Neuroinflammation in Alzheimer’s disease: different molecular targets and potential therapeutic agents including curcumin
Balmiki Ray¹ and Debmoy K Lahiri¹,²

Alzheimer’s disease (AD) is a neurodegenerative disorder of the elderly. Deposition of amyloid β plaque and associated neuroinflammation are the major hallmarks of AD. Whereas reactive oxygen species (ROS) and activated microglial cells contribute to neuronal loss, nuclear factor κB and apolipoprotein E participate in inflammatory process of AD. Current FDA approved drugs provide only symptomatic relief in AD. For broad spectrum of activity, some natural products are also being tested. Turmeric is used as an anti-inflammatory medicine in various regions of Asia. Curcumin, which is a yellow colored polyphenol compound present in turmeric, showed anti-inflammatory properties. Herein, we discuss the neurobiological and neuroinflammatory pathways of AD, evaluate different molecular targets and potential therapeutic agents, including curcumin, for the treatment of AD.

**Addresses**
1 Laboratory of Molecular Neurogenetics, Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, 791 Union Drive, Indianapolis, IN 46202, USA
2 Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN, USA

**Corresponding author:** Lahiri, Debmoy K (dlahiri@iupui.edu)

---

**Current Opinion in Pharmacology** 2009, 9:434–444

This review comes from a themed issue on Cancer
Edited by Bharat B Aggarwal

Available online 3rd August 2009
1471-4892/$ – see front matter Published by Elsevier Ltd.

DOI 10.1016/j.coph.2009.06.012

---

**Alzheimer’s disease—basic pathology**

Alzheimer’s disease (AD) is one of the progressive degenerative diseases of the central nervous system (CNS) and is the most common cause of dementia in the elderly population and the fourth leading cause of death in developed countries, after cancer, cardiovascular diseases, and stroke. Approximately 5 million Americans are suffering from this disease and 14 million are projected to have AD by the middle of the next century if a definite curative or preventive medication is not discovered. Pathologically, dementia in AD patients is caused by at least two distinct characteristic events, deposition of the amyloid beta (Aβ) peptide in the intercellular space and formation of intraneuronal tangle owing to hyperphosphorylation of axonal Tau protein [¹,²**]. Although deposition of Aβ peptide and associated reactive oxygen species (ROS) mediated neuronal damage is one of the major hallmarks of AD, the exact sequence of neuronal loss, synaptic dysfunction, and biochemical cascade of events therein are still unknown.

**Pathological stages**

Staging of AD has been performed by immunocytochemical techniques based on neurofibrillary pathology affecting different regions of the brain [³] and the following pathological stages were described:

- **Stage I:** Mild involvement that is confined to the transentorhinal region.
- **Stage II:** Lesion gradually increases and the pathology extends to entorhinal region.
- **Stage III:** Pathology in entorhinal region worsens and lesions extend to adjoining neocortex.
- **Stage IV:** Neurofibrillary pathology extends up to medial temporal gyrus.
- **Stage V:** Lesion extends up to occipital neocortex.
- **Stage VI:** Lesions visible in striate and parastriate areas of occipital neocortex.

**Early and late onset AD**

Genetics of AD is complex and heterogeneous. Researchers reported mutations and polymorphisms of at least five chromosomes (1, 4, 9, 14, and 21) related to the occurrence of AD [⁴]. On the basis of genetic etiology AD can be broadly divided into two groups: Familial form or Early Onset Alzheimer’s disease (EOAD) that occurs before 65 years of age and Late Onset Alzheimer’s Disease (LOAD) that occurs after 65 years of age and is usually sporadic [⁵]. Amyloid β precursor protein (APP) and presenilin (PS) 1 and 2 are considered closely involved in the etiology of familial AD. Apolipoprotein E (APOE) gene has three common alleles, epsilon 2 (ɛ2), epsilon 3 (ɛ3), and epsilon 4 (ɛ4). The ɛ2 allele is considered protective but presence of the ɛ4 is considered a risk factor for developing LOAD, and presence of ɛ4 allele increases the risk for AD from 20% to 90% and decreases the age of onset from 84 to 68 years depending on gene dose of ɛ4 alleles [⁶].

**Key molecules in the pathogenesis of AD**

**APP:** APP is a mammalian transmembrane protein containing 695–770 amino acid residues that has a large ecto- and endocytic N-terminus domain and a shorter intracytoplasmic carboxyl-terminal region. APP is cleaved by β-site APP cleaving enzyme 1 (BACE1) to produce sAPPβ and a C-terminal fragment containing 99 amino acid residues (C99) [¹]. C99 is further cleaved by γ
secretase to produce Aβ peptides of varying numbers of  
amino acid residues. Aβ with a forty amino acid residue  
Aβ (1–40) is the most abundant form and is less patho-  
genic for the development of AD. However, Aβ with 42  
amino acid residue Aβ (1–42), which is produced in much  
lower quantities, is fibrilar in nature and forms aggregates  
that is pathognomonic in AD.

**BACE-1**: BACE-1, initially termed as β secretase,  
catalyzes the initial step of C99 cleavage to form Aβ  
peptide [1,2]. BACE-1 was identified using Expression  
Sequence Tag (EST) and found to be aspartyl protease in  
nature. A close homolog, BACE-2, was also identified but  
its physiological role is unclear and there is no compelling  
evidence that it has any role in direct β-site cleavage of  
human APP protein. BACE-1 is synthesized in rough  
endoplasmic reticulum and transported to Golgi network  
as pre-BACE-1 that has molecular weight of approxi-  
mately 65 kDa. Pre-BACE-1 undergoes maturation and  
post-translational modifications to form active BACE-1  
that has molecular weight of 75 kDa. Other functions of  
BACE-1 were studied using BACE-1 knockout mice and  
it was found that it might have a potential role in mye-  
ellation of peripheral nerves [7]. BACE-1 may have  
several other functions in the central and peripheral  
nervous system that are yet to be discovered. The  
molecular cascade leading to Aβ processing from APP  
by BACE-1 and other enzymes is shown in Figure 1.

**APOE**: ApoE is a lipoprotein that is part of chylomicrons,  
LDL and HDL and participates in the transport of lipids,  
particularly cholesterol. As already mentioned, the pre-  
sence of APOE e4 allele is related to an increased risk of  
AD. A potential function of ApoE in CNS is to decrease  
Aβ aggregation. However, it was found that inhibitory  
effect of Aβ aggregation is minimal in the people carrying  
APOE e4 allele(s) than the other alleles that ultimately  
leads to more Aβ deposition [8]. Studies on APOE knock-  
out mice revealed that it might have roles in the main-  
tenance of neuritic connections that is significantly lost in  
AD and when compared to the neuritic connections in  
APOEe4 transgenic and APOE knockout mice, Veinbergs  
et al. noted a similar pattern in both the groups indicating  
that APOEe4 has deficient role in neuritic maintenance  
[9]. Furthermore, it was found that APOEe4 has hyperin-  
flammatory properties leading to neuronal damage in AD  
brain [10].

**Inflammations in AD**

**Role of activated glia**

In AD, production of Aβ and concurrent ROS production  
synergistically increases the damage to the neurons [11].
Glial activation in the progression of AD related dementia was initially described by Alois Alzheimer. Glial (astrocyte) reactivation occurs around plaques to take up Aβ and neuronal debris. Not only that, activated astrocytes are also involved in plaque formation [12]. Apart from astrocytes, microglias also have an important role in the inflammatory response in the AD brain. It is thought that interaction of microglia with Aβ peptide gives rise to ROS and several other chemokines and cytokines, which work as inflammatory mediators and eventually may cause neuronal damage [13]. Furthermore, activation of complement cascade forms membrane attack complexes, which not only cause substantial damage to the neurons but also can lead to phosphorylation of Tau protein leading to formation of neurofibrillary tangles [14].

**Role of nuclear factor κ beta (NFκB)**

NFκB is a transcription factor located within the cytoplasm and responsible for regulation in cytokine production. Normally NFκB stays inactivated by an inhibitory protein IκB. Once activated, NFκB enters the nucleus and increases the transcription of different inflammatory mediators. Several molecules are capable of activating NFκB including TNFα, Aβ, and secreted APP [15,16]. Gene mapping shows that NFκB sites are present in the regulatory (promoter) region of APP, PS, and BACE-1 genes. An activation of NFκB increases transcription of APP and BACE-1, which eventually leads to increase in Aβ production. It was also found that the level of NFκB in the brain is significantly increased in the presence of APOE ε4 when compared with activation in the presence of APOE ε3 [10]. Thus, APOE ε4 might have roles in the activation of NFκB leading to further damage. An APOE gene promoter study showed that Aβ can stimulate APOE through NFκB-dependent pathway [17].

**Role of peroxisome proliferator-activated receptor-γ (PPARγ)**

PPARγ is a ligand-dependent nuclear hormone receptor transcription factor, which regulates inflammatory responses in different organ systems including CNS. When activated, PPARγ binds to peroxisome proliferator response element (PPRE) within the promoter regions of targeted genes of inflammatory mediators in T cells, such as TNFα, IL-10, IFN-γ, and IL-4, and regulates their expression [18]. PPARγ activation not only suppresses Aβ mediated induction of microglial cells from producing pro-inflammatory cytokines but also inhibits NFκB mediated inflammatory pathways by reducing its nuclear translocation [19].

**Role of signal transducer and activator of transcription-1 (STAT-1)**

STAT-1 is a transcription factor that has binding sites in the APP and BACE-1 gene promoter regions [20,21]. It is believed that activation of STAT-1 is related to expression of APP and BACE-1, which in turn leads to accelerated Aβ formation.

Figure 2: inflammatory cascade in AD.

**Current FDA approved drugs for the treatment of AD**

**Role of cholinesterase inhibitors (ChEI)**

FDA-approved ChEIs, such as rivastigmine, donepezil, and galantamine, are considered as the first line of treatment in those patients with cognitive dysfunction, including AD. Apart from cognitive improvement, one of the ChEIs, rivastigmine was shown to lower inflammatory infiltration caused by T cells and microglia in CNS of transgenic mouse and to decrease the production of APP and BACE-1, which leads to increased formation of Aβ peptide. APP and BACE-1 transcription is also increased by STAT-1. NFκB is activated by reactive oxygen species (ROS) and APOE. Aβ peptide itself can activate glial cells to produce IL and TNFα.
inflammatory cytokines TNF-α and IFN-γ, which are involved in inflammatory response followed by neuronal damage in AD brain [22]. In another study, peripheral blood mononuclear cells (PBMC) were collected and cultured from AD patients who received donepezil 10 mg/day for 30 days or placebo treated, and from age matched healthy controls. Levels of mRNA expression of IL-1β, IL-6, and TNF-α were measured from PBMC from all groups. A significantly decreased level of these three inflammatory cytokines was observed in the cells collected from donepezil-treated AD patients compared with the same from the untreated group [23]. Furthermore, it was also suggested that cholinesterase promotes the formation of amyloid fibril from Aβ peptide, causing neuroinflammation [24**]. A newly developed ChEI posphen dose-dependently reduced APP, Aβ (1–40) and Aβ (1–42) levels and β secretase activity in vivo [25**]. Inhibition of acetylcholinesterase by acetylcholinesterase inhibitors (AChEIs) not only increases synaptic transmission but also have potent roles in reducing Aβ load and subsequent neuroinflammation. However, a significant number of people experience some adverse effects after being treated with AChEIs, like nausea, vomiting, diarrhea, anorexia, headache, syncope, abdominal pain, and dizziness [26], and therefore, the discovery of more novel drug targets is needed for AD treatment.

Role of NMDA receptor and antagonists

NMDA (N-methyl D-aspartate) is a group of ionotropic glutamate receptors present in CNS that possesses central ion channels, which allow entry of calcium ions into the neurons and can cause excitatory damage. Memantine is a noncompetitive NMDA receptor antagonist approved by the FDA for the treatment of moderate to severe AD. There is evidence that selective NMDA antagonism can protect neurons from neuroinflammation caused by activated macrophage and glutamate excitotoxicity. Rosi et al. infused lipopolysaccharide (LPS) in the fourth ventricle of young rats to produce neuroinflammation [27]. They have found that memantine treatment significantly reduced the number of activated microglia in hippocampus when co-treated with LPS compared with only LPS treated rats. Memantine can also reduce the production of Aβ in rat primary cortical neurons and APP/PS1 transgenic mice (Alley et al., 2009; in press). Paradoxically, memantine has nicotinic antagonistic properties that restrict its use in early phase of AD [28].

Role of non steroidal anti-inflammatory drugs (NSAIDs)

Inflammatory processes in AD brains indicated beneficial use of anti-inflammatory agents like ibuprofen [2**]. Non-steroidal anti-inflammatory drugs or NSAIDs were well studied both in vitro and in vivo to observe their effects to ameliorate the inflammation related to Aβ deposition in AD. Several in vitro studies showed that NSAID like aspirin might have anti-aggregation activity for Aβ [29]. Since prolonged use of NSAIDs with nonselective COX inhibition (both 1 and 2) can have several adverse effects such as gastrointestinal hemorrhage, a selective COX2 inhibitor (Celecoxib) were tested in transgenic mouse model of AD. However, it did not have a significant effect on lowering Aβ load in the brain of transgenic mice [30].

Role of statins

Statins are a group of drugs, that inhibit 3-hydroxy-3 methylglutaryl Coenzyme A reductase (HMG-CoA). HMG-CoA is the rate-limiting enzyme in cholesterol biosynthesis. Though statins are widely used in the treatment of hyperlipidemia, they have wide varieties of other functions like anti-inflammatory, antiapoptotic, immunomodulatory, antithrombotic effects that are collectively termed as ‘pleiotrophic effects’. Researchers have found decreased perfusion in AD brain due to reduced production of a potent vasodilator ‘endothelial nitric oxide synthase’ (eNOS) [31]. Statins can increase cerebrovascular perfusion by upregulating eNOS [32] that might decrease the inflammation related to Aβ deposition. However, a recent clinical trial (Alzheimer’s Disease Anti-inflammatory Prevention Trial or ADAPT) has shown no change in mean MMSE score between statin users and controls [33].

Role of PPARγ agonists

As already mentioned, activation of PPARγ can be beneficial in the treatment of neuroinflammation associated with AD. FDA approved PPARγ agonists (e.g. pioglitazone and rosiglitazone) can be useful for that purpose. However, low penetration of blood brain barrier can be an obstacle in the treatment of neuroinflammation of AD by PPARγ agonists [34].

Use of γ secretase inhibitor to block the Aβ-mediated amyloidogenic pathways

As previously discussed, the enzyme γ secretase is involved in the final cleavage of C99 fragment to produce Aβ peptide [1*]. In a clinical setting at Eli Lilly, Bateman et al. have shown that the γ secretase inhibitor LY450139 dose-dependently decreases Aβ production in human CNS [35]. Another placebo control phase I clinical trial with a γ secretase inhibitor MK0752 has recently been carried out by Merck & Co. Inc., but results of this study have not yet been published.

Alternative strategies

Herbal, natural products, and pineal hormone melatonin

From ancient times, herbal or natural products have been used for treating a wide variety of ailments in different countries. Several life-saving drugs, such as morphine, digitalis and quinine were initially isolated from plant extracts. Within the period 1983–1994, approximately 40% of newly approved drugs in North America were derived from plant products [36]. Several natural products have been used in clinical trials in AD patients with variable success. Some of them are briefly listed below:
1. **Ginkgo biloba**: Several studies were performed to evaluate its efficacy in the treatment of AD. However, meta-analysis of those studies revealed no convincing evidence that *Ginkgo biloba* is effective in the treatment of dementia and cognitive impairment [37].

2. **Huperzine A**: This compound was originally isolated from *Huperzia serrata*, a Chinese herb with AChEI activity. Some preclinical studies showed that it can protect cells from ROS-mediated damage caused by Aβ aggregation [38]. However, a systematic review and meta-analysis study found insufficient evidence about the efficacy of Huperzine A in neuro-inflammation caused by AD [39].

3. **Garlic compounds**: Some encouraging pre-clinical studies were performed with ‘aged garlic extract’ or ‘AGE’ and a garlic derived compound ‘s-allyl cysteine’ or ‘SAC’. Behavioral study on Alzheimer’s transgenic mice that overproduce Aβ (1–42) showed significant improvement in hippocampal-based memory tasks when fed with a diet containing AGE [40]. Molecular study of the brain lysate of these mice showed an increased level of presynaptic protein SNAP25 compared with control mice that did not have AGE in their diet (B Ray, DK Lahiri, unpublished data). Furthermore, cell culture studies revealed that AGE and SAC protects neuronal PC12 cells from ROS-mediated insults (B Ray, DK Lahiri, unpublished data). These studies suggest that garlic compounds possess some anti-inflammatory effects that can be beneficial in the treatment of neuroinflammation seen in AD. Though a useful ‘pleotropic’ property of garlic has been proposed, the exact molecular mechanism of action of the garlic compounds is yet to be discovered. Data from clinical studies of garlic are lacking to determine its efficacy in the treatment of neuroinflammation in AD.

4. **Melatonin**: Several pre-clinical studies have shown melatonin’s efficacy as an Aβ lowering agent and free radical scavenger. Melatonin treatment decreases the levels of both secreted APP (sAPP) and APP mRNA in cell culture and potentiate neuronal differentiation [41]. When studied in an *in vivo* model, melatonin was found to decrease the levels of cortical Aβ in mice and was also shown to protect neuronal cells from ROS mediated generation of superoxide free radicals [42]. Currently melatonin is undergoing phase II clinical trials, and reports about its efficacy in the treatment of neuroinflammation associated with AD are yet to be published.

5. **Turmeric**: Turmeric powder is obtained from the root of the plant *Curcuma longa* and is commonly used as a culinary compound in different regions of India, China, and other Asian countries. Apart from dietary use, turmeric powder is also widely used as an anti-inflammatory medication. External use of turmeric powder paste in cases of trauma and several skin disorders is still practiced in India. A large prospective epidemiological study had shown low incidence of AD in a rural Indian population compared with a reference US population [43]. Researchers concluded that several factors including consumption of natural dietary products could be causative for that finding. Turmeric is one of the natural products that has been studied extensively in the recent years and all active ingredients have been isolated by different extraction techniques. Owing to potent anti-inflammatory property, curcumin can be a useful agent in the treatment of neuroinflammation in AD.

**Curcumin: basic mechanism of action**

Inhibition of NFκB is believed to be the central pathway of curcumin’s mechanism [44**]. As mentioned, NFκB stays inactivated within the cytoplasm by an inhibitory protein, IkB. Curcumin, an active ingredient of turmeric, prevents phosphorylation of IkB and subsequent activation of NFκB [44**].

**Molecular targets of curcumin in the treatment of neuroinflammation in AD**

Several pre-clinical and molecular studies were conducted with curcumin to evaluate its potential roles as an antioxidant and anti-inflammatory. It can be concluded that curcumin may have ‘pleotropic’ properties like many other naturally derived compounds. Outcomes of some extensive research works with curcumin are discussed herein:

1. Lim *et al.* found that curcumin decreases Aβ-ROS related inflammation and Aβ burden in APP transgenic mice (with Swedish mutation) [45]. Both low and high doses of curcumin significantly decrease IL-1β level. IL-1β increases the production and processing of APP, thus favoring deposition of Aβ [46]. A low dose of curcumin significantly decreases activated glial marker GFAP, thus ameliorate glial mediated inflammation. Furthermore, a low dose of curcumin decreases microglial activation in hippocampal and cortical layers of the mouse brain. To determine the effect of curcumin on oxidative stress, western blot analysis of oxidized proteins was performed and the result showed a significant decrease in the oxidized protein levels in different areas of brain with the treatment of both low and high doses of curcumin. They have also noticed that a low dose of curcumin significantly decreases the level of insoluble Aβ and plaque burden in cortex and hippocampus of the transgenic mice.

2. In another study, Frautschy and co-workers used older female Sprague–Dawley rats, and performed intracerebroventricular infusion of Aβ (1–42) for one month [47]. A significant loss of one of the presynaptic proteins, synaptophysin, was observed in the control animals who were fed a normal diet compared with the animals that had curcumin in their diet. They also found an increase in levels of PSD-95,
which is an important post-synaptic protein, in the rats who had curcumin in their diet compared with those who had a normal diet. Increase in both pre-synaptic and post-synaptic protein levels ultimately improved synaptic transmission, which was reflected in the Morris water maze test, and the curcumin group did significantly better than did control rats.

3. Yin et al. observed that Aβ-induced mitochondrial damage and subsequent cell death in mouse cerebral endothelial cell (CEC) is related to BIM (belong to Bcl-2 family of apoptosis regulator) overproduction [48]. BIM expression is regulated by AP-1 transcription factor binding activity and inhibition of AP-1 reduces BIM expression. Curcumin protects CEC from Aβ mediated toxicity because of its potent role in AP-1 inhibition.

4. Several mechanisms behind curcumin’s anti-oxidant and anti-inflammatory properties were proposed by many investigators. Working with male Sprague–Dawley rats, Piper et al. showed that curcumin enhances the activity of detoxifying enzymes like glutathione-S-transferase [49]. It was proposed that bilirubin at physiological concentration protects endothelial cells from hydrogen peroxide (H₂O₂)-mediated injury and prevents plasma protein oxidation [50]. Heme oxygenase 1 (HO-1), a widely distributed enzyme, degrades heme to iron and biliverdin, which later converts to bilirubin. Motterlini et al. showed that low concentrations of curcumin upregulate endothelial HO-1 gene and protein expression and protect them from peroxide mediated toxicity by the mechanism mentioned above [51]. Similarly, inducible nitric oxide synthase (iNOS)-mediated production of reactive nitrogen species (RNS) and ROS can also cause neuronal damage in AD. Notably, curcumin and one of its stable metabolites tetrahydrocurcumin (THC) significantly decrease production of both iNOS protein and mRNA in transgenic mouse brain [52].

5. Previous studies revealed an increase in the concentration of copper, zinc, and iron in Aβ plaque, and some in vitro studies also showed that these metals play an important role in the aggregation of Aβ and subsequent ROS production [53]. Baum and Ng proposed metal chelation activity of curcumin and they showed that curcumin can bind with copper and iron ions and act as a chelator and suggested reduced Aβ plaque and subsequent ROS generation [54**]. Furthermore, induction of NFκB can be prevented in the presence of copper that can be prevented by the chelation activity of curcumin [55].

6. Both Aβ (1–40) and Aβ (1–42) treatment can increase mRNA expression of TNF-α, MIP-1β, IL-1β, MCP-1, and IL-8 in monocytes. It was also found that 125 nM Aβ (1–40) activates ERKs (members of MAPK family) that ultimately increase the production of early growth response-1 (Egr-1), which plays an important role in Aβ mediated production of cytochemokines. Interestingly, it was observed that 12.5–100 μM of curcumin reduces Aβ mediated expression of the cytochemokines, and it also decreases the phosphorylation of ERKs and expression of Egr-1 in a dose-dependent manner [56].

7. Atamna and Boyle showed that Aβ binds with heme with high affinity and this Aβ-heme complex acts like a peroxidase, which can cause oxidative damage in the brain [57]. They also found that curcumin inhibits Aβ-heme peroxidase in a dose-dependent way.

8. Aβ and glutamate-induced neurotoxicity may perhaps result from JNK activation. By suppressing JNK activation, curcumin exerts its role in protecting neurons [58].

9. Peschel et al. showed that curcumin dose dependently increases the expression of LDL-R mRNA in HepG2 cells, which would result in a higher net uptake of LDL-cholesterol to the liver from plasma [59]. Excessive circulatory cholesterol increase brain APOE and as already discussed, APOE may play a role in the pathogenesis of AD.

10. Kin et al. have recently found that a low dose of curcumin stimulates proliferation of neural stem cells in mouse embryonic cortical culture as well as in the hippocampus of adult mouse [60**]. The investigators also concluded that the stimulation of neural stem cells is due to activation of ERK and p38 kinases by the action of curcumin. Importantly, adult neurogenesis by curcumin can enhance neural plasticity and repair.

Figure 3 illustrates molecular targets of curcumin in the context of AD related neuroinflammation.

Curcumin increases neuronal cell viability and neurite formation at a non-toxic dose

We have tested the role of curcumin in neuronal cell viability. For this, rat neuronal pheochromocytoma (PC12) cells were treated with 30 nM of nerve growth factor (NGF) for 15 days to achieve (sympathetic) neuronal differentiation. Differentiated PC12 cells were treated with 250 nM, 500 nM, and 1 μM of curcumin. Cells were harvested after 48 h. To determine cell viability, a sensitive luminescent based Cell Titer Glo (CTG) assay was performed with the lysed cell extracts. The CTG assay measures ATP concentration in the lysate and it corresponds with the cell number. CTG assay showed a significant increase in viability of cells treated with 250 nM and 500 nM doses of curcumin (p = 0.010 and 0.002, respectively) (Figure 4a). Cells treated with 1 μM dose of curcumin did not show any change in cell viability compared to the vehicle. To measure toxicity, LDH assay was performed that revealed that 250 nM and 500 nM curcumin in conditioned media sample was non-toxic to the cells. However, secreted LDH level from the cells treated with 1 μM curcumin is insignificantly higher than that of vehicle and other two doses of curcumin (Figure 4b).
Curcumin increases neurite formation in cultured neuronal cells

To determine the role of curcumin in neurite formation, cells from the previous experiment were fixed by using 4% paraformaldehyde. After permeabilization, the fixed cells were probed with primary antibody α-tubulin (Sigma, MO, USA; 1:5000 dilution) overnight and subsequently with biotinylated goat anti-mouse secondary antibody (Vector laboratories, CA, USA; 1:500 dilution) for one hour. Finally, FITC conjugated streptavidin (Jackson immunoResearch Laboratories, PA, USA; 1:300 dilution) was added to wells. To visualize nuclei, a drop of 4′, 6-diamidino-2-phenylindole (DAPI, Sigma) was added to each well and fluorescence signals were observed under inverted fluorescence microscope (Leica Microsystems GmbH, Wetzler, Germany). Morphology of cells treated with 250 nM and 500 nM clearly showed increased number of neurites compared with vehicle treated cells. Cells treated with 1 μM curcumin also displayed more neurites than those of vehicle but the length of neurites is shorter than all treatment groups, indicating that the highest dose of curcumin tested may have some different effects on the cells (Figure 4c).

Role of curcuminoid compounds in AD

Apart from curcumin, several other compounds have been isolated from crude turmeric. Inoue et al. used high speed countercurrent chromatography (HSCCC) to separate curcumin and curcuminoid compounds from crude turmeric extract [61]. Major constituents of turmeric extract are: curcumin (~4.4%), desmethoxycurcumin (~2.4%), and bisdesmethoxycurcumin (~3.6%). The latter two compounds are termed as ‘curcuminoids’. In addition to curcumin, recent studies have found potential roles of curcuminoids compounds as effective agents against neurodegeneration and neuroinflammation as observed in AD. Ahmed et al. have found potent acetylcholine esterase properties of desmethoxy and bisdesmethoxy curcumin [62]. Fiala et al. have reported that downregulation of genes such as 1, 4-mannosyl-glycoprotein 4-N-acetylgalcosaminytransferase (MGAT3) and toll like receptors (TLR) in mononuclear cells in AD patients is related to insufficient clearance of Aβ from brain interstitial space [63]. They have also shown that one of the curcuminoids, bisdesmethoxy curcumin, increases the transcription of genes for MGAT3 and TLRs and can be a potential therapeutic agent to correct defective immune response in AD patients. Macrophage culture from AD patients...
Curcumin increases viability of neuronal cells: Differentiated PC12 cells were treated with 250 nM, 500 nM and 1 μM of curcumin. After 48 h cells were harvested and CTG (viability) assay was performed. CTG data showed an increase in viability of the cells treated with 250 nM and 500 nM of curcumin ($p = 0.010$ and $0.002$, respectively) (a). Secreted LDH was also measured in the media that indicates an insignificant ($p = 0.065$) toxicity in the cells treated with 1 μM dose of curcumin. The error bars represent mean and standard deviation of the experiments performed in quadruplets (b). Morphology pictures of the cells showed more neurite outgrowth in the cells treated with 250 nM and 500 nM of curcumin (green represents α tubulin/ whole cell body and blue represents DAPI/nuclei) than the control (c).

Curcumin in clinical studies
Clinical trials of curcumin have been performed to evaluate its potential effects in patients with several chronic diseases including cancer. As per our previous discussion, pre-clinical research revealed several potential effects of curcumin as an anti-inflammatory and anti-amyloidogenic drug but data from clinical trials in the treatment of AD are lacking. Ringman et al. in UCLA have performed a double blind phase II placebo control study to evaluate the safety of curcumin at dose of 2000 mg/day and 4000 mg/day in patients suffering from mild to moderate AD vs. age-matched healthy controls [65]. They have also looked at different AD markers like levels of Aβ and tau proteins in CSF samples. Outcome of this study has not yet been published. A six-month placebo control, randomized, double blind trial of curcumin on demented patients revealed an increase in vitamin E in the plasma of curcumin treated patients compared with controls [66]. Previous studies have shown that antioxidants are decreased in AD patient’s plasma [67]. One placebo control phase II clinical trial conducted by the National Institute of Aging (NIA) has been completed and another with the patients with mild cognitive impairment is now being conducted at Louisiana State University. Information about clinical trials of curcumin can be obtained at http://clinicaltrials.gov/ct2/results?term=curcumin.

Bioavailability of curcumin
Curcumin is sparingly soluble in water, which is one of the reasons for its poor absorption through oral route, and this has raised questions about its clinical efficacy. Almost all clinical trials used more than 1000 mg of curcumin tablets or capsules. In a phase I trial, serum concentration of curcumin was 0.51 (±0.11) μM, 0.63 (±0.06) μM, and 1.77 (±1.87) μM after ingestion of 1000 mg, 2000 mg, and 4000 mg of curcumin, respectively [68].

Nanocurcumin: a potential source of more bioavailable form of curcumin
Bisht et al. have developed a novel polymeric encapsulated form of curcumin (nanocurcumin) [69**]. They have demonstrated that nanocurcumin inhibits the expression of NFkB in pancreatic cells and also downregulates inflammatory cytokines, such as IL-6. Because of complete solubility in aqueous medium and extended release of curcumin from the nanoparticles, it can be an appropriate delivery mech-
anism of curcumin in vivo for various brain disorders. A comparative study of the effects of turmeric extracts, curcumin, natural products, and other mechanistic drugs on neuronal cell viability, neurite extension, synaptic proteins, and neuroprotection in primary cortical neuronal cultures are being carried out in our laboratory.

Conclusion

Research on molecular mechanism in different neurodegenerative disorders, including AD, to find new drug targets is expanding rapidly. Novel drug targets, such as the 3'-UTR of APP mRNA and its regulation by some drugs, have already been identified [17*]. Identification of micro RNAs, which bind and regulate 3'-UTR of important genes associated with AD pathogenesis, is another major area of current research. However, these studies are still in the preliminary stage. A role of curcumin has already been established in altering inflammatory and neoplastic disorders. Several trials are ongoing to evaluate the anti-cancer efficacy of curcumin in clinical settings. In this review we have discussed potential roles of curcumin in the treatment of neuroinflammation associated with AD. This is important as neuroinflammation, in addition to Aβ deposition, is one of the major causes of neuronal loss and synaptic dysfunction in AD. The primary aim of FDA-approved medications for the treatment of mild, moderate, and severe AD has been to increase synaptic transmission and reduce Aβ load. None of them proved to have potent anti-inflammatory activities. Unrestricted inflammation causes severe damage to the neurons that is often irreversible. We have discussed outcomes of couple of clinical trials of curcumin in AD and demented patients. Unfortunately, none of them showed a dramatic improvement in the symptoms. Low absorption of curcumin through oral route may be a cause for that. Furthermore, we have also discussed some beneficial anti-inflammatory roles for curcuminoid substances found in turmeric. A mixture of curcumin and curcuminoid compounds can be useful in this particular regard. A carrier mediated transport or nanotechnology based delivery system can increase the bioavailability of curcumin that will potentiate its effect and these are the areas going to bear enormous importance in future research.

Acknowledgements

We thank Bryan Maloney, Jason Bailey, Anirban Maitra, and Savita Bisht. This work was supported by grants from Alzheimer's Associations (Zenith Award) and the National Institutes of Health (AG18379 and AG18884) to DKL.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


A review that summarizes APP processing and its relationship with AD.


A comprehensive review about molecular mechanism and potential drug targets in AD.


This review discussed about the genetic epidemiology of different neurodegenerative disorders like Alzheimer disease, Parkinson disease, Lewy body dementia, frontotemporal dementia, amyotrophic lateral sclerosis, Huntington disease, and prion diseases.


Molecular study showed that Aβ-induced upregulation of APOE is mediated by NFκB element that is present in 5′-flanking region of APOE promoter.


24. Alvarez A, Alarcon R, Opazo C, Campos EO, Munoz FJ, Calderon FH, Dajas F, Gentry MK, Doctor BP, De Mello FG et al.: Stable complexes involving acetylcholinesterase and amyloid-beta peptide change the biochemical properties of the enzyme and increase the neurotoxicity of Alzheimer’s fibrils. J Neurosci 1998, 18:3213-3223. This study first demonstrated that in PC12 and primary retina cell, acetylcholine esterase can bind with Aβ peptide to form complex, which is more toxic than Aβ aggregate alone.


44. Singh S, Aggarwal BB: Activation of transcription factor NF-κappa B is suppressed by curcumin (diferuloylmethane) [corrected]. J Biol Chem 1995, 270:24995-25000. This is the first study that demonstrated that curcumin blocks activation of NFκB induced by TNF and various other agents including H2O2.


This study first documented that curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radic Biol Med 2000, 28:1303-1312.

It has already been documented that adult neurogenesis took place in different regions of adult brain. This study first documented that curcumin treatment increases the number of neuroprogenitor cells not only in vitro but also in the hippocampus of adult mice.

This spectrophotometric study proposed a chelating action of curcumin because of the fact that curcumin binds with copper and iron. Removal of metal ions from Aβ plaque can significantly decrease its toxic effect.

Curcumin stimulates proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus. J Biol Chem 2008, 283:14497-14505. It has already been documented that adult neurogenesis took place in different regions of adult brain. This study first documented that curcumin


60. Kim SJ, Son TG, Park HR, Park M, Kim MS, Kim HS, Chung HY, Mattson MP, Lee J: Curcumin stimulates proliferation of embryonic neural progenitor cells and neurogenesis in the adult hippocampus. J Biol Chem 2008, 283:14497-14505. It has already been documented that adult neurogenesis took place in different regions of adult brain. This study first documented that curcumin


