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MINI-SYMPOSIUM: ENERGY DEMAND AND ENERGY SUPPLY IN ALZHEIMER'S DISEASE

Cerebral Hypoperfusion and the Energy Deficit in Alzheimer's Disease

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Keywords
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Abstract

There is a perfusion deficit in Alzheimer's disease (AD), commencing in the precuneus and spreading to other parts of the cerebral cortex. The deficit anticipates the development of dementia, contributes to brain damage, and is caused by both functional and structural abnormalities of the cerebral vasculature. Most of the abnormalities are probably secondary to the accumulation of Aβ but the consequent hypoperfusion may, in turn, increase Aβ production. In the early stages of disease, abnormalities that cause vasoconstriction predominate. These include cholinergic vascular denervation, inhibition of endothelial nitric oxide synthase, increased production of endothelin-1 production and possibly also of angiotensin II. Patients with AD also have an increased prevalence of structural disease of cerebral microvessels, particularly CAA and capillary damage, and particularly in the later stages of disease these are likely to make an important contribution to the cerebral hypoperfusion. The metabolic abnormalities that cause early vascular dysfunction offer several targets for therapeutic intervention. However, for intervention to be effective it probably needs to be early. Prolonged cerebral hypoperfusion may induce compensatory circulatory changes that are themselves damaging, including hypertension and small vessel disease. This has implications for the use of antihypertensive drugs once there is accumulation of Aβ within the brain.

INTRODUCTION

A major contributor to the energy deficit in Alzheimer's disease (AD) is the reduction in cerebral perfusion that results from dysfunction and structural abnormalities of the cerebral vasculature. As discussed elsewhere in this mini-symposium, there are also other contributors to the energy deficit in AD, including extracranial abnormalities that affect cerebral blood supply and intracellular disturbances of mitochondrial function, but those are not covered in the present review.

Within this review, we consider three questions:

1. Is cerebral hypoperfusion in AD caused by reduced demand or reduced supply?
2. What are the mechanisms of the hypoperfusion?
3. What are the therapeutic implications?

REDUCED DEMAND OR REDUCED SUPPLY?

The relative contributions of falling metabolic demand and reduction in blood supply vary according to the stage of disease. In preclinical and early AD there is evidence from multiple studies employing a range of methods that the cerebral hypoperfusion is pathological rather than physiological, that is, the decline in perfusion exceeds the reduction in metabolic demand, and causes tissue damage. Metabolic demand may, in fact, be increased during the earliest stages of amyloid accumulation (11, 27).

Some of the data comes from imaging studies using arterial spin-labeled perfusion magnetic resonance imaging (ASL-MRI) or 2-deoxy-2-(18F)fluoro-D-glucose positron emission tomography (FDG-PET) (there is a near-perfect topographical correspondence between changes in ASL-MRI and FDG-PET in AD—see, eg, reference 24). In people with mutations that cause autosomal dominant forms of AD, in whom the timing of onset of the AD is highly predictable, a reduction in glucose uptake is demonstrable by FDG-PET at least 10 years before the onset of clinical disease and before there is detectable atrophy (11). The decline shows a consistent pattern of topographical progression, starting in the precuneus (medial parietal cortex) and extending along the cingulate gyrus, lateral part of the parietal lobe and anterior part of the occipital lobe, then into the rest of the cerebrum.

A similar pattern of progression of hypoperfusion was demonstrated by ASL-MRI in sporadic AD (12). In patients with MCI the hypoperfusion was most pronounced in the precuneus and posterior cingulate cortex but also involved lateral parietal, occipital and frontal cortex. Perfusion declined significantly in the temporal cortex only when patients became demented and did not fall in the

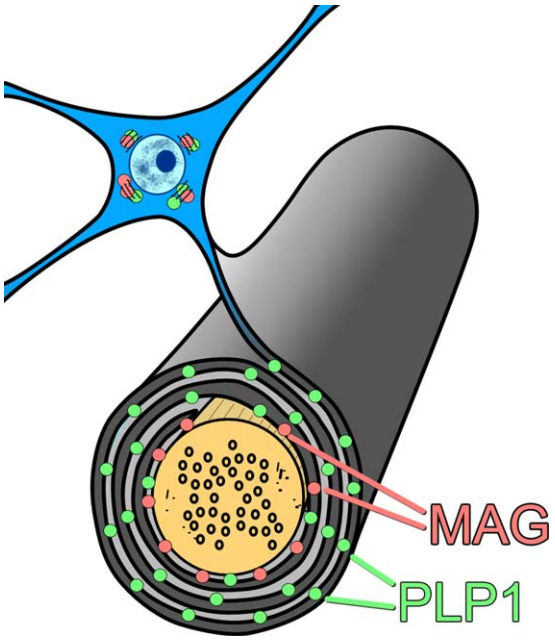

Figure 1. Schematic illustration of the distribution of MAG (pink dots) and PLP1 (green dots) in the myelin sheath. PLP1 is distributed throughout the myelin sheath whereas MAG is inserted only far from the cell body, in the adaxonal loop of myelin, the first part of the sheath to degenerate when blood supply is insufficient to meet the energy demands of the oligodendrocyte. As MAG and PLP1 are stable post mortem and have half-lives of several months, a decline in MAG:PLP1 in post-mortem brain tissue reflects a hypoperfusion-related energy deficit over a relatively long period prior to death. Image adapted from reference 71.

hippocampus. Similar findings were reported in earlier studies on people with MCI (5, 30) and in healthy carriers of the *APOE* ϵ4 allele (65), a strong genetic risk factor for AD. This stereotypical distribution of hypoperfusion does not bear an obvious relationship to the distribution of cerebral atrophy, which correlates with that of neurofibrillary tangle pathology, commencing in the inferomedial part of the temporal lobes before spreading to other parts of the cerebrum (11). The distribution of hypoperfusion/reduced glucose uptake correlates much more closely with that of the preceding accumulation of amyloid (see, eg, references 11, 27).

We recently used a biochemical approach to assess the adequacy of perfusion and oxygenation of the precuneus in early AD. We compared the levels of two myelin proteins, myelin-associated glycoprotein (MAG), which is highly susceptible to reduced tissue oxygenation, and proteolipid protein-1 (PLP1), which is relatively resistant (8, 9). Both myelin proteins are synthesized in the oligodendrocyte cell body and require energy-dependent transport to reach their sites of insertion into the myelin sheath (Figure 1). PLP1 is distributed throughout the myelin sheath whereas MAG is inserted only far from the cell body, in the adaxonal loop of myelin, the first part of the sheath to degenerate when blood supply is insufficient to meet the energy demands of the oligodendrocyte. Both myelin proteins are very stable under post-mortem conditions (9), and as they have half-lives of several months (47, 136), a decline in the MAG:PLP1 ratio in post-mortem brain tissue reflects a pathological reduction in ante-mortem perfusion over a relatively long period prior to death. We showed that the MAG:PLP1 ratio, was reduced in the precuneus by approximately 50% in Braak stage III–IV disease (75), indicating that even in early AD there is a disparity between oligodendrocyte energy demand and supply in the first region of brain to show hypoperfusion.

Tarumi *et al* (131) used near-infrared spectroscopy to compare the tissue oxygenation index (a measure of the saturation of hemoglobin by oxygen) in frontal cortex of people with amnestic MCI and age-matched controls. In amnestic MCI patients the tissue oxygenation index was significantly reduced, both at rest and after a sit–stand maneuver, indicating increased oxygen extraction. Had hypoperfusion been a response to reduced metabolic demand rather than a pathological reduction in blood supply, the tissue oxygenation index would have been higher rather than lower.

Cerebral hypoperfusion predicts the development of dementia in patients with MCI and the rate of cognitive decline in patients with AD (14, 16, 22). The fact that the hypoperfusion actually damages the brain, even in preclinical or early disease, is well demonstrated on imaging of cerebral white matter in people with mutations that cause autosomal dominant forms of AD. A recent study showed that people with such mutations had a greater volume of white matter hyperintensities (WMH) several years before clinical disease, indicating that the hypoperfusion was severe enough to cause tissue damage (67). The increase in WMH was most pronounced in the parietal and occipital lobes; as noted above, this corresponds approximately to the distribution of early amyloid accumulation and reduced perfusion in the overlying cerebral cortex.

The relationship between cortical amyloid and WMH is likely to be relevant to the pathogenesis of the white matter hypoperfusion (see below). Other evidence for a relationship between amyloid burden and WMH comes from an MRI study of 150 cognitively normal people by Scott *et al* (122). The authors found that amyloid burden, as assessed by measuring Aβ42 level in the CSF, was an independent predictor of total WMH volume. Lee *et al* (67) found some correlation between WMH and the presence of microbleeds, suggesting a contribution from cerebral amyloid angiopathy. However, the increase in WMH remained significant after controlling for presence of microbleeds, which were calculated to account for 21% of the association between AD mutation status and WMH.

In later AD, it is likely that the decline in perfusion continues to exceed the reduction in metabolic demand but less so than in early disease. Several functional MRI studies have demonstrated an increase in the regional oxygen extraction fraction (rOEF) in the cerebral cortex and white matter in AD (83, 84, 137), indicating a continued pathological reduction in blood supply. Indeed, in the series of Toghi *et al* (137), rOEF was higher in the cerebral cortex of patients with AD than in those with vascular dementia (in the white matter the increase was more marked in patients with vascular dementia).

Our own studies found the MAG:PLP1 ratio to be reduced in the cerebral cortex in late, as well as early AD (75, 134). However, within the precuneus, the ratio was not as markedly decreased in brain tissue from patients with Braak stage V–VI disease as in those with earlier (Braak stage III–IV) disease and not significantly so in comparison with Braak stage 0–II disease (Figure 2). We interpreted this lessening of the perfusion deficit as being likely to

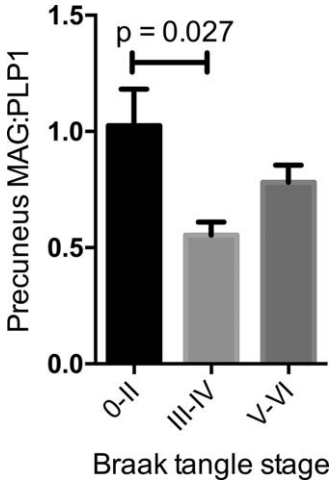

Figure 2. Bar chart showing decline of MAG:PLP1 in the precuneus in AD. The decline is most marked in early disease (Braak tangle stage III–IV). The ratio may rise in late disease as a consequence of falling metabolic demand. Reproduced from reference 75.

reflect falling metabolic demand with increasing synaptic and neuronal damage.

MECHANISMS: METABOLIC VASCULAR DYSFUNCTION

Under normal circumstances, cerebral perfusion is tightly regulated to match the supply of oxygenated blood to metabolic requirements, both of the brain as a whole (through autoregulation—the maintenance of relatively constant blood flow despite changes in perfusion pressure) and of the individual regions within it (through neurovascular coupling) (25, 106). This regulation is effected through multiple neurogenic, myogenic and metabolic pathways. In AD, the activity of several pathways that regulate intracerebral vascular tone and influence neurovascular coupling is abnormally altered. Most of the alterations promote vasoconstriction, acting on smooth muscle cells in the tunica media of arterioles, on pericytes in capillaries or on both types of cell, and reduce tissue oxygenation. Other abnormalities allow inappropriate local vasodilatation, diverting blood away from regions of higher metabolic demand. Both types of alteration have the potential to affect neurological function and, if sustained, to cause permanent damage.

CEREBRAL CORTEX

Cholinergic innervation

Arterioles in the cerebral cortex are innervated by cholinergic nerves, originating in the nucleus basalis of Meynert (139, 142). Stimulation of neurons in the nucleus basalis (see reference 26 for review) or of muscarinic receptors in isolated arterioles (48) causes vasodilatation, partly mediated by stimulation of the production of nitric oxide (NO) (151). Tong and Hamel (139) found a reduction in the cholinergic innervation of cortical blood vessels in AD, mirroring a general loss of cholinergic nerve terminals from the cerebral cortex and in keeping with the loss of neurons from the nucleus basalis from an early stage of disease (see references 31, 68 for review). Cholinergic deafferentation reduces blood flow in the cerebral cortex, as was demonstrated after targeted ablation of cholinergic neurons by administration of 192 IgG-saporin (144). Although interpretation of the findings is complicated by possible effects of cholinergic denervation on neuronal activity and metabolic demand, it seems likely that reduced stimulation of muscarinic receptors in the walls of cortical arterioles contributes to the hypoperfusion of the cortex in AD, and possible beneficial effects of cholinesterase inhibitors in AD may relate partly to augmented cerebral perfusion (13, 26). It should be noted that AD is also associated with alterations in a range of other neurotransmitters that have direct or indirect effects on vascular contractility, including glutamate, γ-aminobutyric acid, noradrenaline (norepinephrine), serotonin and dopamine (28, 34, 44, 63, 113).

Amyloid-β peptide (Aβ)

Perhaps not surprisingly, several of the processes that mediate vascular dysfunction in AD are probably initiated by the accumulation of Aβ. Topical application of Aβ40 or Aβ42 to isolated arteries causes vasoconstriction, Aβ40 being more potent than Aβ42 in this regard (29). The vasoconstriction can be reduced by free radical scavengers and cyclo-oxygenase inhibitors (99, 112, 135, 140). Aβ40 enhances the constriction induced by endothelin-1 (EDN1) and reduces the vasodilatation produced by NO (99, 101).

Aβ40 also induces vasoconstriction *in vivo*, as demonstrated by its application to mouse cortex (89). This can be prevented by administering free radical scavengers or by an M35Nle amino acid substitution in Aβ40 which interferes with its ability to generate reactive oxygen species (89, 90). In a series of studies on mice overexpressing Aβ-precursor protein (APP), Iadecola *et al* showed that elevated endogenous Aβ also caused vasoconstriction from an early age (2 months in Tg2576 mice, well before plaque formation), impaired autoregulation and interfered with neurovascular coupling (in this case the functional hyperemia of the barrel cortex that is normally induced by whisker stimulation) (86–88, 90). Shin *et al* (125) confirmed that Tg2576 mice had an attenuated hyperemic response to hypercapnia and whisker stimulation but were unable to demonstrate this until the mice had reached the age of 9 months, that is, after commencement of vascular deposition of Aβ. The authors suggested that vascular deposition of Aβ was a prerequisite for the vascular dysfunction. It is also possible that the development of vascular dysfunction in these mice simply depends on the concentration of soluble Aβ, which increases with age, although it is not clear why the two research groups found so marked a difference in the timing of onset of the dysfunction. The abnormal cerebral vasoconstriction in Tg2576 mice requires the production of free radicals by nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase), as shown by studies in which NADPH oxidase was either inhibited (103), or inactivated by deletion of Nox2 (104). These findings are in keeping with the studies on isolated arteries, described above.

For simplicity, the various processes that contribute to hypoperfusion in AD are considered under separate headings in the present review. However, as noted below, the different pathways overlap

and interact substantially—particularly insofar as they involve Aβ, for example, in upregulating the production of EDN1 by endothelin-converting enzymes-1 and −2 (ECE1 and ECE2) and that of angiotensin II (Ang II) by angiotensin-converting enzyme (ACE), in reducing NO production by endothelial cells, in binding to and sequestering vascular endothelial growth factor (VEGF) in plaques, and in blocking VEGF receptor 2 (VEGFR-2) signaling in endothelial cells. There is also interaction between the endothelin and renin-angiotensin systems (reviewed in reference 76); Aβ and the cholinergic systems (see references 55, 94, 147 for review); and the cholinergic system and VEGF production (53).

Endothelin system

EDN1 is a potent vasoconstrictor and its concentration is increased significantly in cerebral cortex from patients with AD (75, 97, 134). The increase is demonstrable from an early stage of disease, including within the precuneus (75), the first region in which blood flow declines (see above). Paradoxically, the gene that encodes EDN1 is also upregulated by hypoxia (127). The extent to which EDN1 is increased in AD correlates with the severity of cortical hypoperfusion/tissue hypoxia, as measured by the decline in the MAG:PLP1 ratio.

Both Aβ40 and Aβ42 are capable of increasing EDN1 production *in vitro*: Aβ40 through upregulation of ECE1 in endothelial cells (97, 98), and Aβ42 through upregulation of ECE2 in neurons (96). In cortex from patients with AD the concentration of EDN1 correlates closely with that of Aβ42 but bears no relationship to that of Aβ40 (75), suggesting that the increase in EDN1 and decrease in tissue oxygenation are caused, at least in part, by Aβ42-mediated neuronal upregulation of ECE2. Aβ is a physiological substrate of both ECEs (36–38) and the upregulation of ECE2 and consequent sustained overproduction of EDN1 in AD may simply be an unfortunate side effect of the parenchymal accumulation of substrate in the form of Aβ42 (76). The lack of association between Aβ40 and EDN1 (or MAG:PLP1) does not discount a role for Aβ40 in the vascular dysfunction of AD, but such a role is likely to be predominantly episodic: interfering with autoregulation and neurovascular coupling rather than causing sustained hypoperfusion. Palmer *et al* (98) showed that the enhanced release of EDN1 that follows the addition of Aβ40 to human cerebrovascular endothelial cells in vitro could be prevented by the addition of superoxide dismutase, potentially linking upregulation of ECE1 with the observations of Niwa *et al* (89, 90) on free-radical mediated vasoconstriction, and suggesting that the episodic cerebral vasoconstriction induced by Aβ40 results from a free radical-mediated increase in endothelial ECE-1 activity and EDN1 production.

Angiotensin

Increased production of the vasoconstrictor Ang II may contribute to hypoperfusion of the frontal cortex, where ACE activity (74, 77, 78) and Ang II level (unpublished observations) are elevated in AD, perhaps in response to the accumulation of Aβ42. Miners *et al* (78) showed that ACE activity in SH-SY5Y neuroblastoma cells was upregulated by aggregated Aβ42 (but not Aβ40 or freshly solubilized Aβ42). The relationship between Aβ42, ACE activity, Ang II production and hypoperfusion is, however, less clear-cut than that between Aβ42, ECE1 activity, EDN1 production and hypoperfusion, in that neither ACE activity nor Ang II level was increased in precuneus from patients with AD (75).

Like so many of the dysregulated pathways that are the focus of this review, the renin-angiotensin system has a complex interrelationship with other vasoregulatory processes. ACE cleaves (141) and probably thereby limits the duration of action of the vasodilator bradykinin, the production of which is likely to be elevated in AD, as a result of increased activity of plasma kallikrein (4). Ang II was reported to increase EDN1 production in endothelial (32) and vascular adventitial fibroblasts (1), probably by inducing transcription of the preproendothelin-1 gene (117) and by a mechanism involving NADPH oxidase (2), thereby contributing to EDN1-mediated hypoperfusion. Conversely, hypoxia was shown to upregulate the expression and activation of ACE (62).

Endothelial nitric oxide synthase (eNOS)

NO, a potent vasodilator, is synthesized within the endothelium by eNOS, the activity of which plays an important role in local regulation of the cerebral microcirculation (151). eNOS is activated by a wide range of stimuli, including acetylcholine (40), bradykinin, oxidative stress, shear stress and hypoxia. Both Aβ40 and Aβ42 inhibit eNOS activity. Aβ40 was reported to do so through a mechanism that depends on protein kinase C (46), and Aβ42 through interfering with Akt/GSK-3β signaling and a mechanism involving interaction of eNOS with heat shock protein 90 (64, 128). Mice partially deficient in eNOS develop cognitive impairment associated with a range of neuropathological abnormalities, including cerebral amyloid angiopathy (CAA) and disruption of the blood–brain barrier (BBB), largely confined to the temporoparietal and retrosplenial granular cortex and hippocampus (129). Jeynes and Provias (52) reported a significant negative correlation between the number of eNOS-immunolabeled capillaries and the density of neurofibrillary tangles and Aβ plaques in sections of temporal and calcarine cortex in AD.

WHITE MATTER

As noted above, most of the studies on mechanisms of vascular dysfunction in AD have used rodent models and have focused on cerebral cortex. Whilst several of the local metabolic abnormalities that contribute to hypoperfusion of the cerebral cortex may also apply in the white matter, our studies have highlighted differences that are relevant to our understanding of the pathogenesis of ischemic white matter damage in AD and have implications for treatment.

Whereas we found the concentration of EDN1 to be elevated approximately twofold in the cerebral cortex in AD, presumably in response to Aβ42-induced upregulation of ECE2, EDN1 level was significantly reduced in the underlying white matter (8, 75). This reduction occurred in association with a modest decline in MAG and in MAG:PLP1 in the white matter (9) (in keeping with other evidence of ischemic white matter damage in AD, eg, on neuroimaging, as discussed above). The relationship between MAG:PLP1 and EDN1 in the white matter was the converse of that in the cortex: in the cortex MAG:PLP1 and EDN1 correlated negatively and in the white matter they correlated positively (75). White matter hypoperfusion in AD is not therefore caused by increased white matter EDN1, which falls as would be expected physiologically in

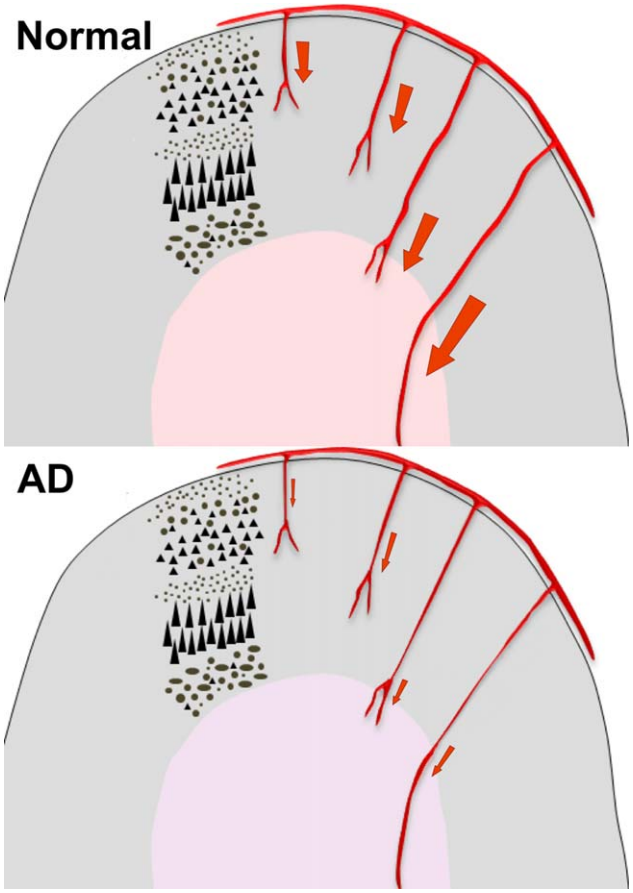

Figure 3. Perforating arterioles that arise from meningeal branches of the major cerebral arteries supply both the cerebral cortex and the underlying white matter. Excessive vasoconstriction within the cerebral cortex in AD affects not only cortical arterioles but also perforating arterioles that traverse the cortex. Thus vasoconstriction within the cerebral cortex contributes to hypoperfusion of the white matter even if arterioles in the white matter are not themselves constricted. Modified from reference 23.

response to hypoperfusion. However, MAG:PLP1 in the white matter did correlate positively with the concentration of EDN1 *in the overlying cortex*, suggesting that hypoperfusion of the white matter in AD results partly from vasoconstriction of perforating arterioles as they traverse the cortex (Figure 3) (23, 75). This mechanism of white matter hypoperfusion is likely to be relevant to other Aβ-dependent processes that increase vasoconstriction within the cortex, including cholinergic denervation, Ang II production and reduction in activity of eNOS.

MECHANISMS: STRUCTURAL ABNORMALITIES OF THE CEREBRAL VASCULATURE

The abnormalities described above affect vascular function but are not associated with long-lasting structural alterations. As recently reviewed (71), patients with AD also have an increased prevalence of structural disease of cerebral microvessels, particularly CAA and capillary damage. They may also have more severe non-amyloid small vessel disease (SVD) than elderly people without AD but most of the cited evidence is indirect, based on the identification of white matter abnormalities on neuroimaging, and as likely to have resulted from CAA or capillary damage as from SVD. An MRI-based study of regional cerebrovascular resistance (CVRi) found this to be increased in several regions of brain that are not affected by CAA and have a predilection for SVD, including thalamus and caudate nucleus (85), but the pathological substrate of the increased CVRi remains to be demonstrated. For further consideration of the possible association of SVD with AD, see reference 71.

CEREBRAL AMYLOID ANGIOPATHY

Most patients with AD have CAA, in some series over 90%, compared with about 30% in elderly controls (21, 42, 69, 72, 73, 143, 146). In many cases the CAA is relatively mild, affecting only occasional arterioles in the leptomeninges, but some patients have widespread involvement of cortical and meningeal arterioles, as well as deposition of Aβ in the adventitia of meningeal venules. In patients with AD, possession of *APOE* ϵ4 is a risk factor for more-severe CAA (21, 111, 121) and is strongly associated with capillary CAA (6, 69, 133) (in controls, arteriolar Aβ amyloid angiopathy is more strongly associated with *APOE* ϵ2). In the majority of patients CAA is restricted to the cerebral cortex and overlying leptomeninges but it may also involve the cerebellum (particularly the meningeal vessels) and occasionally the brain stem. Aβ may also accumulate in the walls of capillaries, sometimes extensively so. Capillary CAA predominantly involves the entorhinal and occipital cortex but can be present in other parts of the neocortex and is often, but not always, associated with severe arteriolar CAA.

CAA has several adverse effects on cerebral perfusion. Perhaps the most widely recognized are cerebral micro-hemorrhages and larger lobar hemorrhages, but there is also extensive documentation of ischemic abnormalities (predominantly cortical microinfarcts) (3, 18, 41, 43, 93, 138), some of which are caused by local thrombosis, some by the marked narrowing of severely affected blood vessels, and some probably by impaired neurovascular coupling. Evidence of neurovascular decoupling in human patients comes from MRI studies of occipital vascular reactivity in response to visual stimulation in patients with probable CAA (35, 107, 126). Peca *et al* (107) also found that impaired neurovascular coupling, as evidenced by lower functional MRI responses to visual stimuli, was associated with more microbleeds and a higher volume of white matter lesions, linking impairment of neurovascular coupling with severity of tissue damage.

CAPILLARY DAMAGE

The capillary bed constitutes much the largest part of the cerebral vasculature and is also the most important in terms of metabolic homeostasis. Yet the contribution of capillary damage to hypoperfusion in AD has been somewhat neglected, perhaps because of the small size and inconspicuous histological appearance of individual capillaries.

Despite the hypoperfusion, the density of capillaries in the cerebral cortex is unchanged or reduced in AD (8, 15, 17, 45, 60, 134),

and more of them show degenerative changes in AD than in age-matched controls (7, 20, 51, 123). Both endothelial cells and pericytes are affected, their degeneration eventually leaving residual "string" vessels consisting solely of tubes of collagen. These degenerative changes occur despite a significant increase in the concentration of VEGF in AD (54, 75, 130, 134), which would be expected to promote angiogenesis, with the formation of new capillaries (92, 150). Several factors may contribute to this lack of angiogenic response. Aβ peptides have direct anti-angiogenic activity (99, 100, 102) and also bind to VEGF receptor 2, blocking VEGF signaling. In addition, Aβ within plaques binds and thereby potentially sequesters VEGF, interfering with its biological availability (105, 148).

At the level of the capillary bed, degeneration of pericytes has emerged as a key contributor to hypoperfusion. Changes in the contractile activity of pericytes modulate capillary caliber and cerebral blood flow and probably play an important role in neurovascular coupling (10, 49, 108). Dore-Duffy *et al* reported that pericytes in primary cultures express both EDN1 and its two receptors (EDNRA and EDNRB) (33). The authors also provided *in vivo* evidence that EDN1 (which is elevated in AD—see above) contributes to the regulation of capillary perfusion through binding to EDNRA receptors in pericytes. Experimental traumatic brain injury in mice caused an increase in the number of smooth muscle actin-positive pericytes around capillaries, a rise in capillary EDN1, and reduced capillary diameter. These changes could be prevented by administration of an EDNRA antagonist. Pericytes are also important for maintenance of the BBB (10).

A series of studies by Zlokovic *et al* have shown that loss of pericytes exacerbates multiple pathological processes in AD (145), including hypoperfusion and disruption of the BBB (10, 123), accumulation of Aβ40 and Aβ42, tau pathology and neuronal loss (120). Montagne *et al* (80) quantified BBB permeability in the hippocampus of young and older adult volunteers by dynamic contrast-enhanced MRI, and showed the degree of increase in permeability to correlate with the CSF:plasma albumin ratio (a marker of BBB breakdown) and the CSF concentration of soluble platelet-derived growth factor receptor β (a marker of pericyte injury). Both BBB permeability and pericyte injury were more pronounced in participants with MCI than in older individuals who were cognitively normal.

THERAPEUTIC IMPLICATIONS

There is therefore overwhelming evidence of a wide range of functional and structural abnormalities of the cerebral microvasculature in AD, that contribute to hypoperfusion and the resulting energy deficit as well as to other aspects of the disease. Implications for therapy are both specific and general. Specific implications concern the potential for targeting of particular pathways or receptors to ameliorate the vascular abnormalities—particularly those that are not the result of structural changes. General implications relate to the timing and broader consequences of intervention.

Several of the pathways implicated in abnormal microvascular function in AD are potentially amenable to treatment. The cholinergic system is, of course, already routinely targeted in AD patients through the administration of cholinesterase inhibitors. These drugs improve cerebral perfusion in mild to moderate disease (19), and several studies found evidence of an association between cognitive response and cerebral blood flow (13, 26, 124). The potential for intervention in the renin-angiotensin system has been extensively reviewed (56–59) and the effects on cognition and cerebral blood flow of losartan, an angiotensin receptor antagonist, are currently being tested in a multicenter UK clinical trial (115).

Another potential target is the endothelin system. Bosentan, a non-selective EDNR antagonist (119), improves pulmonary blood flow and exercise tolerance in patients with pulmonary hypertension, another disease in which there is elevated production of EDN1 (114, 118). Bosentan also preserves endothelium-dependent aortic and carotid vasodilatation in Tg2576 mice (39). Selective EDNRA receptor antagonists such as zibotentan (50, 81) offer theoretical advantages, in that they target the predominant type of EDN1 receptor responsible for mediating vasoconstriction in both smooth muscle cells of cerebral arterioles and pericytes that surround capillaries (33, 91). For discussion see reference 95.

Interventions aimed at reversing functional abnormalities of the vasculature in AD have the potential not only to improve symptoms but also to slow the progression of disease. Hypoperfusion probably increases the production of Aβ42, thereby accelerating the progression of disease. Simulation of neuronal ischemia *in vitro*, or experimental cerebral hypoperfusion in animal models increases Aβ42 production through multiple mechanisms (reviewed in references 70, 71), including upregulation of amyloid-β precursor protein and β-secretase and possibly reduced neprilysin-mediated degradation. Indirect evidence of a hypoperfusion-induced increase in Aβ comes from observations in patients who had survived a recent cardiac arrest (149) or diffuse traumatic brain injury with cerebral edema (and, therefore, almost certainly hypoperfusion) (79). Both groups had elevated serum Aβ42 over several days. In the patients with diffuse traumatic brain injury, Aβ42 was also monitored in the CSF where the level declined, arguing against non-specific leakage of Aβ from damaged brain tissue as the explanation for the rising level in the serum.

The timing of intervention is likely to be critical, as prolonged hypoperfusion causes permanent brain damage (as discussed above), and as the disease progresses the balance tends to shift from metabolic to structural vascular dysfunction (Figure 4). It seems possible too that prolonged cerebral hypoperfusion may induce compensatory changes in the circulation that are themselves damaging, including hypertension and SVD. Mid-life hypertension is significantly associated with AD (109, 110). Several clinical studies have reported an association between hypertension before the age of 65 years (particularly if there is elevation of diastolic blood pressure) and later development of AD (61, 66, 82). Hypertension in cognitively normal adults with at least 1 *APOE* ϵ4 allele was associated with increased binding of the Aβ tracer F18–labeled florbetapir (116). The conventional interpretation is that hypertension increases the risk of developing AD by promoting the accumulation of Aβ. However, another possible explanation is that hypertension is a physiological response to tonic cerebral vasoconstriction induced by mid-life accumulation of Aβ; a means of maintaining cerebral perfusion. There is experimental evidence that this is the case, in that cerebroventricular infusion of Aβ was shown to cause a progressive, highly significant rise in blood pressure in rats (132). This has obvious implications for autoregulation and for the treatment of hypertension once Aβ has begun to accumulate, and suggests that blood pressure in such patients should be lowered only cautiously, ideally with monitoring of the effects on cerebral perfusion.

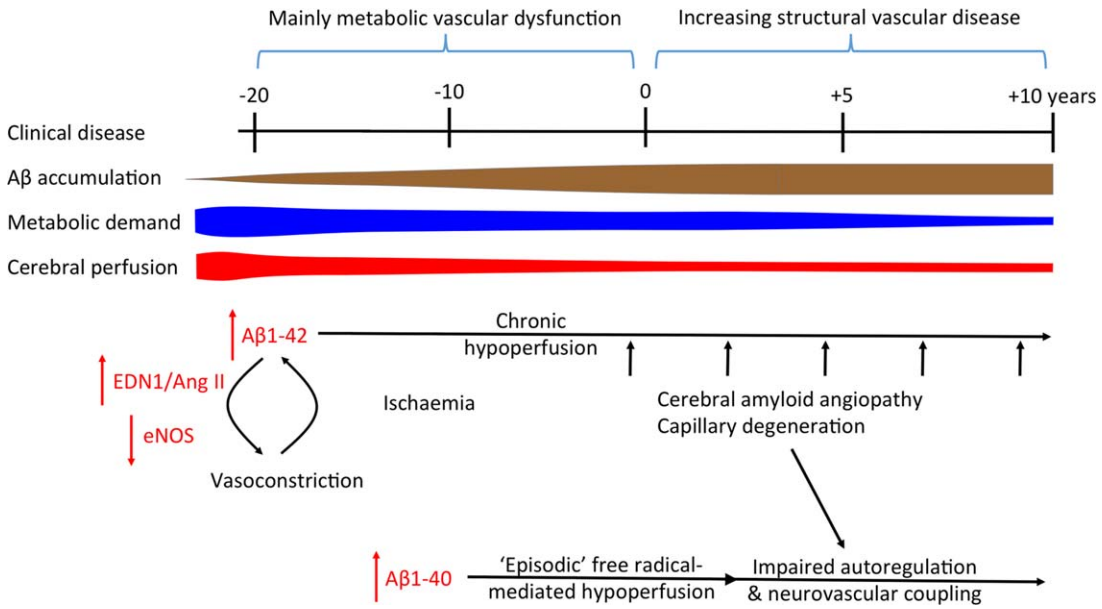

Figure 4. Shift from purely metabolic to structural vascular dysfunction over the course of AD (in relation to the onset of clinical disease, at 0 years). After a brief period of increased metabolic demand and cerebral blood flow, the progressive accumulation of Aβ in early (preclinical) stages of AD drives several metabolic pathways that lead to excessive vasoconstriction and reduced cerebral perfusion. Cerebral perfusion declines faster than metabolic demand. Aβ42-induced metabolic processes may be more important in driving chronic hypoperfusion, and Aβ40-induced processes in impairing vascular responsiveness. As the disease progresses, capillary damage and, in many patients, CAA, become increasingly important contributors to both chronic hypoperfusion and abnormalities of autoregulation and neurovascular coupling.

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