Curcumin labels amyloid pathology in vivo, disrupts existing plaques, and partially restores distorted neurites in an Alzheimer mouse model

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Abstract
Alzheimer’s disease (AD) is characterized by senile plaques and neurodegeneration although the neurotoxic mechanisms have not been completely elucidated. It is clear that both oxidative stress and inflammation play an important role in the illness. The compound curcumin, with a broad spectrum of anti-oxidant, anti-inflammatory, and anti-fibrilogenic activities may represent a promising approach for preventing or treating AD. Curcumin is a small fluorescent compound that binds to amyloid deposits. In the present work we used in vivo multiphoton microscopy (MPM) to demonstrate that curcumin crosses the blood–brain barrier and labels senile plaques and cerebrovascular amyloid angiopathy (CAA) in APPswe/PS1dE9 mice. Moreover, systemic treatment of mice with curcumin for 7 days clears and reduces existing plaques, as monitored with longitudinal imaging, suggesting a potent disaggregation effect. Curcumin also led to a limited, but significant reversal of structural changes in dystrophic dendrites, including abnormal curvature and dystrophy size. Together, these data suggest that curcumin reverses existing amyloid pathology and associated neurotoxicity in a mouse model of AD. This approach could lead to more effective clinical therapies for the prevention of oxidative stress, inflammation and neurotoxicity associated with AD.

Keywords: Alzheimer, curcumin, imaging, multiphoton, neuritic dystrophy, senile plaque.

brain microglial cells (Kim et al. 2003; Jung et al. 2006). Curcumin also inhibits the formation of Aβ oligomers and fibrils in vitro (Ono et al. 2004; Yang et al. 2005). Other studies have shown that curcumin prevents neuronal damage (Shukla et al. 2003), and reduces both oxidative damage (Lim et al. 2001) and amyloid accumulation (Yang et al. 2005) in a transgenic mouse model of AD. Clinical trials with curcumin have shown that the compound is not only safe but may be a chemoprotective (Cheng et al. 2001) and anti-inflammatory (Holt et al. 2005) drug. In the present work, we used multiphoton microscopy (MPM) and longitudinal imaging to evaluate in vivo and in real time the effect of systemic curcumin administration on existing Aβ deposits using aged APPswe/PS1dE9 transgenic mice. We also assessed the effect of curcumin on the dendritic abnormalities associated with dense-core plaques. We found that curcumin clears and reduces plaques, and partially restores the altered neurite structure near and away from plaques, adding evidence that curcumin has beneficial effects in reducing the pathology and neurotoxicity of AD in transgenic mice.

Material and methods

Animals

Adult male and female APPswe/PS1dE9 mice aged 7.5–8.5 months were obtained from Jackson Lab (Bar Harbor, ME, USA). All studies were conducted with approved protocols from the Massachusetts General Hospital Animal Care and Use Committee and in compliance with NIH guidelines for the use of experimental animals.

Materials

Primary antibodies Anti-Iba-1 (Wako Chemicals, Richmond, VA, USA), and anti-smi-32 (Stemberger Monoclonals Inc, Berkeley, CA, USA) were used. Anti-rabbit Alexa Fluor 594, anti-mouse Cy-3 and Texas Red dextran 70 000 Da were from Molecular probes, Eugene, OR, USA. Methoxy-XO4 was a generous gift from Dr Klunk, University of Pittsburgh. ELISA Aβ40-42 kits were from Takeda (Deerfield, IL, USA). Curcumin, thioflavin S, and common chemical reagents where obtained from Sigma (St Louis, MO, USA).

Methods

Curcumin labeling of amyloid deposits

Ex vivo staining. Paraformaldehyde fixed brain sections (30 μm) of APPswe/PS1dE9 mice were used for the ex vivo assays. Mounted tissue was dehydrated and treated for 20 min with different concentrations of curcumin (10 μmol/L–1 mmol/L). Sections were rinsed off, aqueous mounted and imaged with epifluorescence. Sections were also stained with thioflavin S 0.001% for 15 more minutes and the same sites were reimaged for histochemical assessment.

In vivo staining. To assess whether curcumin crosses the blood brain barrier and to establish amyloid staining in vivo, 3 APPswe/PS1dE9 mice were treated with curcumin (7.5 mg/kg/day) for 7 days i.v. via tail vein. APPswe/PS1dE9 mice have been previously shown to develop early Aβ deposits around 4 months of age, whereas by 6–8 months of age senile plaques and amyloid angiopathy are easily detectable with no differences due to gender (Garcia-Alloza et al. 2006b). Therefore animals with a substantial amyloid burden, between 7.5 and 8.5 months were selected for this study. Mice were anesthetized with avertin and 6 mm craniotomies were performed with dura intact as previously described (Bacsakai et al. 2002a; Skoch et al. 2005). A glass coverslip was attached with dental cement providing optical access to the underlying region of the brain. Mice were imaged immediately after surgery and allowed to recover. The animals were imaged 1 h after the first administration of curcumin and after the last day of treatment. Animals received methoxy-XO4 i.p. (~5 mg/kg), a Congo Red derivative that crosses the blood brain barrier and binds fibrillar Aβ, immediately after the second imaging session to avoid any interference with curcumin fluorescence. Animals were re-imaged one last time 24 h later for histochemical confirmation. Angiograms were performed with 12.5 mg/mL i.v. injection of Texas Red dextran proceeding each imaging session.

In vivo treatment with curcumin

Four mice were injected with methoxy-XO4 i.p. (~5 mg/kg) 24 h prior to placing cranial windows as described above, and animals were imaged for the first time. After the first imaging session curcumin was administered via daily tail vein injections [7.5 mg/kg/day in phosphate-buffered saline (PBS)] for 7 days. Methoxy-XO4 was injected i.p. and the same sites were imaged one last time after the treatment. Angiograms were performed with 12.5 mg/mL i.v. injection of Texas Red dextran proceeding each imaging session. Control animals followed similar procedures but received PBS i.v. for 7 days.

Multiphoton imaging and processing

As previously described (McLellan et al. 2003; Garcia-Alloza et al. 2006b; Robbins et al. 2006), two-photon fluorescence was generated with 800-nm excitation from a mode-locked Ti:Sapphire laser (MaiTai, Spectra-Physics, Mountain View, CA, USA) mounted on a multiphoton imaging system (Bio-Rad 1024ES, Bio-Rad, Hercules, CA, USA). A custom-built external detector containing three photomultiplier tubes (Hamamatsu Photonics, Bridgewater, NJ, USA) collected emitted light in the range 380–480, 500–540 and 560–650 nm. Ex vivo imaging was performed using the normal scan speed and multiple z-series were collected after adding curcumin and again after adding thioflavin S using a 20× water immersion objective (615 × 615 μm, z/step 2 μm, depth 30 μm approximately). In vivo imaging was conducted under the same conditions (615 × 615 μm; z/step 5 μm, depth 200 μm approximately). Images were analyzed with ImageJ software (NIH, freeware) and Adobe Photoshop 7.0 (Adobe Systems Incorporated, San Antonio, CA, USA) software packages as previously described to determine plaque size and CAA deposition after every session (Garcia-Alloza et al. 2006b; Robbins et al. 2006).

Aβ ELISA measurements

Soluble and insoluble Aβ40 and Aβ42 were quantified in frozen hemispheres using colorimetric ELISA kits as previously described (Kawarabayashi et al. 2001) with minor modifications. Hemibrains were homogenized for 45 s at speed 20 (BioSpec Tissue-Tearor™);
BioSpec Products Inc., Bartlesville, OK, USA) in extraction buffer (10 µL/mg brain mass) with protease inhibitor (Complete Protease Inhibitor Cocktail, Roche Diagnostics GmbH, Mannheim, Germany). Extraction buffer consisted of deionized water with 50 mmol/L Tris–HCl, 2 mmol/L EDTA 2Na, 0.1% Merthiorate Na, 400 mmol/L NaCl, and 1% bovine serum albumin. One milliliter of each homogenized brain was centrifuged at 50,000 g for 5 min at 4°C. The supernatant was removed (soluble Aβ, 1 : 10 dilution), and the pellet was diluted 1 : 8 and homogenized in 70% formic acid (800 µl formic acid for a 100 mg pellet) and centrifuged at 50,000 g for 5 min at 4°C. Supernatant was removed again (insoluble Aβ, 1 : 10). Prior to plate loading, insoluble Aβ was neutralized and diluted 1 : 25 in Tris buffer with pH = 11 (1 mol/L Tris with 70% formic acid). Final dilution of insoluble Aβ prior to plate loading was 1 : 200. All samples were analyzed in duplicate. Standard curves were made using human Aβ40 and Aβ42 standards provided in the ELISA kit diluted in Triton-X extraction buffer (for soluble Aβ) or Tris–neutralized FA (for insoluble Aβ). Absorbance was measured by Wallac Victor 2 1420 Multilabel Counter (PerkinElmer Life & Analytical Sciences, Shelton, CT, USA) and data expressed as pmol/g wet tissue.

**Immunohistochemistry**

**Microglia staining.** Paraformaldehyde fixed hemibrains were sectioned (30 μm) and immunostained with anti-Iba-1 antibody (1 : 2000) as previously described (Li et al. 2006) and anti-rabbit Alexa Fluor 594 (1 : 200) was used as secondary antibody. Senile plaques were stained with thioflavin S 0.05% in 50% ethanol for 8 min. Micrographs of stained tissue were obtained on an upright Olympus BX51 (Olympus, Center Valley, PA, USA) fluorescence microscope with a DP70 camera using DPController and CPManager software (Olympus).

**Dendritic staining.** Dendritic curvature was determined following SMI-32 (1 : 1000) immunohistochemistry (Fuentes-Santamaria et al. 2006) on one of every 30 paraformaldehyde fixed hemibrain sections (30 μm) under the cranial window as previously described with minor modifications. The sections were pretreated with 10 mmol/L citrate buffer pH 6 at 95°C for 10 min followed by 70% formic acid at 21 ± 2°C for 10 min and anti-mouse conjugated Cy-3 (1 : 200) was used as secondary antibody. Senile plaques were stained with thioflavin S 0.05% in 50% ethanol for 8 min. Micrographs of stained tissue were obtained as described for microglia immunohistochemistry. Dendrite curvature ratio was calculated by dividing the end-to-end distance of a dendrite segment by the total length between the two segment ends (Knowles et al. 1999; D’Amore et al. 2003; Lombardo et al. 2003; Garcia-Alloza et al. 2006a). Dendrite distance to the closest senile plaque was measured at three points along each dendrite and the average distance was taken from these three measurements. Dendrite curvature ratio and distance to senile plaques as well as dystrophy size was measured using ImageJ and Photoshop.

**Statistical analysis**

To assess the evolution of senile plaques we used Student’s t-test for dependent samples. CAA, soluble and insoluble Aβ40 and 42 and dendrite curvature and dystrophy size were analyzed by Student’s t-test for independent samples. To assess the effect of curcumin on CAA progression we used a linear model that allows for mouse-specific and vessel segment-specific effects as previously described (Garcia-Alloza et al. 2006b).

**Results**

**Curcumin labeling of amyloid deposits**

*Ex vivo* treatment of paraformaldehyde fixed brain tissue sections from APP mouse with curcumin showed bright green fluorescent staining after a 20-min incubation. Curcumin staining was histochemically confirmed with thioflavin S, as shown in Fig. 1 (panels a and b). *Ex vivo* staining showed that all thioflavin S-positive plaques were also curcumin positive. *Ex vivo* staining with methoxy-XO4 also showed co-localization, regardless of whether curcumin was added before or after methoxy-XO4, demonstrating that the two compounds do not compete for binding to senile plaques.

We imaged the brains of live mice with MPM 1 h after i.v. administration of curcumin to check for labeling *in vivo* and we did not observe significant amyloid fluorescence. However, after 7 days of daily treatment with curcumin we observed that both senile plaques and CAA were green fluorescent. Histochemical confirmation *in vivo* with methoxy-XO4 demonstrated that not all plaques were labeled with curcumin (Fig. 1 panels c, d and e). Together, these results demonstrate that curcumin crosses blood–brain barrier and binds to amyloid deposits. The ability of curcumin to cross the blood–brain barrier appears weak, but its affinity for Aβ appears strong as cumulative administration leads to detectable labeling. These results are in agreement with a previous study utilizing radiolabeled curcumin (Ryu et al. 2006).

**Effect of curcumin on amyloid beta deposits**

We used longitudinal imaging with MPM *in vivo* to test the effect of curcumin on existing amyloid deposits. Plaques were imaged before, and 7 days following curcumin treatment. Individual plaques were identified, measured, and compared at the two time points using fluorescent angiograms as independent fiduciary markers. Fig. 2 shows representative examples of an imaging volume before and 7 days after the treatment. The control-treated animal looks unchanged within this treatment period, but the curcumin-treated mouse has noticeably fewer and smaller plaques. By examining the populations of measured plaques, a significant reduction in plaque size (~30%) was observed after 7 days of treatment (Fig. 3), suggesting that curcumin is capable of reducing amyloid deposition. This result is comparable with measuring the Aβ burden in the cortex, however, longitudinal imaging with MPM allowed us to dissect the contributions of new plaque formation, plaque clearance, and reductions in plaque size. On average, we observed ~6 new plaques/mm² and ~6

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cleared plaques/mm$^3$ in control mice during the 7 day interval, supporting a dynamic balance between amyloid deposition and clearance. It is known that the number of plaques increases over time in these transgenic mouse models (Garcia-Alloza et al. 2006b), however, in the 1-week interval this rate is relatively slow. In curcumin-treated mice we observed the appearance of $\sim$4 new plaques/mm$^3$ whereas $\sim$21 plaques/mm$^3$ disappeared after 7 days of treatment, demonstrating that the balance can be altered in favor of clearance. These data suggest that curcumin can partially prevent the appearance of new plaques and dramatically clear existing deposits. Remaining plaques, when newly formed or completely cleared plaques were excluded from the analysis, were reduced in size by about 16% in the curcumin-treated animals compared with a trend to increase in size (14%) in vehicle-treated mice (Control: first session = 237 ± 18, second session = 270 ± 22; Curcumin: first session = 262 ± 28, second session = 219 ± 15 $\mu$m$^2$; $n = 60–117$ plaques from 3–4 animals).

Vascular amyloid (CAA) in this mouse model is modest, and limited to small vessels at the age used in this study (Garcia-Alloza et al. 2006b). However, cursory examination of the effect of curcumin treatment revealed a tendency toward reduced progression of CAA that was not statistically significant. There was no evidence for clearance of vascular amyloid during treatment.

**Effect of curcumin on Aβ40 and 42 levels**

At the end of the experiments, the mice were killed, and the brains hemisected. One half was flash frozen, homogenized, and used for measurements of Aβ. ELISA determinations showed that curcumin administered for 7 days led to a tendency to reduce soluble Aβ40 levels and to increase soluble Aβ42 but did not reach statistical significance (Table 1). However, as a consequence, a significant increase in the soluble ratio of Aβ42/40 was detected. No effect was detected on insoluble Aβ40, Aβ42 or in the insoluble ratio of Aβ42/40 after the treatment, and this ratio was similar to those previously described (Jankowsky et al. 2003; Garcia-Alloza et al. 2006b).

**Microglia activation**

The contralateral hemispheres of the brains were fixed in paraformaldehyde and processed for immunohistochemistry. Activated microglia labeled with anti-Iba-1 antibodies were observed surrounding senile plaques, as previously shown.
(Simard et al. 2006), and some parenchymal microglia activation was also observed, probably as a consequence of the cranial window. No visible differences were observed in activated microglia when untreated control and curcumin-treated animals were compared, as shown in Fig. 4, suggesting that curcumin amyloid clearance is not strictly mediated by activation of microglia, although some indirect contribution cannot be excluded.

**Effect of curcumin on dendritic abnormalities**

Neurites are distorted and curvy near senile plaques, and this structural pathology can be measured quantitatively (Knowles et al. 1999; Le et al. 2001; Spires et al. 2005; Garcia-Alloza et al. 2006a). Reversal of amyloid deposits with anti-Aβ antibody treatment leads to recovery of these morphological abnormalities, with straightening of neurites (Lombardo et al. 2003) and improvement of dystrophic swelling (Brendza et al. 2005). We used the same analytical approaches to examine neurite morphology after curcumin treatment. Neurite morphology was examined in postmortem tissue with SMI32 staining, and when we compared the curvature ratios of neurites in very close proximity to the plaques (<50 μm) we observed a statistically significant improvement in the curcumin-treated mice (Fig. 5).

![Fig. 2](image-url) Representative longitudinal imaging of plaques with multiphoton microscopy before and after curcumin treatment in an APPswe/PS1dE9 mouse. (a) Control first session, (b) control second session, (c) curcumin first session, (d) curcumin second session. Angiograms were performed with Texas Red dextran 70 kDa. White arrows point to reduced or disappeared plaques after 7 days of i.v. treatment with curcumin (7.5 mg/kg/day i.v., 7 days). Scale bar = 125 μm.

![Fig. 3](image-url) Curcumin (7.5 mg/kg/day i.v., 7 days) clears or reduces size of senile plaques in APPswe/PS1dE9 mice. Data are representative of 69–175 plaques from three to four mice and results are expressed as percentage of plaque size in the first session. Student’s t-test for independent samples showed no differences for control animals (p = 0.067). A significant reduction in plaque size was observed in curcumin-treated animals (*p < 0.0001 second session vs. first session).
proximal to plaques, probably reflecting toxicity related to a soluble agent. In this case dendrites located at least 50 μm from the plaque border also showed higher curvature ratios (less curvy) in curcumin-treated animals compared with vehicle-treated animals (Table 2). This result suggests that curcumin can reverse neuritic abnormalities far from plaques, probably resulting from soluble toxic species of Aβ. We also analyzed dystrophies normally found in the immediate surrounding of senile plaques, and we observed a statistically significant reduction in dystrophy size after curcumin treatment (Table 2). All together, these results demonstrate that the altered neurite morphologies resulting from Aβ production can be at least partially reversed with curcumin administration.

### Discussion

Alzheimer’s disease is characterized by several pathological features that include senile plaques. Plaques contain a myriad of molecules, apart from Aβ, including apolipoprotein E, phosphorylated tau and many others (Armstrong 2006). Although the exact role of these various bioactive compounds is difficult to dissect, the microenvironment surrounding plaques is a local source of oxidative stress (Behl and Moosmann 2002). Plaque deposition is tightly associated with neurotoxicity, as exhibited by dystrophies and distorted neurites (Knowles et al. 1999; Le et al. 2001; Garcia-Alloza et al. 2006a). However, soluble aggregates of Aβ including oligomers and ADDLs may contribute to neurotoxicity (Selkoe 1994).

In the present study, we show that curcumin stains amyloid deposits ex vivo, confirming previous studies in other transgenic mouse models and in tissue sections from AD patients (Yang et al. 2005). When we assessed the capacity of curcumin to cross the blood–brain barrier and label amyloid deposits in vivo, we observed using MPM that after a single i.v. dose the staining with curcumin was undetectable, despite intravascular fluorescence. However, after administering curcumin i.v. daily for 7 days we observed that senile plaques and CAA were stained with curcumin, demonstrating that it crosses the blood–brain barrier and, importantly, accumulates in the local vicinity of senile plaques. This observation supports a trial where radiolabeled curcumin was evaluated as a ligand for non-invasive amyloid imaging (Ryu et al. 2006). The dose of curcumin that we used (7.5 mg/kg/day) is less than the ‘low dose’ (24 mg/kg formulated in chow) of previous reports (Lim et al. 2001; Yang et al. 2005), and is much...
lower than tolerable doses approaching 2000 mg/kg (Lim et al. 2001). While careful toxicological testing will be needed, the dose used in this study resulted in no observable side effects in the mice.

When we examined the effect of curcumin treatment in vivo on amyloid deposition we observed that curcumin was capable of reducing plaque burden. This occurred by preventing formation of new deposits, clearing existing deposits, and reducing the size of remaining deposits. Together, these data confirm that curcumin can not only suppress new amyloid accumulation, as observed in tissue sections from chronically treated animals (Yang et al. 2005), but it can also remove the previously deposited amyloid. This can be attributed to the capacity of curcumin to disaggregate and inhibit Aβ aggregation as previously described in vitro (Ono et al. 2004; Yang et al. 2005). However, due to the broad range of activities of curcumin, it cannot be excluded that some other actions are contributing to the observed clearance of amyloid. In this sense, other well-known anti-oxidants such as Ginkgo biloba extract (EGb 761) or vitamin E do not seem to affect Aβ deposition or clearance (Garcia-Alloza et al. 2006a) and non-steroidal anti-inflammatory drugs such as ibuprofen show a surprisingly limited effect on inflammatory markers in APPswe transgenic mice when compared with curcumin (Cole et al. 2004). However, no study has systematically

<table>
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<tr>
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<th>Curvature ratio (up to 50 μm)</th>
<th>Curvature ratio (50–125 μm)</th>
<th>Dystrophy size (μm²)</th>
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<tbody>
<tr>
<td>Untreated control</td>
<td>0.928 ± 0.005</td>
<td>0.952 ± 0.006</td>
<td>15.76 ± 1.35</td>
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<tr>
<td>Curcumin</td>
<td>0.948 ± 0.004*</td>
<td>0.966 ± 0.003*</td>
<td>12.35 ± 0.82*</td>
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Dendrite curvature ratio was calculated by dividing the end-to-end distance of a dendrite segment by the total length between the two segment ends. Data are mean ± SDM from three to four animals and differences are detected by Student’s t-test for independent samples. Curcumin significantly reduces dendritic curvature in the proximity of senile plaques (<50 μm) (*p = 0.002 curcumin vs. untreated control group, n = 226–391) and also reduces curvature ratio in dendrites more than 50 μm away from plaques (50–125 μm from the plaque border) (*p < 0.05 curcumin vs. untreated control group, n = 33–79). Curcumin also reduced dystrophy size in curcumin-treated mice (*p = 0.024 curcumin vs. untreated control group, n = 195–409 dystrophies from three to four mice).

Fig. 5 Representative images of the effect of curcumin on curvy neurites and dystrophies. Example of dendritic curvature ratio (a, b) and characteristic dystrophies (c, d) in 30 μm brain sections of APPswe/PS1dE9 mice stained with an anti-smi-32 antibody. Senile plaques are stained with thioflavin S 0.05%. (a, c) vehicle-treated and (b, d) curcumin-treated (7.5 mg/kg/day i.v., 7 days) mouse. Scale bar = 100 μm (a, b) and 10 μm (c, d).

Table 2 Effect of curcumin on neuritic abnormalities in APPswe/PS1dE9 mice

![Representative images of the effect of curcumin on curvy neurites and dystrophies. Example of dendritic curvature ratio (a, b) and characteristic dystrophies (c, d) in 30 μm brain sections of APPswe/PS1dE9 mice stained with an anti-smi-32 antibody. Senile plaques are stained with thioflavin S 0.05%. (a, c) vehicle-treated and (b, d) curcumin-treated (7.5 mg/kg/day i.v., 7 days) mouse. Scale bar = 100 μm (a, b) and 10 μm (c, d).]
assessed a broad range of anti-oxidants and anti-inflammatory drugs in the same paradigm.

With regard to biochemical measures of Aβ, we observed that curcumin led to a tendency to reduce soluble Aβ40 as well as to increase soluble Aβ42, and as a consequence the soluble Aβ42/40 ratio was significantly increased. Although a reduction in both soluble and insoluble Aβ has been previously described (Lim et al. 2001), those animals were chronically treated for 6 months with a different dosing and route. Our experiments were limited to 1 week of treatment, resulting in measurable clearance of deposited Aβ without large effects on biochemical measures of Aβ. It is intriguing that in our study we observed clearance of amyloid and reduced toxicity as measured by examining the structural alterations of neurites near plaques, despite the increase in soluble Aβ42/40 ratio which has been implicated as causative in AD (Walker et al. 2005). The animal model used in this work, APPswe/PS1dE9, has a higher Aβ42/40 ratio probably due to the PS1 mutation (Borchelt et al. 2002; Garcia-Alloza et al. 2006b), and the immediate effect of curcumin tends to exacerbate this tendency. Further studies with mouse models that favor the Aβ40/42 ratio could also help to elucidate this question. It is also possible that no changes in Aβ40 levels were detected because it might be more easily cleared.

We did not detect a statistically significant effect of curcumin on insoluble Aβ levels, despite the fact that a clear reduction in plaque size was observed after the treatment. A precedence exists for the lack of an effect on biochemical measures of Aβ following anti-Aβ therapy, despite improvements in behavioral tests (Janus et al. 2000). In our hands, this effect also suggests that longitudinal imaging provides a very powerful tool to detect amyloid clearance in vivo when other ex vivo or biochemical approaches are not sensitive enough to detect early changes. In total, it seems clear that there is an overall beneficial effect of curcumin in this and previous studies through modulation of Aβ (Shukla et al. 2003; Ono et al. 2004; Yang et al. 2005; Jung et al. 2006). Moreover, studies in rodents have shown that curcumin partially reversed behavioral abnormalities in APP mice (Frautschy et al. 2001) or after heavy metal induced neurotoxicity (Dairam et al. 2007).

Senile plaque deposition can be reduced by anti-Aβ antibodies (Bacsakai et al. 2002b) and this effect seems to be at least partially mediated by microglial activation (Bard et al. 2000; Wilcock et al. 2003). However, previous studies with curcumin have shown its capacity to attenuate inflammatory response of brain microglial cells (Kim et al. 2003). In our hands microglia staining showed a classical distribution of microglial cells surrounding plaques without any apparent differences between curcumin treated and control mice. Although it is possible that the effects observed in cell culture cannot be directly extrapolated to in vivo studies, it can not be excluded that microglia are indirectly implicated in the clearance of plaques. On the other hand curcumin effects are highly dose-dependent in general and therefore it is possible that doses reaching the brain in the present study were not high enough for effects on microglia.

Abnormal neuritic curvature is observed in APPswe/PS1dE9 (Garcia-Alloza et al. 2006a) and in our hands curcumin showed a neuroprotective effect that led to a significant straightening of the distorted dendrites near and away from plaques. These effects suggest that aggregated Aβ as well as soluble Aβ species results in abnormal neuritic morphology that is reversible. However, we can not exclude an effect on inflammation or oxidative stress that may be responsible for the observed straightening effect. Previous studies have shown that abnormal neuronal morphology is correlated with the presence of Aβ and with neuronal dysfunction (Stern et al. 2004) and that anti-Aβ antibodies led to a rapid normalization of neuritic curvature (Lombardo et al. 2003). Other anti-oxidants also show a similar effect in vivo (Garcia-Alloza et al. 2006a), however, in contrast to anti-oxidant treatments, curcumin also reduces dystrophy size in close proximity to the plaques. This effect was demonstrated after anti-Aβ antibody treatment (Brendza et al. 2005), and may be an important determinant of an effective therapy. Although the anti-oxidant effects of curcumin cannot be excluded, other actions or a combination of actions of curcumin may be responsible for the effects observed on dystrophy size. It is also possible that the mechanisms underlying the alterations in trajectories and dystrophies may differ. Altogether, these data show that curcumin can prevent and reduce amyloid deposition in vivo and that it also partially restores dendritic abnormalities, suggesting that multifactorial curcumin effects can reverse a range of pathological features associated with AD. These studies provide support for the further elucidation of the mechanism of action of curcumin as a possible therapeutic treatment of AD.

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References


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radiolabeling, and evaluation for beta-amyloid plaque imaging. 