Acta Neuropathol (1995) 90: 135–141

REGULAR PAPER

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Cerebral β amyloid deposition in patients with malignant neoplasms: its prevalence with aging and effects of radiation therapy on vascular amyloid

Received: 15 July 1994 / Revised: 31 January 1995 / Accepted: 2 March 1995

Abstract We examined immunohistochemically 123 autopsy brains from patients aged between 30 to 59, who died as a result of malignant neoplasms. Using antiserum to amyloid β protein (Aβ), we found that cerebral Aβ deposits began in the subjects' fifth decade; its prevalence was 0%, 9.8% and 21.5% in the fourth, fifth and sixth decades, respectively. The major form of Aβ deposition was diffuse-type plaques, although one third of the brains with Aβ deposition showed amyloid angiopathy. Subpial Aβ deposition is frequently associated with amyloid angiopathy. The prevalence of cerebral Aβ deposits was about two times higher in the patients who had received brain radiation therapy (27.8%) compared to non-radiated patients (14.8%). Amyloid angiopathy was much more prominent ($P < 0.05$) with radiation therapy (22.2%) than without (8.0%). We found that cerebral Aβ deposition is dependent on aging, even in patients with malignant tumors and at beginning in their forties, and that brain radiation therapy is a possible risk factor of Aβ deposition, especially in the form of amyloid angiopathy.

Key words Amyloid β protein · Alzheimer's disease · Dementia · Malignant tumor

Introduction

Amyloid β protein (Aβ), a major component of senile plaque amyloid, is a hydrophobic self-aggregation peptide consisting of 40–42 residues [26], and is derived from a larger membrane-bound protein, amyloid β protein precursor (APP) [13]. Analyses in patients with early-onset type familial Alzheimer's disease have shown point mutations in the APP gene near the Aβ cleavage site [2, 9, 21]. Brains of subjects with these mutations show typical Alzheimer's pathology, both numerous neurofibrillary tangles and senile plaques, supporting the role of Aβ in the pathogenesis of Alzheimer's disease [19]. Recent research has shown the existence of a soluble form of Aβ in the cerebrospinal fluid [28, 29] and in the culture cell medium of neuronal and non-neuronal clones [1, 10, 29], suggesting that soluble Aβ is produced everywhere in the brain. However, Aβ deposition occurs in localized areas as a form of senile plaques and amyloid angiopathy. Therefore, local factors which enhance Aβ biosynthesis or reduce Aβ clearance may play an important role in the mechanism of amyloid fibril formation from soluble Aβ. It is very important to identify amyloid deposition-enhancing factors or risk factors such as head trauma [24, 25, 30] and the ε4 allele of apolipoprotein E [3, 27] to establish therapeutic strategies for the prevention of Aβ deposition, or to develop animal model systems of cerebral Aβ deposition.

In the present study, we examined Aβ deposition in middle-aged patients, who had malignant neoplasms, and showed the following: (1) the prevalence of Aβ deposition with aging, (2) forms and sites of Aβ deposition, and (3) risk factors of Aβ deposition.

Materials and methods

We examined autopsied brains from 136 unselected patients (90 males and 46 females) who died in the Gunma Cancer Center and its associated hospitals at ages between 30 and 59 years. None of the patients showed any signs of dementia. Because most patients (123 of 136) died with malignant tumors, we studied Aβ and tau deposits in the 123 brains from the subjects with malignant tumors. There were 17 in their fourth decades (30–39 years old, 30s groups), 41 in their fifth decades (40–49 years old, 40s group) and 65 in their sixth decades (50–59 years old, 50s group) (Table 1). Whole brains were fixed in 10% formalin for 7–10 days. Tissue samples from the frontal lobe and the temporal lobe (including the

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hippocampal area) were embedded in paraffin, and 3-μm-thick sections were prepared. For the immunostaining, tissue sections were first pretreated with 99% formic acid for 5 min to enhance labeling and then reacted with two antisera against Aβ (1–28, 28K, 1 : 2,000 and 1–40, G42, 1 : 1,000) [34, 38] and antiserum against normal human tau (1 : 500, provided by Dr. Y. Ihara, Tokyo University) [11]. Immunoreaction was visualized using a Vectastain ABC Elite kit (Vector, USA) and diaminobenzidine and H_2O_2 solution. As controls, Aβ antisera were preabsorbed using commercially available synthetic β $peptide_{1-40}$ (Bachem California, USA). Because the amount and the number of Aβ deposits were very small in the brains of the 40s group, they could not generally be found at low magnification, and it was not easy to distinguish them from artifacts. Therefore, we immunolabeled groups of four serial sections: two with the two Aβ antisera and two with preabsorbed antisera, and considered a reaction to be positive when the labeling pattern was the same with the two different Aβ antisera but had disappeared with preabsorption. The density of senile plaques was expressed semiquantitatively: +, less than 1/mm^2; ++, 1–10/mm^2; +++, more than 10/mm^2.

The interrelationship between cerebral Aβ deposition and clinicopathological features such as brain metastasis and brain radiation therapies, was statistically analyzed in 106 cancer-bearing subjects aged between 40 and 59 using *t*-test and comparison of two proportions. Of the 106 brains, 41 were found to have brain metastases; 18 patients had received radiation therapy for metastatic and invasive tumors (see Table 4).

Results

Aβ deposition and aging (Table 1)

Of a total of 123 patients, we found 18 with cerebral Aβ deposition (14.6%). The prevalence of cerebral Aβ deposition was clearly dependent on aging; no cerebral Aβ deposition was found in the 30s group, 4 of 41 brains from 40s group (9.8%) had Aβ deposition, and in the 50s group the prevalence was increased to 21.5% (14 of 65).

Major form and site of senile plaques

Senile plaques were found more frequently in the frontal cortex than in the temporal cortex (Table 2). The hippocampus showed no plaques in the 40s group. The majority of plaques were of the diffuse type in all 4 cases in the 40s group (Fig. 1a). When analyzing the frontal Aβ deposits in 50s group, approximately half of the 14 cases showed a predominance of diffuse plaques, while 2 cases showed mixed-type plaques (both diffuse and cored plaques), 3 cases had predominantly cored plaque (Fig. 1b), and 3 cases showed predominantly amyloid angiopathy (Fig. 1c). The density of the senile plaques in the positive cases tended to increase with aging, although it varied among cases.

Table 1 The prevalence of amyloid β protein (Aβ) deposits and tau accumulation in brains from subjects with malignant neoplasms

Age (years)	*n*	Aβ deposits		Tau accumulation	
30–39	17	0/ 17	(0%)	0/ 17	(0%)
40–49	41	4/ 41	(9.8%)	1/ 41	(2.4%)
50–59	65	14/ 65	(21.5%)	18/ 65	(27.7%)
Total	123	18/123	(14.6%)	19/123	(15.4%)

Amyloid angiopathy and subpial Aβ deposition

Amyloid angiopathy was found in 11 brains from both 40s and 50s groups, whereas subpial deposits were only found in 5 brains from the 50s group. As shown in Table 3 and Fig. 2a, subpial Aβ deposition showed a significant association with amyloid angiopathy ($P < 0.05$). In the cases with amyloid angiopathy, the majority showed predominantly cored plaques or mixed-type plaques (Tables 2, 3). In the meningeal vascular walls, amyloid deposition began at the junction between media and adventitia as very small clusters of amyloid (Fig. 2b). In the cerebral cortex, a small part or the whole wall of arterioles were Aβ immunoreactive, and some were associated with perivascular parenchymal diffuse staining (Fig. 2c). Frontal cortices from 3 brains had amyloid angiopathy without senile plaques.

Effects of brain metastasis and brain radiation therapy

The effects of brain metastasis and radiation therapy were analyzed in 106 subjects aged between 40–59 with malignancy (Table 4). Of the total of 106 subjects, 18 had Aβ deposits. The prevalence of Aβ deposition was 27.8% (5 of 18) in the brains from the subjects treated with radiation therapy, and approximately double that in subjects without brain radiation therapy (13 of 88, 14.8%). In the frontal lobe, the prevalence of the amyloid angiopathy of the irradiated group (4 of 18, 22.2%) was nearly three times higher than that of the non-radiated group (7 of 88, 8.0%), showing significant increase ($P < 0.05$). Capillary amyloid was found in six cases, and was prominent in those with brain radiation therapy (cases 9 and 15 shown in Table 2) (Fig. 1c).

Of the 18 Aβ-positive cases, 5 had received brain radiation therapy, 3 of which had whole brain radiation therapy and 2 had irradiation of the skull base for invasive tumors. The amount of irradiation and the duration from the end of radiation therapy to death (survival time) were not significantly different between cases with and without cerebral Aβ deposition (Table 5). Brain radiation had no effect on tau accumulation (Table 4). Among the 106 subjects, 4 out of 18 irradiated brains (22.2%) showed marked vascular hyalinosis (Table 4), whereas 2 of 88 non-radiated brains (2.3%) had vascular hyalinosis ($P < 0.001$).

In the 88 subjects without brain radiation therapy, the prevalence of Aβ deposition in the subjects with brain metastasis/invasion (3 of 23, 13.0%) was similar to that in the subjects without brain metastasis/invasion (10 of 65, 15.4%) (Table 4).

Table 2 Clinico-pathological data of the 18 Aβ-positive cases (*ca.* carcinoma, *NT* neuropil threads, *NFT* neurofibrillary tangles, *SP, AA* dystrophic neurites associated with senile plaques, and amyloid angiopathy, respectively)

No.	Age (years)	Primary tumor	Brain metastasis	Brain radiation		Frontal lesion					Temporal lesion				
				Dose (Gray)	Survival time (M)	Senile plaques		Amyloid angiopathy	Subpial Aβ	Tau[b]	Plaques[c]		Amyloid angiopathy	Subpial Aβ	Tau[b]
						Types[a]	Density				Types	Density			
1	43	Breast ca.	+	40	6	Diffuse	++	+	–	–	–		–	–	–
2	47	Breast ca.	+	0		Diffuse	+	–	–	–	–		–	–	–
3	48	Breast ca.	–	0		Diffuse	++	+	–	–	Diffuse	++	+	+	–
4	49	Lung ca.	–	0		Diffuse	+	–	–	–	Diffuse	+	–	–	–
5	51	Uterine ca.	–	0		Diffuse	+++	–	–	–	Diffuse	++	–	–	NFT
6	52	Nasal cavity ca.	+	50	3	Diffuse	++	–	–	–	Diffuse	++	–	–	NT
7	53	Hepatoma	–	0		Cored	+	+	+	–	Cored	+	+	+	SP
8	56	Uterine ca.	+	30	1	Mixed	+	+	–	–	–		–	–	–
9	56	Maxillary ca.	+	40	2	Cored	+++	+++	+	SP, AA NFT, NT	Cored	++	++	+	SP, AA
10	56	Pharyngeal ca.	–	0		Mixed	++	+	+	–	Diffuse	++	–	+	NFT, SP
11	57	Gastric ca.	–	0		Diffuse	+++	+	–	–	Diffuse	+++	–	–	–
12	57	Multiple myeloma	+	0		Diffuse	+	–	–	–	–		–	–	NT, NFT
13	58	Lung ca.	+	0		–		+	–	–	–		–	–	–
14	59	Renal cell ca.	–	0		Cored	++	+	+	–	Cored	++	+	+	NT, NFT, SP
15	59	Lung ca.	+	40	1	–		++	+	–	Mixed	++	++	–	–
16	59	Bile duct ca.	–	0		Diffuse	+++	–	–	–	Diffuse	+++	–	–	NFT, NT
17	59	Gastric ca.	–	0		Diffuse	+	–	–	–	Diffuse	++	–	–	NFT, NT
18	59	Lung ca.	–	0		–		+	–	–	–		+	–	NFT, NT

[a] Types, predominant plaque type
[b] Tau, site of tau accumulation
[c] Plaque density, + = < 1/mm^2, ++ = 1–10/mm^2, +++ = > 10/mm^2

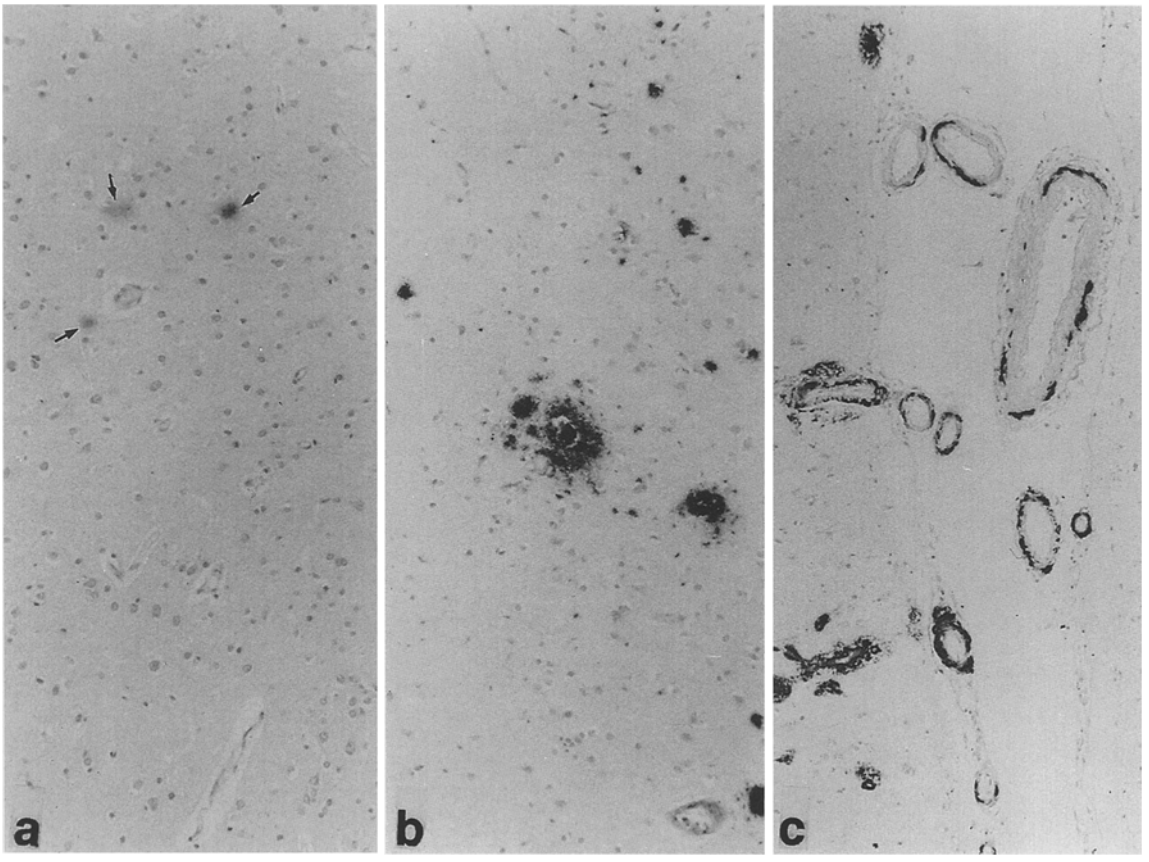

Fig. 1 **a** Amyloid β protein (Aβ) immunostaining of the frontal lobe of a 43-year-old female with breast carcinoma. *Arrows* indicate small diffuse plaques. **b** Immunohistochemistry for Aβ of the frontal lobe of a 59-year-old male with renal carcinoma. Mixture of cored and diffuse plaques. **c** Aβ immunohistochemistry showed mainly amyloid angiopathy of the frontal lobe of a 59-year-old male with lung carcinoma received brain radiation therapy. **a–c** × 100

Table 3 Relationship between amyloid angiopathy and other lesions in the frontal lobe (*n.s.* not significant)

Cases	*n*	Average age (years)	Predominant plaque type	Subpial Aβ deposition			Tau-positive structures		
Without amyloid angiopathy	7	53.4	Diffuse plaque predominance	0/ 7	(0%)	$P < 0.05$	0/ 7	(0%)	n.s.
With amyloid angiopathy	11	54.9	Presence of cored plaques	5/11	(45.5%)		1/11	(9.1%)	

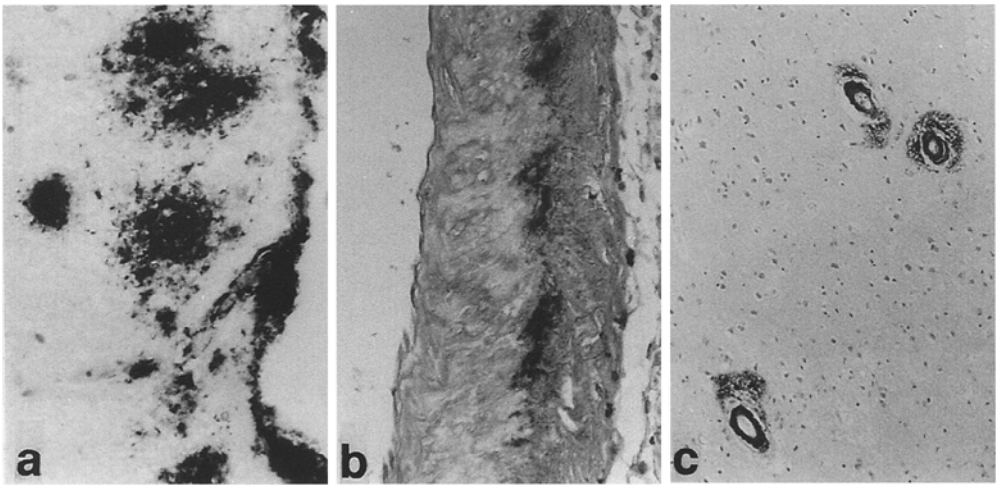

Fig. 2 a–c Amyloid angiopathy shown by Aβ immunohistochemistry. **a** Subpial Aβ deposition was associated with amyloid angiopathy. **b** In the meningeal vascular wall, Aβ deposition was identified as very small aggregates of amyloid. **c** Aβ-positive material in the wall of arterioles and perivascular diffuse deposits. **a** × 192, **b** × 384, **c** × 144

Table 4 Effects of brain irradiation and brain metastasis in subjects aged between 40 and 59 years of age (*n.s. not significant*)

Cases	*n*	Average age (years)		Aβ deposition			Amyloid angiopathy			Vascular hyalinosis			Tau accumulation		
Brain irradiation															
With irradiation	18	53.2	n.s.	5/ 18	(27.8%)	$P < 0.1$	4/ 18	(22.2%)	$P < 0.05$	4/ 18	(22.2%)	$P < 0.001$	2/ 18	(11.1%)	n.s.
Without irradiation	88	54.8		13/ 88	(14.8%)		7/ 88	(8.0%)		2/ 88	(2.3%)		9/ 88	(10.2%)	
Total	106	54.5		18/106	(17.0%)		11/106	(10.4%)		6/106	(5.7%)		11/106	(10.4%)	
Brain metastasis															
With metastasis	23	54.2	n.s.	3/ 23	(13.0%)	n.s.	1/ 23	(4.3%)	n.s.	1/ 23	(4.3%)	n.s.	4/ 23	(18.2%)	$P < 0.1$
Without metastasis	65	55.1		10/ 65	(15.4%)		6/ 65	(9.2%)		1/ 65	(1.5%)		5/ 65	(7.7%)	
Total without irradiation	88	54.5		13/ 88	(14.7%)		7/ 88	(8.0%)		2/ 88	(2.3%)		9/ 88	(10.2%)	

Abnormal accumulation of tau (Table 1)

Using antiserum to normal human tau (not directed to abnormally phosphorylated forms of tau), we found accumulation of this protein in 19 of 123 brains. In the 30s group, no cases were positive for tau, in the 40s group, 1 of 41 (2.4%) had small quantities of neurofibrillary tangles in the subiculum without Aβ deposition, and in the 50s group, 18 out of 65 had tau accumulation; 11 with and 7 without Aβ deposits. In most cases, the site of tau accumulation was restricted to the entorhinal cortex, subiculum and CA1 region. Cored plaques were frequently associated with tau-immunoreactive swollen neurites (Fig. 3a). In one case, amyloid angiopathy was associated with abundant tau-positive neurites (Fig. 3b).

Discussion

In the Down's syndrome brain, Aβ deposition begins in the second decade as a form of diffuse-type plaques, and its prevalence increases up to 100% at the age of 30 [16, 20]. In the course of the normal aging, the cerebral Aβ deposition begins in the sixth decade, as shown by sensitive immunohistochemical methods [4, 17, 18, 23] or the seventh decade with traditional histopathological methods [6, 31, 32]. The prevalence of Aβ deposition increases with aging, and almost all centenarians have cerebral Aβ deposits [7]. In the present study, we found diffuse-type senile plaques in 4 of 41 brains (9.8%) from subjects in their 40s with malignant neoplasms. Compared to the previous studies of non-demented subjects showing the prevalence of 0–1.6% in the fifth decade [4, 15, 17, 18, 23, 30], our results suggest that malignant neoplasms accelerate cerebral aging changes. However, it was difficult to confirm this hypothesis, because we could not obtain sufficient numbers of autopsy brains from middle-aged people without neoplasms.

In 1935, Neubürger and Röusch [22] reported senile plaque-like argyrophilic structures known as "Krebsdrüsen" in 19 of 60 young subjects with a variety of carcinomas. In 1937, however, Yamashita [39] reported that Krebsdrüsen was an artificial product comprised of crystallized formic acid formed during formalin fixation. In 1970, Dayan [6] examined 20 subjects with visceral carcinomas (41–59 years) and found small numbers of senile plaques in 2 cases (aged 51 and 55 years), but could not confirm the result of Neubürger and Röusch [22]. In the present study, we found senile plaques in the brains of subjects with carcinomas that were in their 40s, but not in those that were in their 30s. It seems worthwhile to examine a large series of brains to confirm whether carcinomas accelerate cerebral aging change.

Previously, we reported the massive formation of diffuse plaques in the cerebral cortex of the Alzheimer's brain [34–36]. The diffuse plaques in such brains should be a mixture of mature and immature ones. The brains examined in the present study, which had tiny amounts of Aβ, may represent the very early stages of senile plaque

Table 5 Comparison of the dose of radiation and survival time after radiation between Aβ-positive and negative groups in the 18 cases with brain radiation therapy (*n.s.* not significant)

Cases	*n*	Average age (years)		Dose of radiation (Gray)		Survival time (days)	
				Mean	Range	Mean	Range
With Aβ deposits	5	53.2	n.s.	40.0	30–50	70	10–180
Without Aβ deposits	13	50.4		42.5	12–78	120	30–300

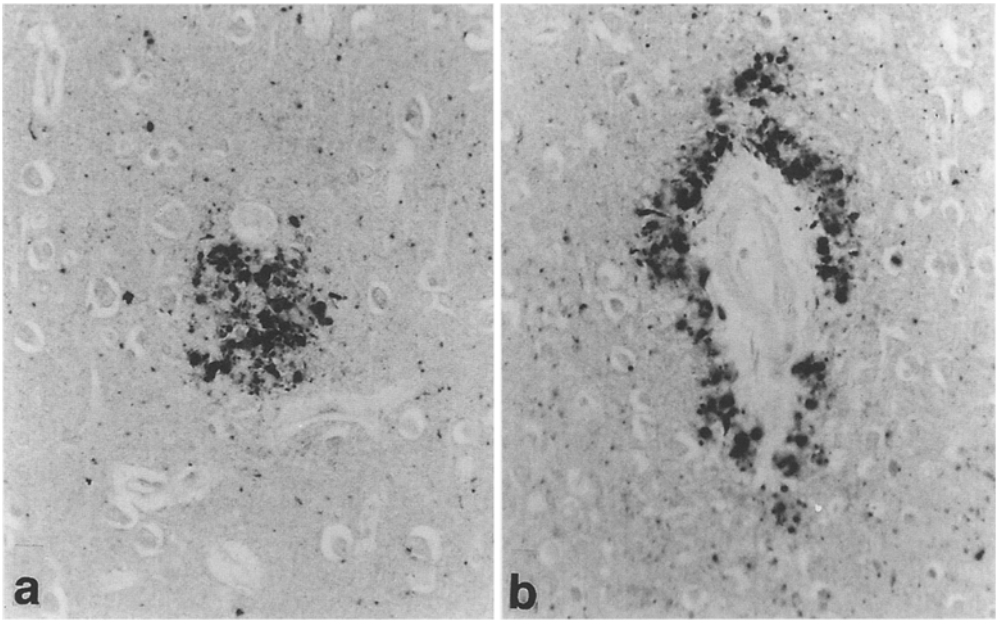

Fig. 3 a, b Tau immunostaining. **a** A senile plaque with tau-immunoreactive neurites. **b** Amyloid angiopathy was associated with tau-positive neurites. **a, b** × 400

formation. As expected, the major form of senile plaques was the diffuse type. However, one third of the subjects showed vascular amyloid predominance, amyloid angiopathy alone or a combination of amyloid angiopathy and cored plaques, suggesting that some different mechanisms of amyloidogenesis or risk factors may exist between Aβ deposition in the form of diffuse plaques and vascular amyloid [33]. In these cases, very small cores seemed to represent an early stage of cored-plaque formation. In the large meningeal vessels, amyloid deposition began at the junction of media and adventitia as very small clusters. We designated such patterns as an early stage of amyloid angiopathy in a previous study of Alzheimer's brains [37], and the results of the present study confirm the finding.

Although amyloid angiopathy is frequently associated with Alzheimer's disease, it is less specific to Alzheimer's disease than senile plaques [12]. Arteriovenous malformation promotes Aβ-immunoreactive amyloid angiopathy [5]. In the present study, we showed that brain radiation therapy may possibly enhance Aβ deposition, especially as amyloid angiopathy. Although amyloid deposits in areas of the brain exposed to radiation therapy have been reported previously, their histochemical definition of amyloid was based merely on its affinity for Congo red, and not on birefringence [14]. However, hyaline deposits in the brain, which exhibit delayed effects following irradiation also have an affinity for Congo red [14]. Radiation injures endothelial cells and breaks the blood-brain barrier [8], and could be an enhancing factor of Aβ deposition, although further study is necessary.

Acknowledgements The authors thank Prof. Yasuo Ihara, Tokyo University, Tokyo, for kind gift of tau antiserum and Mrs. Shigeru Tsuchida, Nobuyuki Shizawa, and Ms. Emiko Kobori, Gunma Cancer Center, Gunma, for preparing excellent staining and photographs. This work was supported by the Grant-in-Aid for Scientific Research on Priority Areas (No. 04268104) from the Ministry of Education, Science and Culture, Japan, and by the Takeda Medical Foundation.

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