Pathological Studies on Cerebral Amyloid Angiopathy, Senile Plaques and Amyloid Deposition in Visceral Organs in Aged Dogs

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ABSTRACT. The relationship between cerebral lesions such as amyloid angiopathy or senile plaques and amyloid deposition in the visceral organs were studied in 90 autopsy cases of dogs, 0 to 19-year-old. Cerebral amyloid angiopathy was detected in 28 aged dogs (mean age: 13.7-year-old) and was found mostly in or around the wall of cerebral meningeal arterioles and capillaries of the neocortex. That condition was often accompanied by cerebral hemorrhage in dogs more than 9 years of age. Senile plaques were detected in the neocortex of the brain of 12 dogs (mean age: 13.2-year-old) and classified into 3 subtypes, i.e., "diffuse plaque", "primitive plaque" and "classical plaque". Among those 3 subtypes of senile plaques, amyloid containing plaques were small in number. In the visceral organs of dogs with cerebral amyloid angiopathy, amyloid deposition was found in the vascular walls or connective tissues of small intestines at a high frequency and sometimes in the vascular walls of the heart, lung, liver and thyroid gland as well as in atrioventricular valves. Amyloid in both cerebral and visceral organs was congophilic and showed green birefringence under polarized light even after potassium permanganate oxidation.—**KEY WORDS:** aged dog, amyloid angiopathy, cerebral hemorrhage, senile plaque, visceral amyloidosis.

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Age-related amyloidosis has been known as a common disorder in aged human beings. This amyloid deposition was found mostly in the brain, heart, aorta, pancreas and seminal vesicles [8, 31]. In the brain, it was found frequently in the cases with amyloid angiopathy and senile plaque such as primitive or classical plaques [29], which is thought to be the important histological diagnostic marker of Alzheimer's disease together with neurofibrillary tangles [1, 6, 13, 20, 23]. The cerebrovascular amyloid and senile plaques were suggested to be formed by the aggregation of a unique protein, amyloid beta-protein or A4 protein [2, 14]. Recently, amyloid beta-protein deposition was found in the skin and intestines of the patients with Alzheimer's disease or non-demented aged people, suggesting that the protein might deposit in various organs besides the brain [10].

In non-human mammals including dogs, cerebral amyloid angiopathy or senile plaque has been described [3, 18, 21, 27, 28], while in those cases senile amyloid deposition in organs other than the brain has not been well examined.

In the present study, we examined organ distribution of amyloid deposition in 90 canine autopsy cases to clarify the relationship between aging,

cerebral lesions such as amyloid angiopathy or senile plaques and visceral amyloid deposition. In addition, cerebral hemorrhage was referred, because the disorder related to amyloid angiopathy might be the direct cause of death in aged people or some human stroke syndrome [4, 5, 9, 12, 15, 22, 24] and aged dogs [3, 21].

MATERIALS AND METHODS

In autopsy cases carried out during 1985 to 1989 in our laboratory, 90 dogs consisting of 43 males and 47 females, 0 to 19-year-old, were collected randomly except for the cases diagnosed as systemic amyloidosis. These cases were classified into 6 groups by age: group 1, 0 to 3-year-old (17 cases); group 2, 3 to 6-year-old (8 cases); group 3, 6 to 9-year-old (17 cases); group 4, 9 to 12-year-old (18 cases); group 5, 12 to 15-year-old (22 cases); and group 6, above 15-year-old (8 cases). The brains of these dogs were histopathologically examined and in cases showing amyloid angiopathy, the heart, lung, liver, spleen, kidneys, intestines, pancreas, thyroid glands and adrenal glands were also investigated. For light microscopy, paraffin sections were made and stained with hematoxylin and eosin (HE). Modified alkaline Congo-red stain [17] was applied to demonstrate amyloid and stained sections were observed under polarized light to confirm the characteristic green

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birefringence of amyloid. Oxidation of the sections by potassium permanganate treatment [30] before alkaline Congo-red stain was also performed to determine the nature of deposited amyloid. For the detection of senile plaque, paraffin sections of 6 to 8 μm thick were subjected to periodic acid-methenamine-silver (PAM) stain.

Formalin-fixed brain samples were also used for electron microscopy. Small blocks of the cerebral cortex were post-fixed with osmium tetroxide and dehydrated in ascending ethanol series. Then, the samples were immersed in propylene oxide and embedded in epoxy resin (Epok 812, Oken Co., Tokyo). Ultrathin sections were made, stained with uranyl acetate and lead citrate, and observed under an electron microscope JEOL 1200EX, at 80 kV.

RESULTS

Grossly, the brain of aged dogs, especially above 10-year-old, commonly showed thickening of the meninges and the wall of cerebro-cortical arterioles, petechiae or hematoma of the cerebral cortex, and slight atrophy of the frontal cortex, but the relationship between these gross features, except for cerebral hemorrhage, and histopathological lesions of the brain such as amyloid angiopathy or senile plaques was unclear.

Among 90 dogs examined, cerebral vascular deposition of amyloid was found in 28 dogs (mean age: 13.7 years old). Figure 1 shows cerebral amyloid angiopathy increased together with age and there was no predisposition of the breed or sex in these dogs.

Histopathologically, amyloid was often found in the wall of meningeal arterioles and capillaries of the cerebral neocortex (Fig. 2) and occasionally arterioles in the cerebellar meninges were involved. The wall of arterioles with amyloid deposition thickened and had hyaline appearance. In the small area around capillaries, homogeneous substance which was faintly stained by eosin and consisted of amyloid spread diffusely. Electron microscopy revealed the accumulation of amyloid fibrils measuring 10–20 nm in diameter in the tunica media of arterioles in the cerebral cortex. The amyloid deposited in those vascular walls was resistant to oxidation with potassium permanganate.

By the examination of the brain sections stained with PAM, 12 dogs (mean age: 13.2 years old) showed senile plaques in the cerebral cortex. Senile

Fig. 1. Incidence of amyloid angiopathy, cerebral hemorrhage and senile plaque in the brain of dogs. The figures in the parentheses represent the number of dogs investigated.

Fig. 2. (a) Cerebral amyloid angiopathy in arterioles, $\times 107$ and (b) a capillary (arrow) $\times 429$. Alkaline Congo-red stain. Case No. 88.

plaque appeared increasingly with age, but not so frequent as cerebral amyloid angiopathy (Fig. 1). The histopathological studies revealed 3 types of senile plaques, i.e., diffuse, primitive, and classical plaques. Diffuse plaques characterized by the diffuse accumulation of PAM-positive fibrillous substance without compact amyloid deposition (Fig. 3) were predominant as compared with other types of senile plaques. Diffuse plaque appeared in the brain of 12 cases having senile plaques. Primitive plaques containing granular hyaline materials and congophilic deposits were found in 2 cases (Fig. 4). Classical plaques having well-defined amyloid core

Fig. 3. Senile plaques (diffuse plaques) in the cerebral cortex. PAM stain. $\times 107$. Case No. 40.

Fig. 5. A classical plaque having well defined amyloid core. (a) PAM stain, (b) alkaline Congo-red stain. $\times 214$. Case No. 84.

Fig. 4. (a) Swollen degenerative neurites, PAM stain, (b) hyalinous granular materials and slight linear amyloid deposition in a primitive plaque. Alkaline Congo-red stain. $\times 214$. Case No. 84.

and neuritic halo were observed in one case (Fig. 5). Amyloid in these 2 types of plaques were resistant to oxidation. In the dogs having primitive or classical plaques, diffuse plaques and cerebral amyloid angiopathy were always detected, and occasionally diffuse plaques appeared in cases without cerebral amyloid angiopathy (Table 1).

Cerebral hemorrhage was found in the cerebral cortex of 17 cases (mean age: 12.1 years old) and seen frequently in the dogs more than 9 years of age (Figs. 1 and 6). Among these dogs, 12 dogs had cerebrovascular amyloid deposition, except for other 4 younger cases (0 to 6 years old) with meningoencephalitis.

Cerebral lesions other than amyloid angiopathy, senile plaques and cortical hemorrhage seen in aged dogs were the accumulation of macrophages containing lipid granules around the cerebral vessels and the deposition of lipofuscin in the cytoplasm of

Table 1. Occurrence of senile plaque and amyloid angiopathy in the brain of dogs

Case No.	Age (Year)	Senile plaque			Amyloid angiopathy
		Diffuse	Primitive	Classical	
40	8	+	—	—	—
55	10	+	—	—	+
57	11	+	—	—	—
67	13	+	—	—	+
70	13	+	—	—	+
72	13	+	—	—	+
76	13	+	—	—	—
80	14	+	—	—	—
82	14	+	—	—	+
84	15	+	+	+	+
86	16	+	+	—	+
88	18	+	—	—	+

*+: Detected. —: Not detected.

Fig. 6. Scattered hemorrhagic foci in the cerebral cortex (arrows). Case No. 88.

Fig. 7. Amyloid deposition in organs other than the brain of dogs having cerebral amyloid angiopathy. The figures in the parentheses represent the number of dogs examined.

neuronal cells. Neurofibrillary tangles could not be observed in any dogs.

Amyloid deposition in organs other than the brain of the cases with cerebral amyloid angiopathy was found in 16 of 28 cases, showing slight or moderate amyloid deposition in or around the vascular walls of arterioles and the connective tissues of submucosa of the small intestines (Figs. 7 and 8). In the heart of 2 cases, massive amyloid deposit was seen in the left atrioventricular valve (Fig. 9) and around the walls of coronal arteries. Pulmonary arteries, interlobular arteries of the liver and arteries of the thyroid glands were occasionally involved. Amyloid in these sites was also as resistant to oxidation as that in the brain was.

Fig. 8. Amyloid deposition around vessels of the intestinal submucosa (arrows). Alkaline Congo-red stain. $\times 81$. Case No. 88.

Fig. 9. Amyloid deposition in the left atrioventricular valve (arrow). Alkaline Congo-red stain. $\times 26$. Case No. 82.

DISCUSSION

Age-related cerebral lesions such as amyloid angiopathy or senile plaques and amyloidosis in visceral organs have not been well examined in dogs and other non-human mammals, though both of them were suggested to be age-dependent disorders in man [8, 31]. In dogs, although the relationship between cerebral amyloid angiopathy and aging has been investigated [3], senile plaques especially diffuse plaques remained to be studied. In the present study, we examined cerebral amyloid angiopathy and senile plaque in dogs 0 to 19 years of age and found that these changes in dogs were as closely correlated to age as those of human beings were.

In addition, the relationship between amyloid angiopathy and cerebral hemorrhage has been documented in aged dogs [3, 21]. In this study, a high incidence of cerebral hemorrhage accompanied by amyloid angiopathy was shown in dogs more than 9 years old. These findings accorded with the previous studies [3] of the cerebral hemorrhage in aged dogs, supporting the pathogenesis of cerebral hemorrhage in aged people.

Senile plaques in the brain of dogs did not always show amyloid deposits in this study. The plaque that has neither compact amyloid cores nor obvious degenerative neurites and is undetectable by HE, Congo-red or Bodian's silver stain has been reported to be very primitive or diffuse plaque and thought to be initial form of senile plaques [11, 29, 32]. In this study, diffuse plaques appeared commonly in the cases without the other types of senile plaques such as primitive or classical plaques and the dogs were usually younger as compared with those having latter two types of plaques except for one case (Case No. 88, Table 1). These results might indicate that diffuse plaques in dogs also arose as initial form of senile plaques. Recently, Yamaguchi *et al.* [32] revealed ultrastructurally that diffuse plaques in the brain of a patient with Alzheimer's disease contained some scattered bundles of amyloid fibrils and proposed that the amount of amyloid fibrils might increase with the development of senile plaques. Similar events might take place in the formation of canine senile plaques.

In dogs with cerebral amyloid angiopathy, intestinal amyloid deposition was also remarkable, while the relationship between intestinal and cerebral amyloid angiopathy in dogs was unclear. In aged human, amyloid deposition occurred frequently in the brain, aorta, heart, pancreas and seminal vesicles but not in the intestines, and the biochemical nature of amyloid protein in these sites was indicated to be different by the organs [7, 16, 25, 26]. In aged dogs, high incidence of intestinal amyloidosis was shown in this study. The results indicate that the intestinal amyloid deposition is a specific feature in dogs and may closely correlate to cerebral amyloid angiopathy. Recently, amyloid beta-protein deposition in the intestines of the aged people and patients with Alzheimer's disease [10] has been reported to be caused by the mechanism identical to cerebral amyloid angiopathy and senile plaque. This report [10] suggests that the amyloid precursor protein may be produced in various organs and deposited in the

form of amyloid beta-protein in the brain and intestines. Similar hypothesis is also suggestable in aged dogs.

Quantitative analysis of amyloid proteins and immunohistochemistry using antibodies against human beta-protein are now in progress on cerebral and visceral amyloid deposition and senile plaques in aged dogs, to elucidate their histopathogenesis.

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