Short report

A comparative study of a silver stain and monoclonal antibody reactions on Alzheimer's neurofibrillary tangles

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SUMMARY Serial sections of brains of eight cases with Alzheimer's disease were stained by methods utilising silver and three different monoclonal antibodies. The numbers of Alzheimer's neurofibrillary tangles (NFT) were counted in the silver stained sections and compared with the numbers detected by three monoclonal anti-NF antibodies: RT97, BF10 and 8D8. The monoclonal antibody (Mab) BF10 demonstrated NFTs more clearly than Mab 8D8, but neither detected as many tangles as the silver staining method. Mab RT97 reacted well with NFTs in freshly processed specimens but not following prolonged fixation.

Some of the most widely-used techniques for the demonstration of Alzheimer's neurofibrillary tangles (NFTs) are those utilising silver impregnation because of their consistent results.¹⁻¹⁰ Recently the possibility of using monoclonal antibodies which react with paired helical filaments or neurofilament epitopes in NFTs has been realised.^{6,11-14} It was hoped that such reactions would simplify the identification of NFTs because of their specificity. Furthermore such stains could be used both on fresh and preserved specimens. However, direct comparisons of silver and monoclonal antibody staining have rarely been performed.¹¹ The aim of this study was to compare the use of three such monoclonal antibodies for the identification of NFTs in sections of recently fixed brains and those preserved in formalin for several years with a reliable and convenient silver method of staining.

Materials and methods

Eight cases of Alzheimer's disease with progressive dementia over several years, and with typical neuropathological

findings, were selected (table 1). Brain tissues had been fixed in formalin for approximately 4 weeks before being sliced and for between 4 weeks and 10 years before being embedded in paraffin, and sections cut and stained. Eight serial 6 μ sections were obtained of the hippocampus and cerebral cortex (association cortex of frontal or temporal lobe). The first and eighth sections were stained with the Cross modification of the Palmgren silver stain.³ The second/third, fourth/fifth, and sixth/seventh were stained with the monoclonal antibodies RT97, BF10 and 8D8 respectively. These antibodies were produced as previously described using as immunogen either Triton X-100-insoluble rat brain protein (RT97), Triton X-100-insoluble human brainstem (8D8), or a crude soluble protein fraction (1h at 150,000g supernatant) from two pooled hippocampi (BF10).¹¹ Previously BF10 had been shown to react predominantly with 155,000 MW bovine neurofilament protein and the corresponding polypeptide in human brain. RT97 and 8D8 have been found to bind most strongly to the 210,000 MW bovine neurofilament protein and equivalent human polypeptide.^{11,12} All three monoclonal antibodies have been shown previously to react with Alzheimer's NFTs as well as with normal neurofilaments.^{11,12}

Identical areas measuring 3 mm² for counting were identified using the silver stained specimens. Under 230 times magnification using a Leitz Micropromar projector, which projected the field of view on to a screen, the number of NFTs in three fields on each section were counted. The fields were selected for having distinguishing vascular landmarks that enabled the same region to be identified in adjacent sections. Counts of NFTs detected by Mab BF10, 8D8

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Table 1 Number of NFTs in three areas of each section

Case No	1N	1N	1N	1N	2N	2H	3N	3N	4N	4H	5N	5H	6N	6H	7N	7H	8N	8H
Age/sex	73/F				86/F		76/F		86/F		88/M		95/M		85/F		64/F	
Time in formalin	3 months				7 mth		2 yr		2.5 yr		5 yr		5.5 yr		9.5 yr		10 yr	
RT97	22	6	35	1	37	4	12	6	/	—	/	/	/	/	/	/	57	4
	30	3	22	5	40	4	12	3	/	—	/	/	/	/	/	/	63	4
	17	4	25	0	21	3	15	5	/	—	/	/	/	/	/	/	49	3
AV	23	4	27	2	33	4	13	5	/	—	/	/	/	/	/	/	56	4
Silver	29	10	62	18	41	7	19	22	2	36	43	16	25	11	45	74	19	7
	35	2	48	12	39	15	6	11	13	15	44	25	29	10	40	42	28	15
	13	1	45	9	38	27	13	14	14	22	50	13	7	15	55	31	26	27
AV	26	4	48	13	39	16	13	16	8	24	46	18	20	12	47	49	24	16
BF10	12	5	20	7	15	10	6	4	2	28	69	4	44	5	13	14	—	10
	28	2	28	2	13	19	7	10	8	54	64	3	34	5	4	7	—	19
	20	3	34	3	16	20	16	8	5	58	58	2	24	4	6	10	—	20
AV	20	3	27	4	15	16	10	7	5	47	64	3	35	5	8	10	—	16
8D8	19	2	32	3	42	6	4	3	4	3	30	8	17	1	17	30	—	6
	20	3	29	2	39	7	3	5	3	4	32	9	29	3	21	28	—	7
	20	3	19	1	41	6	14	4	1	3	26	3	17	1	10	21	—	6
AV	20	3	27	2	41	6	7	4	3	3	29	7	21	2	16	26	—	6

Index: —, no slide; /, poor stain; H, hippocampus; N, neocortex.

and silver were compared using a non parametric Wilcoxon test.

Results

The most satisfactory staining with silver and the Mabs was obtained in the sections prepared from brains that had been stored in formalin for 7 months or less. In these brains most NFTs shown with silver were readily visible with all the Mabs (fig). However, NFTs were most easily seen in the silver stained sections in which there was a sharp contrast between the densely stained NFTs and the unstained background. The Mabs gave a reaction in some neurons without well defined NFTs and reacted with some elements in the rest of the neural tissue thus making interpretation more difficult. In the sections from brains immersed in formalin for 2–10 years the staining with silver progressively deteriorated. When compared with the staining obtained by the same technique in sections prepared from blocks embedded in paraffin immediately after the brains were sliced, reactions with the Mabs after prolonged formalin fixation were even more capricious. Most of these cases showed fewer NFTs with Mabs than with silver, and of the Mabs, RT97 was the least satisfactory and BF10 gave the most reliable reactions (table 1). Taking the results of counting NFTs overall, the median number counted were 18, 10 and 8 per field for the silver stain and Mabs BF10 and 8D8 respectively. The difference in counts between silver and each of the Mabs was significant ($p < 0.002$) but that between the two Mabs was not significant (table 2). RT97 had to be excluded

Table 2 Results of non-parametric Wilcoxon test

Comparison	N	Statistic	p value	Median
(Silver-BF10)	51	915.0	0.003	7.5
(Silver-8D8)	51	1152.0	0.000	10.5
(BF10-8D8)	51	715.0	0.111	2.0

from the Wilcoxon test owing to lack of satisfactory staining in the majority of cases.

Discussion

The present study demonstrated that the use of a silver stain revealed NFTs more clearly and in greater quantity than the three monoclonal antibody stains.¹⁰ Besides reacting with fewer tangles than silver, the monoclonal antibodies reacted with some neuronal perikarya lacking well defined NFTs and this made the distinction of cells with NFTs more difficult than with silver stains.¹³ Problems were experienced more when using the antibodies on brains that had undergone prolonged storage for several years in formalin. Thus, in our opinion a silver stain remains the method of choice for the routine demonstration of NFTs.

A common characteristic of all the staining techniques was that they all stained the NFTs with a greater degree of intensity in the freshly processed brains which had not been stored for long. For example Mab RT97 only reacted well with NFTs in the two freshly processed samples.

Comparison of silver stain and monoclonal antibody reactions on Alzheimer's neurofibrillary tangles

Fig In these sections the more darkly stained regions represent NFTs which have been localised by the use of silver (a) or one of the monoclonal antibodies: (b) RT97, (c) BF10 and (d) 8D8. ($\times 130$)

The difference in stainability of NFTs with various techniques is also an important consideration. It is not only necessary for morphometric evaluation where different stains would yield different data,² but also for analysis of the fundamental structural elements composing NFTs.^{11 12} In the latter case the use of monoclonal antibodies to define neurofilament and other epitopes is essential. Mab reactions also have the advantage of working well on frozen sections which are difficult to stain with silver satisfactorily.

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