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Decreased myelin lipids in Alzheimer's disease and vascular dementia

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ABSTRACT - The lipid composition of white matter and myelin from the semioval centre was studied in autopsy material from cases with Alzheimer's disease (AD) ($n = 11$), vascular dementia (VD) ($n = 7$), and age-matched controls ($n = 11$). In AD and VD the white matter content of phospholipids and cholesterol was reduced to 72–76% of the control values ($P < 0.01$), the diminution of cerebrosides and sulphatides was more pronounced (55–69%) ($P < 0.001$) while the concentration of gangliosides did not change significantly (87–90%). The myelin composition was the same in the 3 groups, suggesting that the white matter involvement is not caused by alteration of the myelin structure. The altered lipid composition in white matter in AD and VD suggests that the myelin sheath is the primary lesion site.

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Binswanger's disease is a relatively rare, neuropathologically defined, subcortical vascular dementia disorder with lacunar and white matter infarcts (1) and diffuse white matter lesions (2) associated with hypertension and hypertensive vascular changes. With the emergence of modern brain imaging techniques, the presence of this disorder is more often suspected (3). Other types of vascular dementia (VD) such as multi-infarct dementia and probable VD are also associated with radiological signs of white matter changes (4–6). Until recently, when Englund & Brun (7, 8) claimed that white matter changes in Alzheimer's disease (AD) are common neuropathological findings (60%), few commented on this issue (9–11). In addition, modern brain imaging techniques show that white matter involvement in AD is relatively common (12–14, 5).

Despite the frequency of morphologically defined white matter changes in dementia, there are few reports on the white matter lipid constituents. Sjögren et al (15) found the same lipid pattern in white matter from cases with AD and age-matched cases with other neuropsychiatric conditions, when the composition was expressed on a dry weight basis. Bowen et al (16) examined compounds characteristic of myelin temporal lobe white matter in AD. Compared with normal elderly controls the cerebrosides in AD patients were not significantly reduced. Gottfries et al (17) reported significant reductions of white matter lipids from the semioval centre in AD. Malone et al (18) found a reduction of myelin in white matter association areas in individuals with AD compared with non-neurological control cases. Englund et al (19, 20, unpublished observations) presented data that suggest a correlation between reduction in gangliosides and cerebrosides and the degree of histopathologically defined lesion in white matter. In a preliminary communica-

Table 1
Major lipids in white matter of brains from AD and VD cases compared with age-matched controls

	Controls ($n = 11$)		AD ($n = 11$)		AD/ control	VD ($n = 7$)		VD/ control
	mean	SD	mean	SD	(%)	mean	SD	(%)
Phospolipid**	94	12	70	14	(74)	70	23	(74)
Cholesterol**	105	13	80	18	(76)	76	14	(72)
Cerebroside***	39	6	27	5	(69)	24	5	(62)
Sulphatide***	15	4	9.9	2.8	(66)	8.3	2.8	(55)
Ganglioside (NS)	1.10	0.13	0.96	0.13	(87)	0.99	0.29	(90)

Expressed in pmol/g wet weight. AD = Alzheimer's disease; VD = vascular dementia. Group differences according to Kruskal-Wallis test: $^{**}P < 0.01$; $^{***}P < 0.001$; NS = not significant.

tion, Svennerholm et al (21) found a significant reduction of myelin in white matter from the semioval centre in AD.

The aim of this study was to extend the analysis of white matter lipid constituents in AD compared with an age-matched control group and to examine whether white matter changes also occur in vascular dementia.

Material and methods

Brain material

The brains of 11 subjects with clinically diagnosed AD (M/F:2/9) were investigated. The mean age was 82.3±5.8 years (range 75–93), and the mean duration of disease was 9.9 years (range 6–19). Two of the 11 cases had early onset AD with dementia appearing at 62 and 65 years of age, respectively. All the patients had dementia with insidious onset, which progressed slowly to an end phase of severe dementia. They had no signs or symptoms of cerebrovascular disease. At autopsy the brains had no macroscopic signs of infarcts or other changes that could account for the dementia. A microscopic investigation confirmed the AD diagnosis.

The brains of 7 subjects with VD (M/F:4/3) were investigated. The mean age at death was 80.7±5.4 (range 74–87) and duration of disease was 4.7 years (range 1–10). A history of stroke and/or fluctuating course was noted in all cases. In the terminal phase all patients were severely demented. At autopsy all brains showed at least one macroscopic brain infarct. The mean number of infarcts was 2.5 (range 1–5) and the mean volume of infarcted tissue was 7.3 ml (range 2–12.5 ml). Neurodegenerative changes of the Alzheimer type were quantitatively insignificant and within the range of controls. The VD material was also investigated for grey matter neurotransmitter deficits, which has been reported previously (22).

Eleven brains from age-matched controls (M/F:8/3; 80.7±5.4 years, range 73–91) were investigated. The patients had died from somatic disorders and they had no history of neuropsychiatric disease. The brains had no macroscopic signs of infarcts or other brain damage. Microscopic investigation showed low levels of neurodegenerative changes of the Alzheimer type.

The AD and VD cases and the controls were stored in a room at 4°C before autopsy, which was performed 44±13 h (range 32–78) in AD, 54±20 h (range 32–81) in VD and 67±30 h (range 19–124) after death in the controls. The autopsy latencies did not differ significantly between the 3 groups. The brains were dissected immediately after being removed from the skulls. Approximately 5 g white matter was cut off from the frontal semioval centre. In the VD cases only tissue specimens without macroscopic infarctions were taken for analysis. The samples were frozen and kept at -80°C until analysed. The specimen were homogenized by being pulverized in liquid nitrogen, and portions of the frozen powder were used for analysis.

Isolation of myelin

Myelin was isolated from 2 g of white matter according to the procedure described by Norton & Poduslo (23). The final myelin pellet was suspended 3 times in 0.156 M KCl and centrifuged

at 20,000 × *g* between each suspension to remove sucrose, and was then lyophilized and dried to constant weight in a vacuum desiccator.

Determination of white matter and myelin components

Duplicate samples of 100 mg of white matter and 30 mg of myelin were homogenized and extracted twice with 5 ml chloroform-methanol water 4:8:3 (by volume) (24). The lipid extract was purified by chromatography on Sephadex G 25 (25) and DEAE-Sepharose (26). Phospholipids and cholesterol were assayed according to Svennerholm & Vanier (27). Cerebrosides and sulphatides were quantified by densitometric TLC scanning at 450 nm after visualization of the lipids with cupric acetate (28). Protein-bound and lipid-bound (ganglioside) sialic acid was measured by the resorcinol method and the ganglioside pattern by densitometric TLC scanning at 620 nm (24).

Statistical analysis

Statistical analysis of group comparisons was performed using the Kruskal-Wallis test.

Results

Lipid composition

The concentrations of the membrane lipids in central semioval white matter were reduced in both AD and VD (Table 1). The phospholipids and cholesterol decreased to 72–76% of the control values in the 2 groups ($P < 0.01$), while the reduction of cerebrosides and sulphatides was more pronounced (55–69% of the control values) ($P < 0.001$). However, the concentrations of gangliosides did not differ between the 3 groups. The ganglioside pattern was similar in all 3 groups (Table 2).

Table 2
Ganglioside pattern in white matter of brains from AD and VD cases and age-matched controls

Ganglioside	Controls ($n = 11$)		AD ($n = 11$)		VD ($n = 7$)	
	mean	SD	mean	SD	mean	SD
GM2	2.8	1.7	3.2	1.4	2.2	1.6
GM1	22.9	2.9	20.5	2.7	19.3	8.8
GD3	6.3	3.5	7.6	4.0	9.7	4.7
GD1a	17.7	2.0	20.3	4.6	22.0	7.3
GD1b	26.0	3.8	24.0	4.9	20.7	3.3
GT1b	22.4	4.3	21.7	4.7	19.3	3.7
GQ1b	3.7	1.4	3.3	1.3	4.0	2.0

All values are percentages. AD = Alzheimer's disease; VD = vascular dementia. Group differences according to Kruskal-Wallis test: NS.

Constituents of myelin

The yield of isolated myelin was lower in AD and VD than in the control group (57 and 70%, respectively) ($P < 0.05$) (Table 3). The composition of isolated myelin did not differ between the 3 groups (Table 3).

Discussion

In white matter in AD and VD, the characteristic myelin constituents, cerebrosides and sulphatides, declined more (55–69% of controls) than phospholipids (74% of controls). The yield of isolated myelin in AD and VD (during the myelin isolation mainly axonic tissue is removed (23)) was reduced to approximately the same extent (57–70%) as the myelin-specific lipids (55–69%). This, together with the fact that the myelin lipid composition was the same in all 3 groups, suggests that the diminution of myelin lipids in white matter is not caused by a selective loss of certain lipids but rather by a reduced amount of myelin of normal composition.

In white matter, the axonal membrane has the highest ganglioside concentration while the cerebrosides are restricted to myelin. Gangliosides were virtually unchanged (87–90% of the control values) and cerebrosides sharply declined (62–69%), indicating that myelin is more affected than axonal membranes. This suggests that the primary site of changes is the myelin sheath.

AD, in the classical sense, is mainly a parieto-temporal grey matter disorder (29, 30), whereas the present concept of AD is used independently of topographic involvement (31). The reductions of myelin and membrane lipids in the semioval centre in our AD material (mainly late onset AD) show that subcortical white matter regions are involved. Brun & Englund (8) also found mild cortical changes combined with severely altered white matter in several AD cases. Thus, the possible role of subcortical white matter lesions in

Table 3
Myelin yield and composition in white matter of brains from AD and VD cases compared with age-matched controls

	Controls ($n = 5$)		AD ($n = 5$)		AD/ control	VD ($n = 6$)		VD/ control
	mean	SD	mean	SD	(%)	mean	SD	(%)
Myelin yield* (mg dry weight/g white matter wet weight)	114	21	65	7	(57)	80	25	(70)
Myelin composition (NS) (μmol/g dry weight)								
phospholipid	362	13	370	6		344	29	
cholesterol	435	23	431	12		411	23	
cerebroside	132	13	119	16		113	13	
sulphatide	50	5	54	5		53	9	
Gangliosides (μmol NeuAc/g tissue)	2.26	0.08	2.11	0.17		nd	nd	
Sialoglycoproteins (μmol NeuAc/g tissues)	0.58	0.05	0.59	0.07		nd	nd	

NeuAc = *N*-acetylneuraminic acid; nd = not determined; AD = Alzheimer's disease; VD = vascular dementia. Group differences according to Kruskal-Wallis test: $*P < 0.05$; NS = not significant.

the pathogenesis of AD, especially late onset AD, has to be considered. Our findings in VD also show that subcortical white matter is affected not only in infarcted areas but more generally. Thus, subcortical white matter lesions may play a role in the pathogenesis of VD, which is in line with the opinion of Roman (6).

Brun et al (8, 19) interpreted their findings of white matter changes in AD to indicate that there was neither pure demyelinating disease, such as multiple sclerosis, nor purely secondary Wallerian degeneration. They suggested instead an independent white matter disorder of cerebrovascular hypoperfusion or hypoxic origin. The neuropathological findings in AD are then very similar to those described by Plum et al (32) in delayed postanoxic encephalopathy. In these cases there was relatively little neuronal damage, but massive destruction of myelin with relative sparing of axis cylinders. The central nervous system myelin is proliferated and maintained by the oligodendroglial cell. The ratio of cell body surface membrane to myelin membrane is not known precisely, but Racine (33) has suggested that it is at least 1:620. These oligodendroglial cells must then have large metabolic requirements, and it seems reasonable that in vascular dementia, hypoperfusion might occur in small areas, leading to hypoxia and hypoglycemia of the cells and subsequent myelin loss. A similar hypoperfusion caused by fibrohyaline sclerosis of white matter arterioles and cardiovascular hypotensive disorder was suggested in AD by Brun & Englund (8). Our findings of a comparable loss of myelin lipids and a normal composition of the remaining myelin in late onset AD and VD support our hypothesis of a similar pathogenesis for the white matter changes in these 2 disorders.

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