Binding of Curcumin to Senile Plaques and Cerebral Amyloid Angiopathy in the Aged Brain of Various Animals and to Neurofibrillary Tangles in Alzheimer's Brain

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ABSTRACT. The binding of curcumin to senile plaques (SPs) and cerebral amyloid angiopathy (CAA) was examined in the aged brain of various animal species and a human patient with Alzheimer's disease (AD), together with its binding to neurofibrillary tangles (NFTs). Brain sections were immunostained with anti-amyloid β protein 1-42 (A β 42) and anti-amyloid β protein 1-40 (A β 40) antibodies. These sections were also stained with alkaline Congo red, periodic acid-methenamine silver (PAM), and curcumin (0.009% curcumin solution) with or without formic acid pretreatment. The sections from the AD brain were also immunostained for anti-paired helical filament-tau (PHF-tau), and were stained with Gallyas silver for NFTs. Some SPs in the AD, monkey, dog, bear, and amyloid precursor protein transgenic mouse (APP Tg-mouse) brains contained congophilic materials, and were intensely positive for curcumin. In addition, curcumin labeled some diffuse SPs negative for Congo red in the AD, monkey, bear, and APP Tg-mouse brains. In all animals, CAA was intensely positive for both Congo red and curcumin. The specific curcumin staining activity was lost by formic acid pretreatment. In the AD brain, NFTs positive for PHF-tau and Gallyas silver were moderately stained with curcumin. These findings indicate that curcumin specifically binds to the aggregated A β molecules in various animals, and further to phosphorylated tau protein, probably according to its conformational nature.

KEY WORDS: β amyloid, cerebral amyloid angiopathy, curcumin, neurofibrillary tangle, senile plaque.

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Senile plaques (SPs), cerebral amyloid angiopathy (CAA), and neurofibrillary tangles (NFTs) are the most characteristic histopathological features in the brains of patients with Alzheimer's disease (AD). SPs and CAA consist of amyloid β protein (A β), and they are detected by Congo red or periodic acid-methenamine silver (PAM) staining. SPs are morphologically classified into two types: diffuse plaques (DPs) and mature plaques (MPs). DPs are the earliest stage of plaque formation, and negative for Congo red [29]. MPs are the progressive stage of A β aggregation and positive for Congo red [28]. MPs also have swollen neurites or glial hallow. On the other hand, NFTs are formed in the cytoplasm of a neuron as aggregates of highly phosphorylated tau, a microtubule-associated protein. NFTs are detected clearly by Gallyas silver staining. These histopathological features are frequently observed in the aged brains of various nonhuman animal species. SPs and CAA have been observed in the brain of aged nonhuman primates [7, 17, 21, 24], dogs [3, 9, 16, 24, 26], cats [18], a camel [19], bears [6, 27], a wolverine [22], and a great spotted woodpecker [20], whereas NFTs have been reported

only in a chimpanzee [23], sheep [5], bears [6], and a wolverine [22].

Curcumin, a yellow phenolic pigment and an ingredient for curry, has potent protective and curative activities against neoplastic-, inflammatory-, amyloid and oxidant-associated disorders [1, 8, 13]. It has been reported that curcumin binds to A β aggregates *in vivo* and inhibits the formation of SPs [2, 10, 11, 30]. Because of its specific binding ability, use of curcumin has been expected to prevent or treat AD. Recent investigations have demonstrated that SPs, CAA, and NFTs are detected by curcumin staining in AD model mice (APPswe/PS1dE9 mice) [10] as well as AD brains [14]. However, such binding of curcumin is not well known in animals other than mouse and human.

In the present study, we examined the binding of curcumin to SPs and CAA in the aged brain of various animal species, and to NFTs in the AD brain.

MATERIALS AND METHODS

Tissue samples and histology: We examined the cerebral cortex from an AD patient, a Japanese macaque, dogs, a cat, an American black bear, a Bactrian camel, a great spotted woodpecker, and an amyloid precursor protein transgenic mouse (APP Tg-mouse) (APP23, The Jackson Laboratory, Bar Harbor, ME, U.S.A.). Sex, age, and breed of the animals are shown in Table 1. Brain tissue samples were fixed

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Table 1. Histopathologic features of the brain of the aged animals examined

Case No.	Species	Breed or remark	Sex	Age	SPs		CAA	NFT
					DP	MP		
1	Human	AD patient	Female	73y	+	+	+	+
2	Japanese macaque	NA	unknown	>26y	+	+	+	–
3	Dog	Miniture.dax	Female	13y	+	–	+	–
4	Dog	Beagle	Male	14y	+	–	+	–
5	Dog	Mongrel	Male	14y	+	+	+	–
6	Dog	unknown	unknown	14y	+	+	+	–
7	Dog	unknown	unknown	18y	+	+	+	–
8	Dog	Maltese	unknown	18y	+	–	+	–
9	Dog	Mongrel	Female	20y	+	–	+	–
10	Cat	Mongrel	Female	20y	+	–	+	–
11	American black bear	NA	Female	20y	+	+	+	–
12	Bactrian camel	NA	Female	>20y	+	–	–	–
13	Great spotted woodpecker	NA	Male	>16y	–	–	+	–
14	Mouse	APP23Tg	Female	30m	+	+	+	–

SP: Senile plaque, DP: Diffuse plaque, MP: Mature plaque, CAA: Cerebral amyloid angiopathy, NFT: Neurofibrillary tangle, y: Years, m: Months, and NA: Not applicable.

in a 10% neutral-buffered formalin solution and embedded in paraffin by a routine procedure.

Paraffin sections (4 to 8 μ m thick) were stained with alkaline Congo red or PAM to detect SPs and CAA. In addition, sections from the AD brain were stained with Gallyas silver [4] to detect NFTs. The sections were preferably compared at the same regions.

Immunohistochemistry: Immunostaining with anti-A β 1-42 (A β 42: BC05, A β -Protein Immunohistostain Kit, Wako, Osaka, Japan) and anti-A β 1-40 (A β 40: BA27, A β -Protein Immunohistostain Kit, Wako) antibodies was carried out in accordance with the manufacturer's procedure for the A β -Protein Immunohistostain Kit (Wako). Immunostaining with anti-PHF-tau antibody (PHF-tau: AT8, 10 μ g/ml, Thermo, Kanagawa, Japan) was carried out in the following methods. Deparaffinized sections were first autoclaved at 120°C for 10 min in 10 mM citrate buffer for antigen retrieval. Tissue sections were then treated with 1% hydrogen peroxide in methanol for 3 min to inhibit endogenous peroxidase activity and further incubated in 8% skimmed milk in tris-buffered saline (TBS) at 37°C for 30 min to block nonspecific reactions. The sections were incubated with a primary antibody at 4°C overnight. Following 3 washings in TBS, sections were then incubated with an HRP-labeled polymer-conjugated secondary antibody against mouse IgG (Dako Japan, Kyoto, Japan) at 37°C for 60 min. Finally, the reaction products were visualized with 0.05% 3-3'-diaminobenzidine and 0.03% hydrogen peroxide in tris-HCl buffer. Counterstaining was carried out with Mayer's hematoxylin.

Curcumin staining: Deparaffinized sections were immersed in 80% ethanol, 0.3% Triton X-100, and 0.1 M TBS (pH 7.4) containing 3% bovine serum albumin (BSA) and 0.5% Tween 20, for 10 min each. Curcumin powder (Cayman Chemical Company, Ann Arbor, MI, U.S.A.) was resolved in 0.1 M TBS (pH 7.4) containing 3% BSA and 0.5% Tween 20 at 25 μ M (0.009% curcumin solution). Sec-

tions were incubated with the curcumin solution at 37°C for 60 min. Following 3 washings in TBS, the sections were rinsed once in distilled water (DW), and coverslipped with a non-fluorescent mounting medium. All sections were examined using a confocal laser scanning microscope (LSM510, Carl Zeiss, Oberkochen, Germany) (green: excitation wavelength 488 nm/emission filter 505 to 550 nm, red: excitation wavelength 488 nm/emission filter over 650 nm) or a fluorescence microscope (DMI3000B, Leica microsystems, Wetzlar, Germany) (green: excitation filter 470 $\pm$ 40 nm/emission filter 525 $\pm$ 50 nm, red: excitation filter 546 $\pm$ 12 nm/emission filter 605 $\pm$ 75 nm). Emission wavelength of curcumin in water is about 550 nm when excited with wavelength 430 nm [15]. In the present study, we used merged pictures of red and green fluorescences to obtain higher-contrasted pictures (Fig. 1). Furthermore, some sections were stained with curcumin after a 5-min pre-treatment with 99% formic acid (Wako).

Image processing: A β 40- or A β 42-positive areas (pixels) were measured in randomly selected regions of the cerebral cortex of each section using the Image J image analysis software (NIH, Bethesda, MD, U.S.A.). The means of the data were analyzed by Student's *t*-test.

RESULTS

Immunohistochemistry: A β 40- or A β 42-positive SPs were found in the brains of all animals except for a woodpecker (Table 1 and Fig. 2). In the brains of an AD patient, a monkey, a bear, a camel, and a APP Tg-mouse, most SPs were distinct, whereas those of dogs and cats had an indistinct boundary. In the brains of an AD patient, dogs, and a bear, A β 42-positive SPs were observed more than A β 40-positive SPs. In the brains of cats and a camel, the majority of SPs were positive for A β 42 and negative for A β 40. In a monkey and a APP Tg-mouse, there was no difference between A β 42- and A β 40-positive areas (Fig. 2).

Fig. 1. A curcumin stained section of a Japanese macaque. A β -positive meningeal vessels are depicted with (a) red fluorescence (excitation filter 546 ± 12 nm/emission filter 605 ± 75 nm) or (b) green fluorescence (excitation filter 470 ± 40 nm/emission filter 525 ± 50 nm) and the pictures are merged (c).

Fig. 2. Immunostain for SPs using antibodies for A β 40 (a, d, g, j) and A β 42 (b, e, h, k), and the semi-quantitative data (c, f, i, l) for A β 40 (□) or A β 42 (■). AD patient (a, b, c), dog No.9 (d, e, f), cat (g, h, i) and APP Tg-mouse (j, k, l).

Fig. 3. Immunostain for CAA using antibodies for Aβ40 (a, d, g) and Aβ42 (b, e, h), and the semi-quantitative data (c, f, i) for Aβ40 (□) or Aβ42 (■). AD patient (a, b, c), Japanese macaque (d, e, f) and great spotted woodpecker (g, h, i).

Fig. 4. Comparison of Congo red stain (a, d, g, j, m, p), PAM stain (b, e, h, k, n, q) and curcumin stain (c, f, i, l, o, r) for SPs. AD patient (a, b, c), Japanese macaque (d, e, f), dog No. 5 (g, h, i), American black bear (j, k, l), Bactrian camel (m, n, o) and APP Tg-mouse (p, q, r).

CAA was observed in the meningeal and parenchymal vessels and capillaries of all animal species excluding a camel by both Aβ42 and Aβ40 immunostains (Table 1 and Fig. 3). CAA was labeled more intensely for Aβ40 compared with that for Aβ42 (Fig. 3).

NFTs were found only in the AD brain by PHF-tau immunostain (Table 1).

Curcumin stain: SPs (Fig. 4): Both congophilic MPs and Congo red-negative DPs were observed in the AD, monkey, bear, and APP Tg-mouse brains. Curcumin stained all these

SPs. DPs were found in all dogs examined, whereas a few MPs were found only in three dogs (Nos. 5, 6, and 7). Such canine MPs were intensely stained with curcumin, but canine DPs were negative. In the camel, only DPs were found and they were positive for curcumin. Aβ42-positive deposits were found in the cat brain, but they were negative for curcumin as well as Congo red and PAM.

CAA (Fig. 5): In all animals except for a camel, CAA was intensely stained with curcumin. In a woodpecker and some dogs, perivascular Aβ deposits were also intensely positive

Fig. 5. Comparison of Congo red stain (a, c, e, g, i, k, m, o) and curcumin stain (b, d, f, h, j, l, n, p) for CAA. AD patient (a,b), Japanese macaque (c, d), dog No. 5 (e, f), cat (g, h), American black bear (i, j), APP Tg-mouse (k, l), great spotted woodpecker (m, n), and perivascular A β -positive deposits in the great spotted woodpecker (o, p).

Fig. 6. NFTs in the cerebral cortex of an AD patient. Immunostain for PHF-tau (a), Gallyas silver stain (b) and curcumin stain (c).

for Congo red and curcumin (Fig. 5-o, p).

NFTs (Fig. 6): NFTs found in the AD brain were positive for Gallyas silver and curcumin. Gallyas silver stain was more sensitive than curcumin stain.

Formic acid pretreatment (Fig. 7): By formic acid pretreatment, curcumin-positive SPs and CAA in all animal species became negative.

DISCUSSION

DPs are thought to be the initial stage of plaque forma-

tion, and they are negative for Congo red [29]. In contrast, MPs are plaques of the more progressive stages, and they are positive for Congo red [28]. Both DPs and MPs can be detected by PAM stain and A β immunostain [25, 29]. The present study revealed that DPs were found in all animals other than the woodpecker, and MPs were found in the AD patient, monkey, dog, bear, and APP Tg-mouse (Table 1), indicating the difference of A β aggregation status among stages and animal species.

The shape of SPs in the APP Tg-mouse was flower-like and different from that in other animals. Artificially overexpressed APP may induce such abnormal A β aggregates in the mouse. In addition, SPs in the APP Tg-mouse were more strongly stained with curcumin than those in AD patients and other animal species. These results indicate that highly aggregated A β is apt to bind to curcumin. On the other hand, curcumin did not stain canine and feline DPs. Generally, most canine and all feline SPs are DPs [26], and they had indistinct boundaries. Feline SPs are formed in very old animals, suggesting that such feline A β aggregates occur at the very early stage of SP formation [18]. Thus, A β in canine and feline DPs may be less aggregated than that in AD patient and monkey. These findings also indicate that curcumin may bind to A β aggregates at later stages. Further-

Fig. 7. Curcumin stain without formic acid pretreatment (a, c) and Curcumin stain with formic acid pretreatment (b, d). SPs of an AD patient (a, b) and CAA of a Japanese macaque (c, d).

more, DP in the AD patient, monkey, bear, camel and APP Tg-mouse were stained with curcumin. It is thought that curcumin binds to SPs more sensitively than Congo red, since Congo red-negative DPs were positive for curcumin.

By formic acid pretreatment, the binding of curcumin to SPs, CAA, and NFTs was lost. For the A β immunostain, formic acid pretreatment is used to restore and reinforce A β immunoreactivity [12]. Formic acid more likely solubilizes A β -derived amyloid fibrils and therefore disrupts the polymer fibrillar configuration while exposing the A β epitopes. The disrupted structures enhance the immunoreactivity of the antibody to A β . However, A β could bind to neither Congo red nor curcumin when its protein structure was lost. Since the chemical structure of curcumin is partly similar to that of Congo red [30], curcumin may bind to the A β aggregates by the recognition of their structure by a similar manner to that of Congo red.

Generally, it is thought that SPs in human mainly consist of A β 42, and CAA consists of A β 40. It has also been shown that DPs of AD patients and dogs mainly consist of A β 42, and their MPs mainly consist of A β 40 [16]. The present study indicated that most SPs consist of A β 42 in AD, dog, bear, camel, and cat brains, whereas most SPs consist of both A β 42 and A β 40 in monkey and APP Tg-mouse brains. Regarding CAA, it mainly consists of A β 40 and A β 42 in the AD, monkey, cat, APP Tg-mouse, and woodpecker brains, whereas CAA consists of A β 40 in the dog and bear. Curcumin stained both SPs and CAA, which were positive for A β 40 and/or A β 42 in the present study. These results suggest that the binding of curcumin to A β does not depend on the C-terminal structure of A β .

In the present study, NFTs in the AD brain were stained

with curcumin as described in a previous report [14]. However, the Gallyas silver stains labeled NFTs more sensitively than curcumin stain. This result indicates that curcumin binds to abnormal tau protein, but the binding activity is weaker than that to A β , and reveals the limited utility of curcumin stain for the detection of NFTs.

In conclusion, curcumin can bind specifically to abnormal tau proteins as well as to highly aggregated A β . Such curcumin binding is more sensitively than Congo red. Furthermore, curcumin stain is more simple and less nonspecific than Congo red stain. The present results clearly indicate the utility of curcumin as a specific marker for A β detection in various animals.

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REFERENCES

1. Aggarwal, B. B., Kumar, A. and Bharti, A. C. 2003. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res.* **23**: 363–398.
2. Begum, A. N., Jones, M. R., Lim, G. P., Morihara, T., Kim, P., Heath, D. D., Rock, C. L., Pruitt, M. A., Yang, F., Hudspeth, B., Hu, S., Faull, K. F., Teter, B., Cole, G. M. and Frautschy, S. A. 2008. Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. *J. Pharmacol. Exp. Ther.* **326**: 196–208.
3. Borrás, D., Ferrer, I. and Pumarola, M. 1999. Age-related

- changes in the brain of the dog. *Vet. Pathol.* **36**: 202–211.
4. Braak, H., Braak, E., Ohm, T. and Bohl, J. 1988. Silver impregnation of Alzheimer's neurofibrillary changes counterstained for basophilic material and lipofuscin pigment. *Stain Technol.* **63**: 197–200.
5. Braak, H., Braak, E. and Strothjohann, M. 1994. Abnormally phosphorylated tau protein related to the formation of neurofibrillary tangles and neuropil threads in the cerebral cortex of sheep and goat. *Neurosci. Lett.* **171**: 1–4.
6. Cork, L. C., Powers, R. E., Selkoe, D. J., Davies, P., Geyer, J. J. and Price, D. L. 1988. Neurofibrillary tangles and senile plaques in aged bears. *J. Neuropathol. Exp. Neurol.* **47**: 629–641.
7. Elfenbein, H. A., Rosen, R. F., Stephens, S. L., Switzer, R. C., Smith, Y., Pare, J., Mehta, P. D., Warzok, R. and Walker, L. C. 2007. Cerebral beta-amyloid angiopathy in aged squirrel monkeys. *Histol. Histopathol.* **22**: 155–167.
8. Frautschy, S. A., Hu, W., Kim, P., Miller, S. A., Chu, T., Harris-White, M. E. and Cole, G. M. 2001. Phenolic anti-inflammatory antioxidant reversal of Abeta-induced cognitive deficits and neuropathology. *Neurobiol. Aging* **22**: 993–1005.
9. Fukuoka, A., Nakayama, H. and Doi, K. 2004. Immunohistochemical detection of beta-amyloid and beta-amyloid precursor protein in the canine brain and non-neuronal epithelial tissues. *Amyloid* **11**: 173–178.
10. Garcia-Alloza, M., Borrelli, L. A., Rozkalne, A., Hyman, B. T. and Bacskaï, B. J. 2007. Curcumin labels amyloid pathology in vivo, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model. *J. Neurochem.* **102**: 1095–1104.
11. Hamaguchi, T., Ono, K., Murase, A. and Yamada, M. 2009. Phenolic compounds prevent Alzheimer's pathology through different effects on the amyloid-beta aggregation pathway. *Am. J. Pathol.* **175**: 2557–2565.
12. Kitamoto, T., Ogomori, K., Tateishi, J. and Prusiner, S. B. 1987. Formic acid pretreatment enhances immunostaining of cerebral and systemic amyloids. *Lab. Invest.* **57**: 230–236.
13. Lim, G. P., Chu, T., Yang, F., Beech, W., Frautschy, S. A. and Cole, G. M. 2001. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. *J. Neurosci.* **21**: 8370–8377.
14. Mohorko, N., Repovs, G., Popović, M., Kovacs, G. G. and Bresjanac, M. 2010. Curcumin labeling of neuronal fibrillar tau inclusions in human brain samples. *J. Neuropathol. Exp. Neurol.* **69**: 405–414.
15. Mukerjee, A., Sørensen, T. J., Ranjan, A. P., Raut, S., Gryczynski, I., Vishwanatha, J. K. and Gryczynski, Z. 2010. Spectroscopic properties of curcumin: orientation of transition moments. *J. Phys. Chem. B* **114**: 12679–12684.
16. Nakamura, S., Tamaoka, A., Sawamura, N., Kiatipattanasakul, W., Nakayama, H., Shoji, S., Yoshikawa, Y. and Doi, K. 1997. Deposition of amyloid beta protein (A β) subtypes [A β 40 and A β 42(43)] in canine senile plaques and cerebral amyloid angiopathy. *Acta Neuropathol.* **94**: 323–328.
17. Nakamura, S., Nakayama, H., Goto, N., Ono, F., Sakakibara, I. and Yoshikawa, Y. 1998. Histopathological studies of senile plaques and cerebral amyloidosis in cynomolgus monkeys. *J. Med. Primatol.* **27**: 244–252.
18. Nakamura, S., Nakayama, H., Kiatipattanasakul, W., Uetsuka, K., Uchida, K. and Goto, N. 1996. Senile plaques in very aged cats. *Acta Neuropathol.* **91**: 437–439.
19. Nakamura, S., Nakayama, H., Uetsuka, K., Sasaki, N., Uchida, K. and Goto, N. 1995. Senile plaques in an aged two-humped (Bactrian) camel (*Camelus bactrianus*). *Acta Neuropathol.* **90**: 415–418.
20. Nakayama, H., Katayama, K., Ikawa, A., Miyawaki, K., Shinozuka, J., Uetsuka, K., Nakamura, S., Kimura, N., Yoshikawa, Y. and Doi, K. 1999. Cerebral amyloid angiopathy in an aged great spotted woodpecker (*Picoides major*). *Neurobiol. Aging* **20**: 53–56.
21. Podlisny, M. B., Tolan, D. R. and Selkoe, D. J. 1991. Homology of the amyloid beta protein precursor in monkey and human supports a primate model for beta amyloidosis in Alzheimer's disease. *Am. J. Pathol.* **138**: 1423–1435.
22. Roertgen, K. E., Parisi, J. E., Clark, H. B., Barnes, D. L., O'Brien, T. D. and Johnson, K. H. 1996. A beta-associated cerebral angiopathy and senile plaques with neurofibrillary tangles and cerebral hemorrhage in an aged wolverine (*Gulo gulo*). *Neurobiol. Aging* **17**: 243–247.
23. Rosen, R.F., Farberg, A.S., Gearing, M., Dooyema, J., Long, P. M., Anderson, D. C., Davis-Turak, J., Coppola, G., Geschwind, D. H., Paré, J. F., Duong, T. Q., Hopkins, W. D., Preuss, T. M. and Walker, L. C. 2008. Tauopathy with paired helical filaments in an aged chimpanzee. *J. Comp. Neurol.* **509**: 259–270.
24. Selkoe, D. J., Bell, D. S., Podlisny, M. B., Price, D. L. and Cork, L. C. 1987. Conservation of brain amyloid proteins in aged mammals and humans with Alzheimer's disease. *Science* **235**: 873–877.
25. Tagliavini, F., Giaccone, G., Frangione, B. and Bugiani, O. 1988. Pre-amyloid deposits in the cerebral cortex of patients with Alzheimer's disease and nondemented individuals. *Neurosci. Lett.* **93**: 191–196.
26. Uchida, K., Nakayama, H. and Goto, N. 1991. Pathological studies on cerebral amyloid angiopathy, senile plaques and amyloid deposition in visceral organs in aged dogs. *J. Vet. Med. Sci.* **53**: 1037–1042.
27. Uchida, K., Yoshino, T., Yamaguchi, R., Tateyama, S., Kimoto, Y., Nakayama, H. and Goto, N. 1995. Senile plaques and other senile changes in the brain of an aged American black bear. *Vet. Pathol.* **32**: 412–414.
28. Wisniewski, H. M. and Terry, R. D. 1973. Reexamination of the pathogenesis of the senile plaques. *Prog. Neuropathol.* **2**: 1–26.
29. Yamaguchi, H., Hirai, S., Morimatsu, M., Shoji, M. and Horigaya, Y. 1988. Diffuse type of senile plaques in the brains of Alzheimer-type dementia. *Acta Neuropathol.* **77**: 113–119.
30. Yang, F., Lim, G. P., Begum, A. N., Ubeda, O. J., Simmons, M. R., Ambegaokar, S. S., Chen, P. P., Kayed, R., Glabe, C. G., Frautschy, S. A. and Cole, G. M. 2005. Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid *in vivo*. *J. Biol. Chem.* **280**: 5892–5901.