhage.

The exact etiology of cerebral SVD remains unclear; however, it is known that cerebral ischemia promotes its onset. Garry et al showed<sup>42</sup> that endothelial release of endogenous nitric oxide (NO) is an obligatory condition for optimal CBF. In the case of cerebral SVD, the concentration of asymmetric dimethylarginine (ADMA) increases. ADMA is NO synthase (NOS) inhibitor that blocks positive vasodilation and endothelioprotective effects of NO.<sup>43</sup> Therefore, there is a link between ED and CBF reduction. Furthermore, the increased level of ADMA is considered a risk factor for atherosclerosis and a key factor in cardiovascular disease development.<sup>44</sup>

### Cognitive impairment and serum uric acid

Increased serum uric acid (UA) is an additional cardiovascular risk factor, regardless of EH or diabetes presence.<sup>45</sup> It is considered a predictor of cardiovascular events, even when serum UA is high normal.<sup>46</sup> In contrast, the pathophysiological relationship between serum UA and cognitive decline in patients with and without history of

dementia needs further investigation.<sup>46–48</sup> UA's pathophysiological contribution to different types of dementia (eg, AD, Parkinson's disease, and VCI) differs, and also remains unclear.

Interesting results were obtained in the population-based Rotterdam Scan Study,<sup>47</sup> which included 814 participants (mean age 62.0 years). This study aimed to evaluate a relationship among UA levels, brain atrophy, and cognitive functioning. Higher UA levels were associated with white-matter atrophy (difference in Z-score of white-matter volume per SD increase in uric acid  $-0.07$  [95% CI  $-0.12$  to  $-0.01$ ]). This was particularly marked when comparing participants with elevated and normal serum UA (Z-score difference  $-0.27$  [ $-0.43$  to  $-0.11$ ]). Persons with elevated UA also had worse cognitive scores ( $-0.28$  [ $-0.48$  to  $-0.08$ ]).

Nevertheless, recent data allow us to consider that UA after all has a positive impact on cognition, rather than triggers its deterioration. Engel et al<sup>48</sup> assessed the relationship between hyperuricemia and dementia in regard to antihyperuricemic treatment. This case-control study included 27,528 patients diagnosed with dementia and 110,112 controls. Among all the participants, 22% had hyperuricemia or gout and 17% received antihyperuricemic treatment. The minimum follow-up was 3 years. Authors reported slightly lower dementia risk in patients with hyperuricemia (OR 0.94, 95% CI 0.89–0.98), and this risk reduction was even more marked among patients receiving antihyperuricemic treatment (OR 0.89, 95% CI 0.85–0.94).

There can be multiple pathophysiological mechanisms for a UA neuroprotective effect. Primarily, its antioxidant activity deserves attention.<sup>46</sup> A recent meta-analysis<sup>49</sup> showed a significant reduction in antioxidant-system activity and serum UA in patients with dementia. Furthermore, essential antioxidant concentrations, including  $\alpha$ - and  $\beta$ -carotene, lycopene, lutein, and vitamins A, C, and E, which can facilitate oxidative stress, tend to decrease in cases of low serum UA.<sup>50</sup> UA has effects similar to ascorbate in the body, which is another important antioxidant. In addition, UA has the ability to eliminate oxygen and hydroperoxyl radicals, singlet oxygen, and oxoheme oxidants, and can make stable complexes with iron ions.<sup>46</sup> Several authors have found a linear association between serum and cerebrospinal fluid (CSF) UA and between impaired BBB and UA concentration in CSF blood, which supports the hypothesis that UA can have an impact on the central nervous system (CNS) and cognition.<sup>51</sup>

At the same time, prooxidative properties of UA have been described.<sup>46</sup> Such dual (pleiotropic/ambiguous) chemical properties of UA are assumed to be due to the

influence of the environment in which this substance is included in biochemical processes, including the presence of metal ions.

UA may affect A $\beta$  metabolism, but direct mechanisms are not yet known. Some authors have explained UA neurotoxicity by its ability to potentiate proapoptotic A $\beta$  effects and expression,<sup>52,53</sup> while others<sup>54</sup> have found that increased UA levels lessen the harmful effects of CSF A $\beta$ <sub>1–42</sub> and higher UA reduce the harmful effects of A $\beta$ <sub>1–42</sub>, a CSF biomarker of cognitive function, and observed an improvement in Mini–Mental State Examination (MMSE) and Alzheimer's Disease Assessment Scale — cognitive subscale scores.

Impaired cerebrovascular microcirculation can also provide a pathophysiological link between UA and cognitive functioning, especially in patients with VCI. Elevated serum UA levels may trigger inflammatory responses to oxidative stress, endothelium dysfunction, and cerebral microvasculature damage and remodeling, which in turn can explain an increased risk of vascular dementia.<sup>46,55,56</sup> Recent data also support the relationship between high levels of UA, inflammation, and vascular dementia. Positive correlations between higher levels of CRP, IL6, and serum UA,<sup>57</sup> and between inflammatory markers and WMH, lower gray matter, and hippocampal volume, which are indirect markers of cerebral atrophy, have been observed.<sup>58</sup> In animal studies, inhibition of NF $\kappa$ B-signaling pathways, which causes a reduction in UA-related hippocampal inflammation, improved cognitive functioning. Moreover, hippocampal gliosis in both humans and rats was associated with serum UA levels. The authors have also found a significant increase in hippocampal gliosis related to serum-UA levels both in humans and rats.<sup>59</sup>

## Impaired biochemical processes in nervous tissue: the S100 protein family

VCI is also considered an outcome of impaired biochemical processes in neurons, including synaptic transmission failure. Therefore, recent studies have focused on the broad family of Ca<sup>2+</sup>-binding proteins with the EF-hand structural motif called the S100 proteins, and in particular on the S100B brain protein involved in synaptic processes.<sup>60</sup> S100 proteins were discovered by Moore in 1965.<sup>61</sup> They dissolve completely in 100% saturated ammonium sulfate solution of at pH 7.2, which explains the name of this group.

S100-family proteins are expressed in various tissue types and perform diverse functions. S100 proteins interact with intracellular effectors in tissue, regulate contraction,

mobility, growth, and differentiation of cells, play a role in membrane organization, cytoskeleton dynamic composition, and protein phosphorylation and secretion, and protect against oxidation.<sup>60</sup>

S100 proteins are considered “calcium sensors” like calmodulin and troponin C, without any internal catalytic activity. Upon binding to calcium or (less often) to copper and zinc ions, S100 proteins undergo conformational changes.<sup>62</sup> For instance, the recognition of target proteins (ie,  $\tau$ ) is not a calcium-dependent but a zinc-dependent process, and the implementation of neuroprotective properties requires copper ions.<sup>60,63</sup> Some of the S100 proteins, including S100B, act like cytokines. S100B may cause both neurotrophic (in physiological nanomolar concentrations) and neurotoxic (in micromolar concentrations) effects.

Astroglia, Schwann cells, neurons, and satellite glial cells, as well as melanocytes, chondrocytes, adipocytes, skeletal muscle fibers, dendritic cells, and some populations of lymphocytes, express S100B.<sup>64</sup> This protein stimulates proliferation and migration of cells and inhibits apoptosis and differentiation. Therefore, S100B plays an important role in synaptic process modulation, tissue development and repair, astrocyte activation in neurodegenerative processes, and glioma formation.<sup>64</sup> S100B regulates cell proliferation, which may have a positive effect on tissue regeneration and may promote carcinogenesis. An association between chronically elevated S100B levels and Parkinson's disease has been observed. The mechanisms underlying this association are probably related to a downregulation in the expression of dopamine D<sub>2</sub> receptors and G protein-coupled receptor kinase 2, in the acceleration of dopamine metabolism, and in reduction in serotonin concentration.<sup>65</sup>

In neurotoxic micromolar concentrations, extracellular homo- and heterodimer forms of S100B affect neurons, glial apoptosis, and cell necrosis.<sup>66,67</sup> This effect is based on S100B's ability to induce proinflammatory cytokines and oxidative stress-related enzymes, and to amplify other signals directed at neurons and glial cells.<sup>66–70</sup>

S100B in neurotoxic concentrations enhances the expression of IL1 and interleukin-6 (IL6) in microglia and neurons, changes neuronal metabolism, activates  $\tau$ -protein hyperphosphorylation, reduces levels of synaptic proteins, and elevates the synthesis and activity of acetylcholinesterase.<sup>68</sup> S100B also increases the expression of the A $\beta$  precursor protein in neuronal cell cultures<sup>71</sup> and enhances astrocyte activation caused by the A $\beta$  peptide.<sup>67</sup> In turn, IL1 induces S100B expression,<sup>69</sup> perpetuating the vicious cycle of S100B neurotoxic effects.

Transgenic mice overexpressing S100B have hippocampal dementia-like and behavioral impairment, such as short-term-memory disturbances, partial disability in spatial task solving, spatial and nonspatial memory problems, hyperactivity, ie, exploratory hyperactivity, adaptation disorder, and reduced anxiety.<sup>65,72</sup> Recent studies consider S100B as an early and easily measurable marker of cerebral ischemia. S100B can be detected in blood after the release from injured astrocytes into the extracellular space.<sup>73</sup> In the study by Gao et al the serum level of S100 protein was measured using enzyme-linked immunosorbent assay in patients with cerebral SVD (n=210) and VCI. Authors provided evidence that plasma level of S100 protein was significantly higher in cerebral SVD patients compared to control group ( $P<0.05$ ). Significant cognitive impairment was found in cerebral SVD patients, especially in patients with leukoaraiosis ( $P<0.05$ ; comparing to control group). Significant correlation was found between increased S100 protein level and cognitive decline in patients with leukoaraiosis ( $P<0.05$ ).<sup>7</sup>

Concentration of S100B in CSF elevates in acute cerebrovascular events<sup>74,75</sup> and correlates with the size of the ischemic area and the clinical outcome.<sup>76</sup> It has been shown that S100B concentration reaches a maximum on day 2–3 after ischemic stroke. The concentration of S100B reaches a peak in 2–24 hours after cerebral hypoxia, due to cardiac arrest, and correlates with outcome and coma levels.<sup>66</sup> There are data showing S100B concentration increase in CBVD outcomes: subarachnoid hemorrhages and hemorrhagic and ischemic stroke.<sup>77,78</sup>

Studies have shown possible involvement of S100B in the pathogenesis of AD.<sup>69</sup> In AD patients, the level of S100B in the brain is increased, due to activated astrocytes, which are cellular components of amyloid plaques and contain an increased amount of S100B.<sup>70</sup> Since S100B stimulates axon growth and neuroprotection,<sup>79</sup> its increase in the brain of AD patients is probably initially a compensatory response component. However, overexpression of this protein may have adverse effects. Neurotrophic activity of S100B also promotes aberrant axonal hypertrophy and the formation of large dystrophic neurites, which are found in and near amyloid plaques.<sup>80</sup> Chronically elevated levels of S100B in the brain lead to enhanced expression of the A $\beta$  precursor protein,<sup>81</sup> which is a source of additional A $\beta$ -peptide accumulation.

An increase in S100B in the brain of AD patients is directly related to  $\tau$ -positive neuritic pathology.<sup>82</sup> There is a parallel overexpression of S100B and the proinflammatory cytokine IL1 in AD and vascular dementia, which plays an important role in the pathogenesis of neuropathological

changes.<sup>66,68–70</sup> A connection between glial cells overexpressing IL1 and S100B, and an increase in neurofibrillary  $\tau$ -protein tangles has been found.<sup>82</sup>

## Role of the neurovascular unit in central nervous system diseases

The brain consumes up to 20% of the total amount of oxygen and nutrients (mainly glucose) contained in the blood.<sup>83</sup> Neural homeostasis depends on the complex vascular cerebral network. It provides the essential distribution of nutrients and oxygen in the brain in accordance with local metabolic rate.<sup>84</sup> Therefore, proper cerebral blood flow is the key factor in neuronal functioning. The brain tissue–blood boundary, referred to as the BBB, plays a decisive role in CNS homeostasis.<sup>85</sup> The BBB is formed by endothelial cells with tight junctions between them, constituting an isolating structure that separates circulating blood components from brain tissue. Tight junctions determine the isolating properties of the BBB, as well as contribute to its polarization, leading to different functional features of the internal and external sides, which face the blood flow and brain tissue, respectively.<sup>85</sup> The concept of the neurovascular unit (NVU) is closely related to the BBB. Interest in this topic increased significantly in the early 2000s after the publication of the Stroke Progress Review Group report on progression of the increase in stroke incidence.<sup>86</sup> The NVU consists of neurons, glial cells (astrocytes, microglia, oligodendrocytes), vascular elements (endothelial and smooth-muscle cells, pericytes, basal membrane), and extracellular matrix.<sup>87</sup> The NVU integrates neuronal activity with local cerebral perfusion, modulates functional characteristics of the BBB, and interacts with extracellular matrix proteins.<sup>88</sup> In addition, the NVU underlies the pathogenesis of several CNS diseases (cerebral stroke, vascular cognitive disorders, dementia, AD, Parkinson's disease, amyotrophic lateral sclerosis, and multiple sclerosis).<sup>89</sup>

In the structure of the BBB, highly organized and specialized transport systems (ATP-binding cassette transporters, in particular the A<sub>1</sub> subtype, the multidrug resistance protein), perform a detoxifying function and also eliminate the A $\beta$  peptide.<sup>90</sup> These transporters also ensure maintenance of CNS homeostasis.<sup>88</sup>

The other component of the NVU — astrocytes — constitute approximately 50% of brain cells. Studies have shown that astrocytes are involved in all CNS diseases.<sup>87</sup> Thousands of processes occur in a single astrocyte, allowing the proper functioning of cerebral microcirculation and synapses and

supporting the structure of neuropils. In addition, astrocytes control the ionic balance in the extracellular matrix, as well as development of the vasculature, and synthesize biologically active substances (including neurotrophic factors) that transmit signals to other cells (communicative function). At the same time, astrocytes are able to transform into a reactive state, initiate the production of proinflammatory cytokines and the formation of astroglial scars (gliosis), and suppress axonal regeneration.<sup>87</sup>

Despite the fact that the concept of the NVU is most applicable for studying processes occurring in gray matter, intercellular interactions are equally important for white matter. As another structural component of the NVU, oligodendrocytes are one of the main subtypes of cells that synthesize myelin, a substance rich in phospholipids that covers axons and is essential for effectively conducting a nerve impulse. Studies have shown that oligodendrocyte–endothelial cell couplings (the so-called oligovascular niche) potentiate angiogenesis and oligodendrogenesis in white matter.<sup>87</sup> Once the acute phase of trauma has passed, oligodendrocytes are able to release MMP9, inducing vascular remodeling in white matter.<sup>91</sup> The activity of oligodendrocyte-progenitor cells is aimed at the remyelination of damaged white-matter zones in demyelinating diseases, including multiple sclerosis, leukodystrophy, and vascular dementia.<sup>92</sup>

Another important component of the NVU in terms of VCI pathogenesis are pericytes, which are located around the endothelial layer of capillaries and embrace endothelial cells with their processes. Pericytes perform extremely important functions: integrating, coordinating, and realizing effects on the NVU. Pericytes regulate permeability of the BBB, cerebral perfusion, and eliminate cellular debris. Moreover, pericytes serve as a source of pluripotent stem cells for the CNS.<sup>87</sup> As such, pericytes are closely related to endothelial cells and thus support normal functioning of the NVU.

Because the NVU is a vital structure in cerebral homeostasis, the dysfunction of its components may lead to acute conditions, such as traumatic brain injury,<sup>94–98</sup> subarachnoid hemorrhage,<sup>98</sup> and chronic conditions, such as dementia<sup>99,100</sup> and AD.<sup>101,102</sup>

There are specific morphofunctional changes in all of these pathological processes. The loss of selectivity of the BBB, inflammation, and degradation of the extracellular matrix and basal lamina components are common features observed in NVU dysfunction. A recent study showed that

pericyte degeneration due to ischemia contributes to cerebral homeostasis failure.<sup>103</sup>

Under the concept of the NVU, a focus on neuron pathology as a central link in the pathogenesis of nervous system dysfunction has been transformed into a more integrated view, allowing the creation of a new basis for experimental and clinical research in the field of CNS diseases, including VCI.

## Endothelial dysfunction and VCI biomarkers

Several studies have suggest the role of ED in NVU failure and VCI development. In most cases, ED is associated with oxidative stress and results from both ischemia and inflammation. It is known that vascular risk factors enhance ED progression. Under normal conditions, endothelium-derived vasoactive factors take part in the coordination of vasodilatation/vasoconstriction and CBF. Therefore, it is believed that ED leads to diminution of CBF and alteration in BBB stability.<sup>104</sup>

Under ischemia, the endothelium expresses the adhesion molecules *P*-selectin, *E*-selectin, ICAM1, and (VCAM1, which are crucial for leukocyte migration into the perivascular space. Accordingly, ED can be detected using these adhesion molecules, as well as homocysteine, VWF), and MCP1, as biomarkers.<sup>105</sup> The detection of these substances has potential utility for early diagnosis and prognosis of CVBD and VCI in particular.<sup>106</sup>

Under physiological conditions, low concentration of VEGF, which contributes to angiogenesis can be found in the brain. It is known that ischemia promotes VEGF overproduction.<sup>107</sup> Tarkowski et al showed increased level of markers of inflammation (TGF $\beta$ , VEGF) in VCI, suggesting these substances as potential biomarkers.<sup>108,73</sup>

VWF is derived mostly from the endothelium. Vasoactive hormones, cytokines, hypoxia, and shear stress induce the production of VWF, and NO indirectly inhibits its secretion in vitro.<sup>109</sup> Some studies have reported a correlation between vWF levels and CBVD outcomes (ie, stroke).<sup>110,111</sup>

Results from a meta-analysis by Quinn et al indicated a relationship between increased levels of ED biomarkers associated with coagulation: thrombin-generation markers (D–dimer and prothrombin fragment 1+2) and VCI.<sup>112</sup>

MCP1 acts as a key attractant for mononuclear cells and plays a role in collateral vessel formation and blood-flow regulation in response to ischemia in vivo.<sup>113,114</sup> There is evidence that MCP1 exerts neuroprotective properties in glial–neuronal cocultures.<sup>115</sup> Increased MCP1 levels in

CSF and serum have been found in stroke patients, suggesting an association between MCP1 and cerebral ischemia pathogenesis.<sup>116,117</sup>

Acute-phase CRP is produced in chronic or acute inflammation. Interestingly, studies have shown the role of CRP in MCP1, E-selectin, VCAM1, and ICAM1 expression in cerebral ischemia exaggeration.<sup>118,119</sup> Elevated levels of CRP have been found in clinical studies in stroke patients.<sup>120,121</sup> Therefore, there is a likelihood that CRP might be also a biomarker of VCI.

## Increased arterial stiffness in the development of cerebral vascular disorders

Increased vascular stiffness is another mechanism in cerebrovascular disorder development. Van Sloten et al<sup>122</sup> showed that a decrease in elasticity of the arteries is a predictor of cerebral stroke, regardless of other cardiovascular risk factors or aortic stiffness. Increased stiffness of the carotid arteries may lead to the development of cerebral complications through a variety of mechanisms. Vascular stiffness as a part of CBVD and VCI pathogenesis contributes to pulse pressure that increases the stress on the cerebrovascular system.<sup>123–125</sup> The cerebrovascular system is very vulnerable to hemodynamic changes, since it has low resistance potential, allowing high blood pressure to affect the microcirculatory bed. Ultimately, microcirculatory dysfunction manifests with ischemia and hemorrhage. Compensatory remodeling and thickening of the cerebral vascular walls occurs to withstand high pressure in the microcirculatory bed.<sup>123,124</sup> Over time, this kind of protective mechanism is transformed into pathology, contributing to vascular reactivity disturbances, hypoperfusion, chronic cerebral ischemia, and VCI. On the other hand, the increased stiffness of elastic arteries (including the carotid arteries) leads to excessive variability in blood pressure,<sup>126,127</sup> which increases the sensitivity of organs with high blood flow, including the brain, to pressure fluctuations in the presence of altered reactivity of the microcirculatory system.<sup>123</sup> Last but not least, increased stiffness of the carotid arteries mediates the development of CBVD, potentiating the formation of atherosclerotic plaques prone to rupture.<sup>125,128</sup>

## Vascular aging and cognitive dysfunction

Complex and in some cases completely unexplored processes of vascular aging play a role in VCI pathogenesis.<sup>129</sup>

Oxidative stress and inflammation are pathogenic factors responsible for both CBVD and VCI.<sup>127</sup> With aging,

the generation of reactive oxygen species (ROS) and hyperactivation of NADPH oxidase lead to oxidative stress and ED.<sup>130</sup> Furthermore, the resulting oxidative stress potentiates coronary artery damage, as well as the development of stroke.

ROS generation potentiates the damage of arteries and vasomotor disturbances inhibiting production of NO, the most powerful vasodilator and a crucial factor required for proper endothelial functioning.<sup>131</sup> Vasomotor disturbances present as flux-dependent and shear stress–vasodilation impairment that leads to a mismatch between oxygen-supply capacity and tissue demand, causing the development of ischemia. NO exerts vaso- and cardioprotective actions, inhibiting platelet- and inflammatory-cell adhesion to endothelial cells, blocking signaling pathways triggered by proinflammatory cytokines, protecting endothelial progenitor cells by suppressing apoptosis, and regulating tissue metabolism.<sup>129</sup> Severe NO deficiency is exacerbated by a lack of tetrahydrobiopterin<sup>132</sup> and intracellular L-arginine<sup>130</sup> as much as by the age-dependent reduction in endothelial NO synthase (eNOS) expression.<sup>131</sup> All these pathological changes promote an intracellular energy deficit, vascular inflammation, atherogenesis, and CBF failure.

A number of experimental studies and clinical data provide evidence that mild chronic inflammatory processes predispose older people to atherosclerosis.<sup>133</sup> Studies on experimental models of aging discovered a proinflammatory shift in vascular gene expression resulting in elevations in proinflammatory cytokines, adhesion molecules, and inducible NOS levels.<sup>129</sup> In humans, there is a correlation between age and concentration of several inflammatory markers (such as TNF $\alpha$ , VCAM1, E-selectin, IL6, IL18, and MCP1), independently of other cardiovascular risk factors.<sup>134–137</sup> Increased concentration of these cytokines creates a proinflammatory microenvironment promoting apoptosis of endothelial cells and vascular dysfunction, and contributes to cognitive decline.<sup>138</sup>

## Activation of RAAS and oxidative stress

Activation of the renin–angiotensin–aldosterone system (RAAS) provokes oxidative stress and mild chronic vascular inflammation and raises vulnerability of cerebral vessels to atherosclerotic lesions. Recent studies have described thickening of the intima–media complex, as well as the remodeling of main arteries under activation of the RAAS in older people.<sup>129</sup> Angiotensin II–mediated signal pathways involving Capn1 and MMP2 are associated with migration of vascular smooth-muscle cells<sup>139</sup> and artery remodeling in adulthood.

Oxidative stress and inflammation are the key pathogenetic factors responsible for the development of cardiovascular diseases, neurovascular dysfunction, VCI, and dementia.<sup>132</sup> Cerebral perfusion autoregulation can be altered in response to changes in systemic blood pressure, particularly in patients with hypertension. This leads to activation of aberrant signaling pathways, by which angiotensin II realizes its adverse vasoactive effects, primarily contributing to the remodeling of blood vessels in the presence of existing blood-pressure dysregulation.<sup>140</sup>

In addition, angiotensin II might potentiate inflammation by activating leukocytes, cell-adhesion molecules, NADPH oxidase, proinflammatory cytokines, and ROS generation.<sup>140,141</sup> A number of experimental studies have shown that generation of ROS activates Toll-like receptors and triggers the inflammatory response. The cascade of inflammatory reactions, for its part, increases oxidative stress by inhibiting antioxidant defense systems.<sup>142</sup>

It is believed that in this inflammatory–oxidative vicious circle, disruption of the permeability of the BBB is also important, since in this case plasma-complement components and A $\beta$ , which penetrate brain tissue, serve as potential activators of inflammation and production of free radicals.<sup>143</sup> As such, regardless of the cause, progressive vascular damage caused by oxidative stress and inflammation probably disrupts the NVU and exacerbates tissue hypoxia, thereby damaging neurons and white matter.

Furthermore, oxidative stress suppresses production of BDNF by the endothelium of vessels,<sup>144</sup> which (with the participation of TRKB) provides neuroprotection.<sup>145</sup> Cardiovascular risk factors and disruption of the BBB result in inhibition of proliferation, migration, and differentiation of oligodendrocyte-progenitor cells and also interfere with reparative processes in white matter, thus promoting demyelination and local hypoxia.<sup>146</sup>

## Reduced capillary-network density and cerebral perfusion in terms of cognitive dysfunction and dementia

Apoptosis is a possible cause of CBVD-associated cognitive decline.<sup>129</sup> The relationship between vascular aging and apoptosis remains unclear. Research has demonstrated age-related increase in the number of endothelial cells undergoing apoptosis.<sup>129</sup> NO deficiency, mitochondrial oxidative stress, and elevated TNF $\alpha$  concentration predispose to apoptosis.<sup>147</sup> It is thought that apoptosis contributes to age-dependent decrease in capillary-network density in most

organs.<sup>129</sup> With aging, the decreased microcirculatory network density in certain cerebral areas (eg, the hippocampus) and altered structure of the remaining functioning capillaries are observed. In older patients, these processes either precede or promote cognitive dysfunction in the absence of neurodegeneration.<sup>148</sup>

Angiogenesis disruption is the mechanism for age-related lesions affecting the capillary network and CBF failure.<sup>149</sup> The decline in cerebral microcirculatory network density with aging reduces cerebral perfusion. This leads to a decrease in trophic support of neurotransmitter-signaling pathways, especially those with high neuronal activity. Aging mediates the decrease in microvascular plasticity and adequate responsiveness of cerebral capillary blood flow to changes in oxygen and energy-substrate demands. In adults, the development of nervous tissue is coordinated with angiogenesis, and cerebral microvascular plasticity is decreased.<sup>148</sup>

At any age, EH contributes to cerebral microcirculation disturbances. Patients with EH also have insufficient capillary-network density, vascular stiffness, diminished CBF, and impaired collateral CBF compensation.<sup>41</sup>

Along with other studies, the results of our open comparative clinical trial confirm the association between EH, cognitive decline, and diminished CBF.<sup>150</sup> We enrolled untreated middle-aged patients with stage 1 and 2 EH, and evaluated cerebral perfusion using the arterial spin-labeling technique. We have shown that in contrast to a control group with normal blood pressure (<140/90 mmHg), patients with uncomplicated EH had executive dysfunction and reduced CBF. The observations correlated with vascular age previously estimated in the Framingham study.<sup>151</sup> Therefore, early-onset vascular aging plays a role in EH-associated brain damage in middle-aged patients, even at initial stages of the disease.

The cerebral vasculature is the most vulnerable target for elevated blood pressure in the brain. The vast majority of negative EH effects on cerebral vessels ultimately lead to the hypoperfusion, white-matter lesions, and severe CBVD presentations, such as stroke and VCI.<sup>41</sup>

In experimental animal models and EH patients, vascular wall hypertrophy leads to thickening of walls of arteries and arterioles, internal vascular remodeling, and lumen narrowing.<sup>41</sup> Constantly increased hydrostatic blood pressure contributes to collagen and fibronectin deposition, elastin fragmentation, and cerebral artery–wall stiffness. BBB dysfunction results in inflammation, ROS generation, and protease activation.<sup>132</sup> Decreased elastic properties of the aorta



and stiffness of large cerebral arteries are significant predictors of certain cerebrovascular events and VCI.<sup>152–154</sup>

In summary, oxidative stress and inflammation resulting from the influence of various pathological vascular factors impaired biochemical processes in nervous tissue and the BBB failure diminish CBF and inhibit the proper functioning of the NVU components, thus promoting severe local hypoxia and VCI progression.<sup>146</sup>

## Treatment of VCI and perspectives of a novel preparation

Due to a wide diversity of vascular factors contributing to VCI, basic antihypertensive, anticoagulant/antiaggregant, and antihyperlipidemic therapies are primal to cognitive impairment prevention. Nevertheless, these types of basic treatment do not significantly affect the already-existing cognitive deficits nor are directly related to restoration of biochemical processes in neurons and glia. The usage of acetylcholinesterase inhibitors in VCI is not a proper choice either, because of the lack of benefit in global functioning seen in patients with VCI and vascular dementia.<sup>155</sup> Consequently, attention is drawn to neurotrophic preparations with antioxidant and neuroprotective action.

The combination of released–active antibodies (RAF Abs) to S100 and RAF Abs to eNOS is a novel nootropic preparation for VCI treatment with antioxidant and neuroprotective properties. Released activity is a combination of new properties that forms in an intact solvent during its processing in the presence of the original substance. The technological process is the multiple transfer of a part of the treated solution into an intact solvent accompanied by an external physical action.<sup>156</sup> It has been shown that drugs of this class have a fundamentally novel modifying action, since the RAF Abs alter the interaction of the specific antigen (molecule) with its target by a mechanism of conformation modification.<sup>156</sup> Compared to other nootropic drugs, the combination of RAF Abs to S100 and RAF Abs to eNOS exerts not only nootropic action but also positively impacts vascular homeostasis and endothelial function, due to RAF Abs to eNOS. The combination of endotheliotropic and neurotropic effects provides new opportunities for VCI treatment.

## Treatment perspectives: preclinical trials of novel preparation

Development of the combination preparation RAF Abs to S100 and RAF Abs to eNOS was based on previously

discovered pharmacological effects of each separate component (including their different technological versions). A number of studies on in vivo, ex vivo, and in vitro models (Table 1) not only elucidated the pharmacodynamics of RAF Abs to S100, RAF Abs to eNOS and its combination but also provided insight into its mode of action.

RAF Abs to S100 modifies the effects of Abs to S100 ex vivo.<sup>157</sup> In vitro and in vivo, RAF Abs to S100 has been shown to modify synaptic plasticity and electrical properties of plasma membranes prepared from isolated neurons,<sup>158</sup> and exhibited GABA-modulating activity<sup>159–161</sup> and effects on the serotonergic,<sup>161,162</sup> dopaminergic<sup>161</sup> and glutamatergic<sup>163</sup> systems.

In addition, RAF Abs to S100 in vitro influence on ligand–receptor interaction of pentazocine (standard) with the  $\sigma_1$  receptor<sup>161</sup> might indicate its ability to interfere with other mediator systems that cooperate with these receptors. For example, it is already known that  $\sigma_1$  receptors interact with noradrenergic<sup>182</sup> and cholinergic systems.<sup>183,184</sup> Also,  $\sigma_1$  receptors exert neuroprotective action,<sup>185</sup> and influencing their activity can be considered one of the possible mechanisms of RAF Abs to S100 nootropic effects.

RAF Abs to S100 anti-amnesic activity has been demonstrated in vivo in models of amnesia induced by electric shock<sup>164</sup> or scopolamine<sup>164,165</sup> and on a model of incompletely conditioned passive-avoidance reflex.<sup>166</sup> RAF Abs to S100 effects were comparable in strength to those of the conventional nootropic drug piracetam.

Neuroprotective effects of RAF Abs to S100 have been identified in in vivo models of brain injury: ischemic (photothrombosis-induced)<sup>167</sup> and hemorrhagic stroke<sup>168</sup> models. The observed effects of RAF Abs to S100 did not differ from those of piracetam, cavinton, or nimodipine.

RAF Abs to S100 psychotropic activity (anxiolytic and antidepressant effects) has been observed in both healthy animals exposed to stress conditions<sup>159,162,169–172</sup> and various disease models, eg, cholinergic deficit.<sup>165</sup> The anxiolytic and antidepressant activities of RAF Abs to S100 were similar to the effects of diazepam and amitriptyline.<sup>169,170,172,186</sup> Noteworthy, RAF Abs to S100 did not cause sedation and/or muscle relaxation.<sup>171</sup>

Therefore, results of experimental studies of RAF Abs to S100 demonstrate that the drug has neurotropic activity and is able to improve CNS functions under brain injury, as well as in the absence of pathology but under stressful conditions.

Table 1 Experimental studies of mechanisms of action and pharmacological activity of RAF Abs to S100, RAF Abs to eNOS and combination drug Divaza

Drug/type of study	Test system	Results	Reference
<b>RAF Abs to S100</b>			
<b>Mechanisms of action</b>			
Influence on LPT	Hippocampal slices (400 µm) of mature Wistar rats <i>ex vivo</i>	Anti-S100 (final dilution 1:50) inhibited the induction of LPT, whereas RAF Abs to S100 added to anti-S100 (final dilution 1:50) canceled inhibiting activity of the latter	157
Influence on electrical properties of cell membranes	Isolated neurons of <i>Helix pomatia</i> <i>in vitro</i>	RAF Abs to S100 suppressed generation of action potential in a dose-dependent manner and increased the maximal speed of its growth via changing the volt-ampere characteristics of the incoming current channels	158
Involvement of GABA <sub>A</sub> -ergic system in the realization of RAF Abs to S100 effects	Adult outbred male albino rats, <i>in vivo</i>	Bicuculline and picrotoxin (GABA <sub>A</sub> -receptors antagonists) decreased the anxiolytic effect of RAF Abs to S100 in Vogel conflict test by 1.8- and 1.6-times, respectively	159
Involvement of GABA <sub>B</sub> -ergic system in the realization of RAF Abs to S100 effects	Adult outbred male rats <i>in vivo</i>	Baclofen (GABA <sub>B</sub> -receptor agonist) decreased the anxiolytic effect of RAF Abs to S100 in Vogel conflict test 2.2-fold, whereas phaclofen (GABA <sub>B</sub> -receptor antagonist) increased it 1.4-fold; both baclofen and phaclofen decreased antidepressive effect of RAF Abs to S100 in Nomura's forced swimming test 1.5- and 1.7-fold, respectively	160
Involvement of serotonergic system in the realization of RAF Abs to S100 effects	CHO cells expressing human GABA receptors <i>in vitro</i>	RAF Abs to S100 exerted antagonism on GABA <sub>BI/AB2</sub> receptors inhibiting agonist-induced responses by 30.2% and also inhibited specific binding of [ <sup>3</sup> H]-[3,4- <sup>3</sup> H]-cyclohexylmethyl)phosphinic acid ([ <sup>3</sup> H]-CGP54626) to GABA <sub>BI/AB2</sub> -receptors by 25.8%	161
	Adult outbred male rats <i>in vivo</i>	Ketanserin (5-HT <sub>2</sub> receptors antagonist) decreased both the anxiolytic effect of RAF Abs to S100 in Vogel conflict test and antidepressive effect of RAF Abs to S100 in Nomura forced swimming test 1.9- and twofold, respectively	162
	CHO and CHO-K1 cells <i>in vitro</i>	RAF Abs to S100 increased specific radioligands binding to 5HT <sub>1F</sub> , 5HT <sub>2B</sub> , 5HT <sub>2C</sub> , and 5HT <sub>3</sub> -receptors 142.0%, 131.9%, 149.3%, and 120.7%, respectively; also RAF Abs to S100 exerted antagonist effect on 5HT <sub>1B</sub> receptors, inhibiting their functional activity by 23.2%, and agonist effect on 5HT <sub>1A</sub> receptors, enhancing their functional activity by 28.0%	161
Involvement of dopaminergic system in the realization of RAF Abs to S100 effects	CHO and CHO-K1 cells <i>in vitro</i>	RAF Abs to S100 increased specific radioligand binding to D <sub>3</sub> receptors by 126.3% and exerted antagonism at D <sub>3</sub> receptors inhibiting their functional activity by 32.8%	161
Involvement of glutamatergic system in the realization of RAF Abs to S100 effects	Rat brain cortex neuronal cells <i>in vitro</i>	RAF Abs to S100 decreased specific radioligand binding to NMDA receptors by 39.1%	163

(Continued)

Table 1 (Continued).

Drug/type of study	Test system	Results	Reference
Involvement of $\sigma_1$ -receptor in the realization of RAF Abs to S100 effects	Human leukemic T lymphocytes (Jurkat line), MCF-7 cells, in vitro	RAF Abs to S100 decreased specific radioligand binding to native and recombinant human $\sigma_1$ receptors by 75.3% and 40.3%, respectively	161
<b>Pharmacodynamics</b>			
<b>Anti-amnesic activity</b>	Adult outbred male albino rats with amnesia induced by electric shock in vivo	RAF Abs to S100 increased the latency of CPAR and the number of animals with CPAR by 1.2- and 1.6-fold, respectively	164
	Adult outbred male rats with scopolamine-induced amnesia in vivo	RAF Abs to S100 increased the latency of CPAR and the number of animals with CPAR 1.5- and 1.8-fold, respectively	165
	Immature outbred albino male and female rats with incompletely conditioned passive-avoidance reflex in vivo	RAF Abs to S100 increased the number of active avoidance responses 2.4-fold (up to level of healthy animals)	166
<b>Neuroprotective activity</b>	Adult outbred male albino rats with experimental ischemic stroke in vivo	RAF Abs to S100 increased the latent period of CPAR 1.7-fold	167
	Adult outbred male albino rats with experimental hemorrhagic stroke in vivo	RAF Abs to S100 reduced the area of stroke penumbra by 40% and improved CPAR performance 2.2-fold	168
	Adult outbred male albino rats with experimental stroke in vivo	RAF Abs to S100 increased rat survivability 20%; decreased the number of rats with mild and severe neurological disorders, motor coordination disorders, and myorelaxation 1.4-, 1.5-, 1.7-, and twofold, respectively; improved the CPAR performance twofold; increased the time spent in open arms of EPM 1.6-fold	176
<b>Anxiolytic activity</b>	Adult outbred male albino rats in vivo	RAF Abs to S100 increased punished water intake in Vogel conflict test 1.4–3.2-fold	159,162,169–171
	Adult outbred male albino rats in vivo	RAF Abs to S100 increased the number of entries into open arms of EPM, time spent in open arms, and leaning over the edge of the maze 1.9-, 5.4-, and 4.9-fold, respectively	169,171
	Adult outbred male albino rats in vivo	RAF Abs to S100 increased the number of entries into the center of the open field to 2.4 $\pm$ 0.7 vs $0 \pm 0$ in control group	169,171
	Adult Rj:Wistar (Han) male rats in vivo	RAF Abs to S100 increased punished water intake in Vogel conflict test by 1.5-times	170

(Continued)

Table 1 (Continued).

Drug/type of study	Test system	Results	Reference
<b>Antidepressive activity</b>	Adult outbred male albino rats in vivo	RAF Abs to S100 increased the number of wheel turns in Nomura forced-swimming test 1.8–2.2-fold	20, 162, 169
	Adult outbred male albino rats in vivo	RAF Abs to S100 decreased the duration of immobility in Porsolt forced swimming test by 1.6-times	172
<b>Sedative activity</b>	Adult outbred male albino rats in vivo	RAF Abs to S100 did not decrease horizontal activity in open-field test	171
<b>Myorelaxant activity</b>	Adult outbred male albino rats in vivo	RAF Abs to S100 did not reduce the ability of rats to keep balance in the rotarod test	171
<b>RAF Abs to eNOS</b>			
<b>Mechanisms of action</b>			
Effects on vascular endothelial function	Cavernous bodies of adult Wistar male rats in vivo	RAF Abs to eNOS increased eNOS activity, content of NO derivatives, and content of cGMP 2.4-, 1.3-, and fourfold, respectively	173
<b>Pharmacodynamics</b>			
<b>Endothelioprotective activity</b>	Adult Wistar male rats with NO deficiency induced by L-NAME in vivo	RAF Abs to eNOS reduced arterial blood pressure (184.3±70 mmHg vs 190.3±6.7 in L-NAME group) and exhaustion of myocardial fractional flow reserve by 11%	174
	Adult Wistar male rats with NO deficiency induced by L-NAME in vivo	RAF Abs to eNOS improved microcirculation in the ischemic area, stimulated neoangiogenesis, and promoted inclusion of additional capillaries into general circulation; RAF Abs to eNOS improved the metabolism of endothelial capillaries and significantly decreased the number of desquamated endotheliocytes, which was the unique morphological criterion for endothelium-damage degree	175
<b>Influence on the cardiovascular system</b>	Adult Wistar male rats with hypoestrogen-induced NO deficiency in vivo	RAF Abs to eNOS reduced arterial blood pressure (158.5±15.0 mmHg vs 160.3±0.2 in hypoestrogen group) and the exhaustion of myocardial fractional flow reserve by 26.9%	174
	Normotensive adult Wistar male rats in vivo	RAF Abs to eNOS did not affect systemic hemodynamics, did not augment nitroglycerine effects	176
<b>Divaza</b>	Hypertensive adult NISAG male rats in vivo	RAF Abs to eNOS reduced arterial blood pressure by 5.7%	177
<b>Mechanisms of action</b>			
Involvement of $\sigma_1$ -receptor in the realization of Divaza effects	Segments of vas deferens of male albino Dunkin Hartley guinea-pigs ex vivo	Divaza doubled the amplitude of tissue contraction induced by standard agonist	178

(Continued)

Table 1 (Continued).

Drug/type of study	Test system	Results	Reference
<b>Pharmacodynamics</b>			
<b>Antiamnesic activity</b>	Adult Wistar male rats with $\beta$ -amyloid-induced amnesia in vivo	Divaza tripled the latency of entry into the dark compartment of the CPAR experimental chamber	179
<b>Neuroprotective (antioxidant) activity</b>	Adult outbred male rats with experimental acute hemic hypoxia in vivo	Divaza decreased the content of diene conjugates in the cerebral hemispheres by 9.7%–27.8% in the heptane fraction and 7.5%–47.4% in isopropanol fraction. The accumulation of 2-thiobarbituric acid–reactive products was reduced by 20.1–27.5%	180
<b>Anxiolytic activity</b>	Adult outbred male rats in vivo	Divaza increased the number of punished water intakes in Vogel conflict test by 2.5-times	181
<b>Antidepressive activity</b>	Adult outbred male rats in vivo	Divaza increased the number of the wheel turns in Nomura forced-swimming test 1.8-fold	181

**Abbreviations:** CPAR, conditioned passive-avoidance reflex; eNOS, endothelial NO synthase; EPM, elevated plus maze; LPT, long-term potentiation; RAF, released-active form.

## Effects of RAF Abs to eNOS

In vitro biochemical studies, RAF Abs to eNOS stimulated the eNOS–NO–GC–cGMP cascade,<sup>173,187</sup> which is responsible for relaxation of vascular smooth muscles and regulation of regional blood flow.

The ability of RAF Abs to eNOS to prevent endothelial damage (endothelioprotective effect) has been observed in in vivo NO deficiency models induced by L-N-nitroarginine methyl ester or hypoestrogen conditions.<sup>174,175</sup>

A study on RAF Abs to eNOS influence on the cardiovascular system in rats showed that the drug's administration did not affect main hemodynamic parameters in normotensive rats,<sup>176</sup> but added to losartan, it decreased arterial pressure in hypertensive rats,<sup>177</sup> which makes the use of RAF Abs to eNOS for treatment of cardiovascular diseases promising. Also, RAF Abs to eNOS was shown to cause no additional blood-pressure decrease when combined with nitroglycerin.<sup>176</sup>

## Effects of combination of RAF Abs to eNOS and RAF Abs to S100

The combination of RAF Abs to S100 and RAF Abs to eNOS synthase along with memantine demonstrated anti-amnesic effects in a model of A $\beta$ -induced amnesia.<sup>179</sup> The drug's neuroprotective effect, similar to that of RAF Abs to S100 and might be linked to its influence on  $\sigma_1$  receptors,<sup>180</sup> was demonstrated in an in vivo model of sodium nitrite-induced acute hypoxia: the combination of RAF Abs to S100 and RAF Abs to eNOS, as well as the reference compound mexidol, prevented or reduced activation of lipid peroxidation in the brains of experimental animals, suggesting an antioxidant-like effect.<sup>180</sup>

Anxiolytic and antidepressant effects of the combination of RAF Abs to S100 and RAF Abs to eNOS have been demonstrated in healthy animals using the Vogel conflict test and Nomura forced-swim test,<sup>181</sup> and were similar to amitriptyline activity.

## Safety

Toxicological studies of RAF Abs to S100, RAF Abs to eNOS, and their combination were conducted in accordance with national<sup>188,189</sup> and international (ICH M3R2) Guidance on Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals, (2009) guidelines.

The following assessments were performed: single-dose (acute) toxicity, chronic toxicity, reproductive

toxicity, genotoxicity, immunotoxicity, local tolerance, and allergenicity. Additionally, local tolerability studies were performed.

Safety studies did not reveal any toxic effects (even with doses >100 times recommended human doses; unpublished data). Based on these results, RAF Abs to S100, RAF Abs to eNOS, and their combination can be considered class 4 low-hazard substances (according to the Russian GOST 12.1.007-76).

## Treatment perspectives: clinical trials of novel preparation

Clinical studies of the combination of RAF Abs to S100 and RAF Abs to eNOS – were conducted in accordance with the principles of good clinical practice and Declaration of Helsinki requirements. The total number of participants in all studies was 696, and 545 of them received the combination RAF Abs to S100 and RAF Abs to eNOS. The purpose of the clinical trials was to evaluate the efficacy and safety of preparation for CBVD and cognitive impairment treatment.

In the study of the combination of RAF Abs to S100 and RAF Abs to eNOS in patients with CBVD, the 1.9-fold decrease in eNOS ( $927.5 \pm 11.2$  to  $478.6 \pm 13.4$  pg/mL,  $P < 0.05$  compared to baseline [reference value 450 pg/mL]) and the 1.5-fold decrease in number of circulating (desquamated) endothelial cells ( $6.98 \pm 0.52$  to  $4.62 \pm 0.75$  cells/100  $\mu$ L,  $P < 0.05$  compared to baseline [reference value two to four cells/100  $\mu$ L]) were shown after 12 weeks of therapy. These results indicated that the preparation exerts endothelioprotective action.<sup>190</sup>

Administration of the combination of RAF Abs to S100 and RAF Abs to eNOS in patients with chronic CBVD led to normalization of ischemia and inflammation biomarkers, such as fibrinogen ( $-1.6$  g/L,  $P < 0.01$  compared to baseline) and VWF in plasma ( $1.5$  g/L,  $P < 0.01$  compared to baseline) after 3 months of therapy. In addition, statistically significant decreases in CRP ( $1.7$  mg/L;  $P < 0.05$  compared to baseline) and the ED biomarker MCP1 ( $30.1$  pg/mL) concentrations were shown, suggesting the endothelioprotective effect of the preparation and the ability of RAF Abs to eNOS + RAF Abs to S100 to reduce the severity of the inflammatory process in vessel walls. No significant fluctuations in VEGF or ET1 concentrations were found, indicating the ability of the combination of RAF Abs to S100 and RAF Abs to eNOS to affect angiogenesis and prevent the progression of CBVD. In addition, the 31.3% decrease in S100 level was found, suggesting the deceleration of neurodegeneration.

Several clinical trials in patients with asthenia and mild cognitive impairment have provided evidence of the nootropic effect of the combination of RAF Abs to S100 and RAF Abs to eNOS. The cognitive improvement manifested in average MMSE-score increase, clock-drawing test, and verbal association test performance. Significant improvement in cognitive functions (according to the MMSE) was shown by the end of the 3-month treatment course with the combination of RAF Abs to S100 and RAF Abs to eNOS.<sup>191</sup>

Neuroprotective action of the combination of RAF Abs to S100 and RAF Abs to eNOS was shown in a trial, conducted by Parfenov et al: a 10% decrease in asthenia severity in 61% of patients, threefold decrease in 25% of patients, and improvement in sleep and quality of life (according to the SF36 questionnaire) were shown.<sup>192</sup>

A study on the effect of the combination of RAF Abs to S100 and RAF Abs to eNOS on potentially reversible vascular factors, which play one of the leading roles in the development of cognitive impairment, was performed in the course of the noninterventional observational program Diamant.<sup>193</sup> The program was conducted in 30 cities in Russia between 2016 and 2017. Patients with CBVD attending outpatient clinics were treated with the combination of RAF Abs to S100 and RAF Abs to eNOS (two tablets, three times per day). The Montreal Cognitive Assessment (MoCA) scale was used for cognitive ability analysis in patients before and after 3 months of therapy. The study included 2,583 participants with CBVD, and the majority of them (90.7%) experienced symptoms of cognitive impairment (<26 MoCA score). At the end of treatment with the combination RAF Abs to S100 and RAF Abs to eNOS, the mean MoCA score improved from 19.58±5.13 to 23.99±4.21 ( $P<0.0001$ ). The percentage of patients with normal cognitive function ( $\geq 26$  MoCA score) increased by 32%. Older and senile patients tolerated the treatment well: <0.6% of adverse events (AEs). The vast majority of doctors (88.4%) noted the effect of the drug as a significant improvement or improvement, and 89.6% of patients evaluated the effect of treatment as excellent or good. The authors concluded that use of the combination of RAF Abs to S100 and RAF Abs to eNOS in patients with CBVD and cognitive impairment was substantiated and promising.

The safety of the combination of RAF Abs to S100 and RAF Abs to eNOS was also evaluated. In total, investigators detected 48 AEs in 43 patients. All of them, according to World Health Organization guidelines, were rare. AEs were not severe, and were related to different organ

systems.<sup>194</sup> All patients with AEs were monitored until complete resolution (patient recovery). No AEs have been determined to have a certain or probable relationship to the study drug. There were no serious AEs.

A new multicenter, double-blind, placebo-controlled randomized clinical trial of the efficacy and safety of the combination of RAF Abs to S100 and RAF Abs to eNOS in the correction of oxidant disorders in patients with cerebral atherosclerosis (resolution 42 of the Ministry of Health of the Russian Federation, February 5, 2018) has been proposed. The inclusion of at least 124 outpatients (32 in each group) with mild cognitive impairment (MoCA score <26) taking antihypertensive and hypolipidemic therapy at a constant dose and without significant disability (modified Rankin Scale score  $\leq 1$ ) is planned. Within 12 weeks, evaluation of cognitive impairment severity (MoCA scale), oxidative and antioxidant-system laboratory tests, and compensatory endothelial capacity and its ability to regulate vascular tone are going to be performed. Resistance capacity to lipid peroxidation, concentration of lipid peroxidation products (mainly lipid hydroperoxides) and the ability of lipoproteins to be oxidized will be assessed using  $\text{Fe}^{2+}$ -induced chemiluminescence. Using standard laboratory techniques, the concentration of NO products in serum, platelet aggregation, and thickness of the intima-media complex will be measured. The safety of the combination RAF Abs to S100 and RAF Abs to eNOS will be assessed by the severity of AEs and their relationship to the study drug. Study results will be available at ClinicalTrials.gov (NCT03485495).

## Conclusion

In this review, we have considered the wide range of pathophysiological VCI mechanisms. Changes in cerebral vessels in the form of cerebral SVD, ED, a decrease in cerebral capillary-network density, increased stiffness of arterial walls mediated by aging processes, oxidative stress, impact of the RAAS, and systemic blood pressure are the main causes of VCI. We emphasized that VCI, with the advent of the NVU concept, should be considered not only a vascular disorder but also a result of failed interaction between vascular and cellular (primarily neuronal) factors leading to impaired cerebral function. This view is certainly more rational and more correct, since the CNS is an extremely complex structure and its normal functioning is provided by the integrative interaction of vascular and cellular components. It should be mentioned again that one of the important factors associated with the neuronal

causes of VCI is S100B, which can affect the expression of cytokines in the brain, support homeostasis, and regulate the processes of differentiation, repair, and apoptosis of nervous tissue.

Since the pathological basis of VCI is complex and diverse and specifically targeted treatment has not yet been found, new methods of treatment affecting all mechanisms of cognitive disorders should be developed.

## Highlights

- VCI refers to all forms of cognitive disorder associated with cerebrovascular disease, and its pathogenetic mechanisms are complex and diverse.
- VCI should be considered a result of failed interaction between vascular and cellular (primarily neurotropic) factors leading to impaired cerebral function.
- S100B is an important neurotropic factor associated with VCI.
- The combination of RAF Abs to S100B protein and RAF Abs to eNOS is a safe novel preparation with endotheliotropic and neurotropic effects, providing new opportunities for VCI treatment.

## Acknowledgments

The studies of the combination of released–active form of antibodies to S100 protein and released–active form of antibodies to endothelial NO synthase mentioned in this review were funded by a grant from Materia Medica Holding (Moscow, Russia). The statistical analysis was provided by Materia Medica Holding.

## Disclosure

VVF, KKK, GRK are employees and OIE is the founder of Materia Medica Holding. Divaza is a preparation manufactured and marketed by Materia Medica Holding. Patents on Divaza belong to OIE. VAP received an investigator grant from Materia Medica Holding to conduct the clinical trials of Divaza mentioned in this review. VVF, KKK and GRK report personal fees from Materia Medica Holding during the conduct of the study and outside the submitted work. OIE reports personal fees from Materia Medica Holding, during the conduct of the study and outside the submitted work. In addition, OIE has patent 1302925.1/GB2496342 (licensed UK patent 2496342). ODO, TMO, and AIK report no conflicts of interest.

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