Vascular Contributions to Cognitive Impairment and Dementia

Topical Review of Animal Models

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Cerebrovascular disease and Alzheimer disease (AD) lesions are common in older people and accumulate with age. Cerebrovascular lesions can directly reduce cognitive status: vascular dementia is the second most common cause of clinical dementia after AD. In addition, cerebrovascular lesions worsen the impact of AD and other dementia pathologies and may contribute to AD pathogenesis. This spectrum is reflected in the concept of vascular contributions to cognitive impairment and dementia (VCID).

There are numerous vascular pathologies underlying VCID. The most prevalent is cerebral small vessel disease (SVD), or arteriolosclerosis, in small arteries (outer diameter up to ≈200 μm) that supply deep nuclei and deep white matter areas in the human brain. Parenchymal lesions associated with SVD vesselopathy are small focal infarcts (lacunes), diffuse white matter lesions (WML), and microhemorrhages. Other VCID-related vascular pathologies include microatheroma, venous collagensesis, and cerebral amyloid angiopathy.

The limitations of animal models for VCID are well-known. Experimental species differ from humans in terms of lifespan, relative white matter abundance, large artery dimensions, and in size and morphology of deep penetrating arteries (Figure 2). Nevertheless, animal paradigms provide valuable insights into mechanisms, progression, and possible therapies in VCID. All experimental use of animals for human health-related research carries ethical responsibilities and must be governed by internationally-agreed Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (http://www.nc3rs.org.uk/arrive-guidelines). Here, we update a previous systematic review of VCID-relevant models (online-only Data Supplement) and summarize instructive examples (Table).

**Hydroperfusion: Rats and Mice**

Bilateral surgical ligation of the common carotid arteries (2-vessel occlusion) in rats remains the most frequently used model (online-only Data Supplement). Bilateral carotid artery stenosis (BCAS) in mice, using metal coils to narrow the arteries by 50%, produces a less-severe, chronic global hypoperfusion. BCAS mice develop some white matter damage and increased blood–brain barrier (BBB) permeability and cognitive impairment. Fludeoxyglucose (F-18) positron emission tomography indicates a decrease in hippocampal glucose utilization 6 months post BCAS. In the radial maze and Barnes maze tasks, working memory was impaired at 30 days. Impaired reference memory was also detected at 5 to 6 months post surgery.

After 6 months of stenosis, the animals display significantly (30%) reduced fractional anisotropy on diffusion tensor imaging in white matter areas. Histologically, they exhibit thickened basement membrane collagen IV (relative to 1 month post BCAS and sham-operated animals) and hippocampal atrophy with pyknotic and apoptotic cells from 6 to 8 months post surgery. An unexpected finding in BCAS mice is the incidence at 6 months of subcortical hemorrhagic lesions, detected on magnetic resonance imaging (MRI) and confirmed histologically. The hemorrhagic lesions, and an astrogial response with unusual distribution of aquaporin-4, suggest a pathological process additional to global hypoperfusion.

To produce more gradual cerebral blood flow (CBF) reduction, ameroid microconstrictor cuffs filled with casein (which swells on absorbing water) are placed around the carotid arteries of rats. In rats gradual bilateral occlusion (2-vessel gradual occlusion) over 2 to 3 days leads to comparable CBF reduction and white matter damage, with lower mortality and hippocampal neuronal death, relative to the standard 2-vessel occlusion rat model. 2-vessel gradual occlusion in hypertensive (SHR) rats produced a gradual reduction in global CBF (to 68% of baseline values, after 7 days) and cognitive impairment in the Y-maze. Mice with an ameroid constrictor placed on 1 common carotid artery and a microcoil causing 50% stenosis on the other (so-called ameroid constrictor, arterial stenosis, or ACAS mice) exhibit subcortical infarcts in addition to diffuse...
white matter damage. ACAS mice exhibited gradual reduction of CBF ≥28 days, and multiple infarct damage in subcortical regions ipsilateral to the ameredoid constrictor cuff, observed in 81% of the mice. At day 28 post surgery, ACAS mice showed significant decrease in spatial working memory.

**Hypoperfusion: Baboons**

In adult baboons (Papio anubis; age ≥12 years) occluding 1 vertebral and both internal carotid arteries (termed 3-vessel occlusion), led to a severe hypoperfusion state. Activation of microglia was marked at 3 days post occlusion, and plasma extravasation at 7 to 14 days, both being resolved by 28 days. From 7 days post occlusion, these animals developed progressive white matter pallor and vacuolation in the corpus callosum, deep subcortical and periventricular white matter areas, with some demyelination, up to euthanasia at 28 days. Although a primate surgical model poses substantial logistic challenges, data from a human-like experimental species with extensive white matter are uniquely valuable. For VCID-relevant research, it is notable that aging baboons exhibit both β-amyloid and tau neuropathology.

**Hypoperfusion Paradigms in Relation to Clinical SVD and VCID**

Regional CBF in white matter is universally low across species (Figure 1). This is generally considered to explain the white matter predilection for diffuse hypoperfusion lesions. In human brain, the deep subcortical white matter is supplied by the distal fields of deep penetrating medullary arteries (length ≥50 mm) arising from the leptomeningeal branches of the anterior, middle, and posterior cerebral arteries. Thus, even under normal circumstances, this deep white matter is subject to relatively low perfusion pressure. Although there are some anastomoses between these vessels, an episode of profound global hypoperfusion (eg, acute internal carotid artery occlusion) causes white matter infarcts in a characteristic deep or internal borderzone distribution. Experimental induction of abnormally low perfusion pressure in an animal (eg, 2-vessel occlusion or 3-vessel occlusion models) would be expected to cause ischemic white matter damage with a similar pattern.

A caveat is that pathogenesis of WML in these hypoperfusion models is different from human SVD. The majority of WML and lacunes in humans are thought to arise as a direct result of local small vessel wall changes not of embolic events or episodes of global hypoperfusion. Hence, although experimental proximal large vessel occlusion will cause white matter changes, the distribution of lesions is likely to be more confined and stereotyped, and other features contributing to the local milieu in chronic hypertensive arteriopathy, such as BBB dysfunction are likely to be different in such models, or absent. Furthermore, any vascular adaptions such as ischemic preconditioning are unlikely, except where occlusion is more gradual (eg, 2-vessel gradual occlusion).

**Hypertensive Rodents With Comorbidities**

Spontaneously hypertensive stroke prone rats (SHRSP) develop severe hypertension from 9 to 12 weeks of age and typically exhibit stroke lesions at 9 to 12 months, with 90% mortality by 12 months of age. Stroke lesions are frequently hemorrhagic in nature and are unpredictable in timing, severity, location, and behavioral outcome. In the absence of comorbidities, stroke-free SHRSP exhibit little white matter change on MRI or histologically. In SHRSP subjected to unilateral carotid artery occlusion, then a combination of low-protein, high salt diet (so-called Japanese permissive diet), and NaCl (1%wt/vol)–supplemented drinking water, diffuse WML were seen on MRI. These were accompanied by impaired performance in the Morris water maze. Histologically there was loss of myelin, signs of inflammatory response, and matrix metalloproteinase (MMP)–mediated BBB disruption. Although mature oligodendrocytes were depleted in white matter of SHRSP, oligodendrocyte progenitor cells paradoxically increased in density. The WML were accompanied by hypoperfusion, determined by arterial spin labeling MRI, and reduced brain tissue pO2 measured by electron paramagnetic resonance. Hypoxia-inducible factor-1α, activating MMP2, may be the pathway for BBB disruption. The antibiotic minocycline has both anti-inflammatory and antiapoptotic activity. Young SHRSP were treated with this drug (50 mg/kg IP, every 2 days) for 9 weeks, after the unilateral carotid artery occlusion surgery and transfer to Japanese permissive diet. Minocycline-treated animals showed an impressive protection from WML on MRI, modest improvement in the Morris water maze, and increased lifespan, relative to vehicle-treated animals.
Although SHRSP develop severe hypertension, milder chronic hypertension is induced by supplementing drinking water with the NOS-inhibitor L-N-Nitroarginine methyl ester, or chronic infusion of angiotensin II by minipump. Mice receiving a subpressor infusion of angiotensin II develop mild hypertension (mean arterial blood pressure 90 mm Hg, relative to 70 mm Hg in saline-infused controls). In addition to vascular actions, subpressor concentrations of the hypertensive agent may have direct effects on neural organization and metabolism.

Hyperhomocysteinemia in Mice and Rats
Elevated plasma concentration of the nonessential amino acid homocysteine, termed hyperhomocysteinemia, is a risk factor for VCID. In wild-type mice, a diet deficient in B vitamins (B6, B9-folate, and B12) resulted in hyperhomocysteinemia within 10 weeks, accompanied by reduced capillary density in brain tissue and impaired performance in Morris water maze. The same dietary regime also exacerbated cognitive impairment in amyloid precursor protein (APP) transgenic mice. Maintaining wild-type mice for 12 weeks on a diet enriched for the homocysteinemia precursor methionine, in addition to B6/B9/B12 deficiency, resulted in plasma (homocysteine) in the range of 70 to 90 µmol/L classified as moderate hyperhomocysteinemia in mice (physiological range for plasma [homocysteine] in healthy mice and humans, 5–10 µmol/L). These mice exhibited cognitive impairment on the 2-day radial arm water maze, increased metalloproteinase (MMP2, MMP9) activity in brain tissue, and small focal cerebral hemorrhages. The methionine-enriched, B6/B9/B12-deficient diet was also applied to dual mutant APP/PS1 mice. In these animals, cerebral microhemorrhages (evident on MRI and histology) were accompanied by redistribution of β-amyloid deposits from brain parenchyma to the microvasculature.

In rats, B9-folate deficiency alone was sufficient to induce hyperhomocysteinemia and cognitive impairment and to

Table. Overview of Selected Models Relevant to VCID

<table>
<thead>
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<th>Model</th>
<th>Cognitive Impairment</th>
<th>Brain Pathology</th>
<th>Selected References</th>
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<tbody>
<tr>
<td>Global hypoperfusion: rat 2VO, 2VGO; mouse BCAS, ACAS</td>
<td>Working memory deficits; later, RM deficits (MWM, Barnes maze, Y-maze)</td>
<td>Diffuse WML; some BBB deficit, microglial activation; microhemorrhages at 6 mo</td>
<td>9–12</td>
</tr>
<tr>
<td>Global hypoperfusion: baboon 3VO</td>
<td>Not reported</td>
<td>Progressive, diffuse WML; transient microglial activation; transient global BBB opening</td>
<td>13</td>
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<tr>
<td>SHRSP, with JPD and UCCAo</td>
<td>Memory deficits (MWM)</td>
<td>Diffuse WML; neuroinflammation, BBB deficit</td>
<td>14–16</td>
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<tr>
<td>HHCy in mice, rats</td>
<td>Learning deficits (MWM)</td>
<td>Micro-hemorrhages; CAA</td>
<td>17–20</td>
</tr>
<tr>
<td>Notch3 transgenic mice</td>
<td>Not reported</td>
<td>Vessel fibrosis; later, WML; reduced CBF. No BBB deficit</td>
<td>21–24</td>
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<tr>
<td>ApoE-deficient mice</td>
<td>Learning deficits (MWM, Barnes maze)</td>
<td>BBB deficit (from 2 wk); CAA</td>
<td>25–28</td>
</tr>
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2VGO indicates 2-vessel gradual occlusion; 2VO, 2-vessel occlusion; 3VO, 3-vessel occlusion; ACAS, ameroid constrictor, arterial stenosis; BBB, blood–brain barrier; BCAS, bilateral carotid artery stenosis; CAA, cerebral amyloid angiopathy; CBF, cerebral blood flow; HHCy, hyperhomocysteinemia; JPD, Japanese permissive diet; MWM, Morris water maze; RM, reference memory; SHRSP, spontaneously hypertensive stroke prone rats; UCCAo, unilateral common carotid artery occlusion; VCID, vascular contributions to cognitive impairment and dementia; and WML, white matter lesions.
reduce cerebral blood volume and reactivity measured by absolute, noninvasive, near-infrared spectroscopy. Although the molecular mechanism of hyperhomocysteinemia-induced VCID is unclear, the locus of pathology seems to be vascular, rather than neuronal.

**Animal Models of BBB Dysfunction**

**Pdgfrα−/−** mice deficient in pericytes, the contractile cells that ensheathe capillary vessels, showed progressive BBB breakdown from 1 month of age, with increasing extravasation of plasma proteins in the hippocampus and cerebral cortex. This was accompanied by reduced capillary density and age-dependent reduction in baseline CBF and response to a vasogenic stimulus (whisker twitch). By 16 months of age, the mice exhibited pronounced neuronal loss within the hippocampus, accompanied by impaired performance in a simple assay of learning (novel object recognition task).

APOE genotype is a risk factor for sporadic AD. The APOE ε4 allele increases risk, possibly via a toxic effect of the APOE ε4 gene product, or via loss of physiological APOE function. In an elegant series of target replacement (TR) studies, mice lacking native ApoE expressed the human alleles APOE 2, 3, or 4, under an astrocyte-specific promoter. TR mice carrying only the APOE ε4 allele (like ApoE−/− null mice) exhibited enhanced BBB permeability that was evident by 2 weeks of postnatal age. This was dependent on MMP9 activity, induced via the proinflammatory cytokine cyclophilin-A. None of these changes was evident in APOE2 or APOE3 TR mice. APOE4 TR mice exhibited worse spatial memory relative to age-matched APOE3 TR animals not only at older ages (12 and 24 months) but also in young adulthood (3 months).

Regional CBF was much reduced in the APOE4 TR or ApoE−/− null animals at 9 months of age. CBF could be restored and was at normal levels in double knockout animals, lacking ApoE and the gene for cyclophilin-A. These well-defined transgenic animal systems allow specific biochemical pathways to be explored. The gene product of APOE3 binds to the membrane transporter LRP1, and this suppresses the harmful effects of cyclophilin-A on MMP9 activation and BBB breach. These experiments also suggest that the harmful effect of APOE4 is loss of function, rather than a toxic action of the APOE4 gene product. A functional APOE and LRP1 transport system stimulates clearance of amyloid peptides and possibly other brain parenchymal debris. Furthermore, when APOE4 TR mice are crossed to the APP transgenic mouse models of amyloid deposition, cerebral amyloid angiopathy is significantly increased, suggesting a potential role for ApoE4 in the vascular accumulation of amyloid.

Another molecular participant in β-amyloid clearance from brain tissue is PICALM, a phosphoinositide-binding protein associated with clathrin that is required for endocytosis and internalization of cell surface receptors. PICALM interacts with endothelial LRP1 to mediate β-amyloid clearance from brain tissue. Heterozygous Picalm−/− mice, expressing sub-physiological levels of PICALM protein in brain endothelium, exhibited increased β-amyloid neuropathology and some cognitive impairment and assessed with measures of nest-building and burrowing. PICALM has emerged as a candidate in genome-wide association studies for AD, suggesting a key role in the pathogenesis of AD and dementia.

**CADASIL and CARASIL Mice**

CADASIL (Cerebral Autosomal Dominant Arteriopathy With Sub-Cortical Infarcts and Leukoencephalopathy) and CARASIL (Cerebral Autosomal Recessive Arteriopathy With Sub-Cortical Infarcts and Leukoencephalopathy) are rare monogenic forms of SVD, leading to early onset VCID. In CADASIL, the underlying gene is NOTCH3; in CARASIL, the gene is HTRA1.

Notch3R169C transgenic mice have 4-fold overexpression of CADASIL-associated mutant Notch3. These mice exhibit defective CBF reactivity from 5 months of age, reduced CBF from 12 months, and progressive WML from 18 months. The main WML were microvacuoles within the myelin sheath, suggested to reflect defective ion-water homeostasis. There was no apparent loss of oligodendrocyte density, and axons were intact. The extracellular matrix proteins vitronectin and tissue inhibitor of metalloproteinases-3 (TIMP-3) accumulated in the vascular granular osmiophilic material deposits that are characteristic of CADASIL. Double-transgenic mice that express CADASIL-causing Notch3 mutations, in addition to being heterozygous null for vitronectin, exhibit rescue from WML at 12 to 20 months of age, but not rescue of impaired CBF. Stroke lesions have not been reported for these mice (up to age 24 months). Another transgenic strain has recently been reported, carrying the human genomic NOTCH3 sequence. Knockin Notch3Arg170Cys mouse models, with a mutation in the endogenous Notch3 gene, developed a CADASIL-like vessel pathology and, in addition, some incidence of parenchymal lesions (from 20 months of age). Microinfarcts, microhemorrhages, and behavioral motor deficits were seen in a minority (up to 12%) of these mutant mice up to the age of 13 months.

HTRA1 encodes a secreted serine protease that is involved in transforming growth factor-β signaling. CARASIL-causing mutations result in loss of HtrA1 activity. Brain tissue from Htra1−/− null mice, and fibroblasts from CARASIL patients, exhibited reduced transforming growth factor-β signaling and dysregulation of an extracellular transforming growth factor-β-binding protein that is a novel HtrA1 target. In brain tissue from Htra1 null mice, LTB1 levels were augmented and transforming growth factor-β signaling was depressed.

**Discussion**

**Comorbid Models**

Greater understanding of interactions between risk factors, genotype, and specific vascular lesions (Figure 3) may come from animals with multiple pathologies and comorbidities. Examples are hypertensive rats with Japanese permissive diet and brain hypoperfusion or diet-induced hyperhomocysteinemia combined with AD pathology. Molecular understanding is more advanced in AD than in VCID, and transgenic AD models are well established. VCID–AD overlap and interaction may therefore be explored using vascular challenges combined with brain-injected Aβ peptides or in APP transgenic animals.
Larger Species
Larger animals (primates, dogs, sheep, and swine) have longer natural life span than rodents and offer valuable data relevant to the human brain gyrencephalic anatomy, abundant white matter, and arterial morphology (Figure 2), even though cohort sizes may necessarily be limited. They can be subjected to VCI-relevant risk factors (old age, hypertension, high-fat diet, and physical exercise status). Rhesus macaques 20 to 30 years of age are considered analogous to older people 60 to 90 years of age. Quantitative MRI of these animals shows a highly significant reduction in white matter volume with increasing age. In old dogs, a cognitive dysfunction syndrome, featuring some aspects of VCI, has been described. Experimental sheep models have recently been developed to simulate acute ischemic stroke. Sophisticated cognitive testing paradigms are available for primates. By contrast, cognitive paradigms for large domestic species are currently rudimentary.

Small Species: Zebrafish
Perturbation of FOXC1 (which encodes a forkhead-like transcription factor) in Danio rerio led to cerebral hemorrhages. Genome-wide association studies suggested possible linkage of the FOXC1 locus with SVD phenotype (white matter hyperintensities). Suppression of FOXC1 also affected platelet-derived growth factor (PDGF) signaling and central nervous system development. The zebrafish offers a rapid screening platform for genetic alterations.

Summary
Animal models have great potential to increase our understanding of specific vessel pathologies, how these cause parenchymal lesions, how known risk factors influence vessel and parenchymal changes, and the mechanisms that link them all to VCI (Figure 3).

For example, transgenic animals permit well-controlled testing of molecular hypotheses on a functional pathway, such as ApoE-mediated clearance. CADASIL mice carrying Notch3 mutations combine a known molecular cause with biologically appropriate vessel pathology and parenchymal lesions reminiscent of human SVD. The risk factor hyperhomocysteinemia is induced by dietary manipulation in rodents, which exhibit vessel fibrosis, microhemorrhages, and cognitive deficits. Hyperhomocysteinemia mice and rats offer a valuable platform for identifying the currently unknown molecular targets of hyperhomocysteinemia-related brain disease. Diffuse WML can be induced in rodents after chronic hypoperfusion and also in SHRSP with dietary and surgical comorbidities, in both conditions with some concomitant cognitive deficit. As noted, the vascular pathology in these animals is likely to differ from human VCI (Figure 3).

There are several directions for future progress. In our view, experimental species with closer metabolic and immunologic similarity to humans (primates and larger domestic species) will make preclinical testing of interventions more translational. Given the multifactorial nature of the VCI spectrum, comorbid animals may also accelerate discovery biology for VCI treatments. Although the models discussed here clearly do not reflect the full pathogenic pathway of human disease (Figure 3), they represent a pragmatic test-bed for interventions. VCID is a broad concept, and there is no one optimal VCI model. We hope that this review will assist...
selection of experimental models most relevant to the aspect of VCID under study.

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Disclosures
None.

References


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